THE TROPHIC ECOLOGY OF GUPPIES (*Poecilia reticulata*) FROM THE STREAMS OF TRINIDAD

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DEDICATION

To the memory of the William Beebe Tropical Research Center, aka Simla,
and all the scientists that worked there between 1949 and 2010

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ABSTRACT

The Trophic Ecology of Guppies (*Poecilia reticulata*) from the Streams of Trinidad

Eugenia Zandonà
Susan S. Kilham, Phd, Supervisor

Several factors may influence intraspecific niche differentiation, such as the different levels of resources or degrees of competition and predation organisms experience. Resource use polymorphism can lead to morphological differentiation and to the evolution of different life history traits. Trinidadian Guppies (*Poecilia reticulata*) inhabit streams where they experience high or low predation pressure. Guppies living in high (HP) and low predation (LP) sites have evolved different life history traits: HP guppies mature earlier, produce more and smaller offspring, and have higher overall fecundity and reproductive allotment than their LP counterparts.

The objective of this dissertation was to investigate the trophic ecology of guppies and its correlation with their life histories patterns. Three methodologies were employed: gut content analysis, gut length measurement, and stable isotope analysis. In the dry season, guppies in HP sites had a higher quality diet, which consisted of a greater proportion of invertebrates. Guppies in HP sites were more selective than guppies in LP sites, avoiding invertebrates of poor quality, as measured by a high carbon:nitrogen ratio. Gut morphology data confirmed these results, as guppies with lower quality diets (from LP sites) had longer guts. Comparisons between dry and wet seasons revealed that the diet of guppies shifted during the wet season, thereby eliminating dietary differences between HP and LP guppies found during the dry season.

A survey of HP and LP population pairs from six different rivers conducted in the wet season showed some similarity within stream types in their environmental and biological characteristics but with some differences between rivers. $\delta^{15}N$ and $\delta^{13}C$ stable isotope analysis across the surveyed sites showed that LP guppies generally occupied a higher trophic position than HP guppies, but that this relationship was highly influenced by the river of origin. Stable isotopes also indicated that guppies assimilated invertebrates into their tissues more than other dietary items and that the variation in diet composition between sites was high.

The results of this dissertation help distinguish the mechanisms by which guppy phenotypes (HP vs. LP) affect their environment, improving the understanding of the feedback between evolutionary and ecological processes in nature.

The Guppy

‘Whales have calves,

Cats have kittens,

Bears have cubs,

Bats have bittens,

Swans have cygnets,

Seals have puppies,

But guppies just have little guppies’

Ogden Nash

CHAPTER 1: General introduction

The guppy (*Poecilia reticulata*) is a very interesting and widely studied species, representing one of the most important models in evolutionary ecology, mostly due its rapid evolutionary response to natural selection (Reznick and Bryga 1987, Reznick et al. 1990, Reznick et al. 1997, Magurran 2005). Guppies are found in an incredible variety of habitats, encompassing a wide range of fish assemblages where they show adaptations correlated to the degree of predation (Haskins et al. 1961). Guppies living in presence of predators have evolved differences in their morphology, coloration, behavior, and life history traits compared to sites where the predation pressure is relaxed. These characteristics make guppies and the streams where they live an ideal natural laboratory to test key questions in evolutionary biology.

The guppy is a small fish, around 15-20 mm, which naturally occurs in northeastern South America and some Caribbean islands (Magurran and Seghers 1994). Among these, Trinidad populations have been thus far the most studied (Reznick and Endler 1982, Reznick et al. 1990, Reznick et al. 1997, Magurran 2005). Guppies have been introduced in many countries mostly for mosquito control purposes and now occur worldwide (FAO 1997). Guppies are highly prolific and ovoviviparous (Parenti 1981) and can live in many different habitats, from pristine headwaters, to more polluted and turbid large streams. These characteristics have made them a very successful invasive species. The males are more colorful and smaller than females, and have a large gonopodium formed by anal rays used for internal fertilization (Berra 2001). Females are heavily harassed by males, whose mating attempts are very frequent (Pilastro and Bisazza 1999). Male guppies have determinate growth, while females grow continuously throughout their lives. Guppy sex ratio, even if close to 1 at birth, is often female-biased (Magurran 2005). At birth they are fully developed and independent. With no need of parental care, they often form schools with other baby guppies as a way to avoid predation and cannibalism (Magurran and Seghers 1990).

GUPPY LIFE HISTORIES

Localities with high or low predation pressure on guppies are found in the same stream on Trinidad and are separated by barriers, such as waterfalls that cause a drop in the number of fish species preventing upstream migration (Gilliam et al. 1993). In these upstream sites, guppies are found with only one other fish species, the Hart's killifish (*Rivulus hartii*), which is a competitor and can occasionally prey on small guppies, but does not create a high predation environment. Differences in life history traits in guppy populations that experience high or low predation pressures by predatory fish are widespread throughout many drainages in the Northern Range of Trinidad (Reznick 1989, Reznick and Bryga 1996, Reznick et al. 1996b) and have independently evolved, as shown by genetic data (Fajen and Breden 1992). Guppies exposed to high predation pressure mature earlier and at smaller sizes, produce smaller offspring, have higher fecundity, have shorter interbrood intervals, have higher reproductive allotment, and have lower rates of aging compared to guppies exposed to low predation pressure (Reznick 1982b, a, Reznick and Endler 1982, Reznick 1989, Reznick et al. 2004). Guppies' life histories respond very rapidly to changes in selective pressures, as shown in transplant experiments where guppies from high predation localities were introduced into guppy-free sites (with only the Hart's killifish present) (Reznick 1982b, Reznick et al. 1990, Reznick et al. 1997). Introduced guppies changed some of their life history traits (offspring size and reproductive allotment) into those typical of low predation guppies within 2 years, which corresponds to 3-5 generations.

These patterns in life histories were first considered to be the result of differences in mortality rates and age-specific mortality between guppies in high and low predation environments, as indicated by early demographic models (Gadgil and Bossert 1970, Law 1979). These models, which were density-independent, predicted that an increase in adult mortality would lead to the evolution of early age at maturity and high reproductive effort, while an increase in mortality of young individuals would lead to the evolution of a delayed age at maturity and lower reproductive effort. However, later studies showed that in high predation sites, even if mortality rate was overall higher than in low predation sites, there was no age-specific mortality but guppies of all size classes were equally preyed upon (Mattingly and Butler 1994, Reznick et al. 1996a). In a scenario where mortality rate is not size selective, density-independent theories do not predict changes in reproductive effort or age at maturation (Charlesworth 1980). It then became clear that factors other than age-specific mortality were implicated in the evolution of life history traits in guppies, such as density-dependent population regulation, competition, resource abundance, and environmental fluctuations (Reznick et al. 2002). Indirect effects of predation are a likely mechanism driving the divergences in observed life history traits. The presence of predators reduces the density of guppies, increasing the amount of per capita resource available, and consequently decreasing intraspecific competition (Wootton 1994). The interplay of direct and indirect effects of predation, mediated through resource availability, is now considered an important aspect in the evolution of life history traits as shown by theories (Abrams and Rowe 1996) and some experimental data (Walsh and Reznick 2008), but there is yet no consensus (Gadgil and Bossert 1970, Kozlowski and Wiegert 1987) on how resource availability influences life history evolution.

Even if today resource availability is considered a potentially important selective force in the evolution of life history traits in guppies, and many studies have been conducted altering the amount of food levels in experimental trials, there have not been any studies investigating differences in guppy diets or prey selectivity between high and low predation sites. Remarkably only a few studies investigated guppies feeding behavior (Murdoch et al. 1975, Dussault and Kramer 1981, Morrell et al. 2007) and, to my knowledge there is only one study that looked at guppy diet in natural populations (Dussault and Kramer 1981). This study however was limited to comparison between sexes and did not address the role of predators in shaping guppy diet. No study addressed adaptive questions related to guppy diet and use of resources. Guppies have thus always been considered as opportunistic omnivores and their trophic ecology has been overlooked. Often studies referred to ‘high resource’ and ‘low resource’ available to guppies, but we do not really know which the resources are that the guppy uses or prefers. In my thesis I filled this important gap in the knowledge of guppy ecology by investigating guppy trophic ecology identifying what were their most important food sources (these so called ‘resources’). I employed gut content analysis and stable isotope analysis comparing guppies from many high and low predation localities. Gut content analysis provided detailed taxonomic information on prey consumption by the fish, while stable isotopes supplied information on the long-term diet and what was assimilated into the organism’s tissues. Each technique complemented the other, providing a complete picture of the guppy’s diet.

STABLE ISOTOPES IN FOOD WEB STUDIES

Stable isotopes are used in ecosystem ecology to assess nutrient transfers through habitats and to detect trophic relations in food web studies (Peterson and Fry 1987, Karasov and Martinez del Rio 2007). The basic concept is that a consumer’s isotopic signature - its distinct isotopic ratio – reflects that of its diet, because animals assimilate the isotopic composition of their food sources into their tissues (Karasov and Martinez del Rio 2007). Thus, the isotopic signature of an organism represents the integration over time of its diet and not just a snapshot of what it is eating in a precise moment, as gut content or fecal analysis show (Peterson and Fry 1987). Stable isotopes are useful because they also provide information on diet of organisms that are difficult to process with standard analysis, hard to observe, or even those that are extinct.

The most used isotopes in food web studies are those of nitrogen ($^{15}N/^{14}N$ ratio compared to atmospheric nitrogen, referred to as $\delta^{15}N$), which gives information on trophic steps among individuals; and carbon isotopes ($^{13}C/^{12}C$ ratio compared to a standard fixed on PeeDee Belemnite, referred to as $\delta^{13}C$), which provide insights on the ultimate sources of carbon (De Niro and Epstein 1981). Typically, $\delta^{15}N$ increases with the position of an organism in the food web: predators have higher signals than their prey. During the assimilation process of proteins (deamination and transamination of amino acids), the lighter isotope is removed and the heavier is retained. As a consequence, organisms excrete the lighter isotope and their tissues become enriched in ^{15}N compared to their diet (Minagawa and Wada 1984, Gannes et al. 1997). On the opposite, ^{13}C content is thought not to vary much between trophic levels, but it is useful in differentiating among carbon sources. For instance, $\delta^{13}C$ is used in terrestrial habitats to distinguish between diets based on plants with different photosynthesis pathways, such as C_3 and C_4; in lakes to distinguish among energy generated in pelagic or littoral habitat; and in streams to determine if basal resources have autochthonous (algae) or allochthonous (leaves) origin (Peterson and Fry 1987, Post 2002).

The isotopic difference between a consumer and its diet is referred to as fractionation (or as discrimination factor or enrichment; Karasov and Martinez del Rio 2007):

$\Delta = \delta^{15}X_{consumer} - \delta^{15}X_{diet}$,

where X is the isotope of interest (see also Fry 2006).

Fractionation is typically considered to be equal to 3.4‰ for $\delta^{15}N$ and ~ 0 ‰ for $\delta^{13}C$ (De Niro and Epstein 1981, Vander Zanden and Rasmussen 1999, Post 2002), but many studies have reported a large variations in these values (Vander Zanden and Rasmussen 2001, McCutchan et al. 2003, Vanderklift and Ponsard 2003, Kilham et al. 2008). Vanderklift and Ponsard (2003) conducted a meta-analysis of 134 previous estimates on the sources of variations in $\Delta\ \delta^{15}N$, finding that enrichment varied according to several factors: excretion forms, type of diet, taxonomic group, ecosystem, and tissue sampled. For instance, ureotelic and uricotelic organisms show higher fractionation values than ammonotelic, guanicotelic and species excreting amino acids. Also different tissues show variations in fractionation values: in birds, muscle enrichment is lower than in feathers, blood, or liver (Hobson and Clark 1992b), while in mammals, brain shows high $\Delta\ \delta^{15}N$ values and kidneys low values

(Vanderklift and Ponsard 2003). Peterson and Fry (1987) also documented variations in $\Delta\ \delta^{13}C$ values among tissues of the same organisms, in particular, bone collagen is enriched compared to diet while tissues with high lipid content are depleted. The different fractionation of both $\delta^{15}N$ and $\delta^{13}C$ is the result of different physiological and biochemical processes that tissues undergo, such as enzymatic reactions and turnover rates (Peterson and Fry 1987, Hobson and Clark 1992a, b, MacAvoy et al. 2001, McIntyre and Flecker 2006). Turnover rate is a measure of how quickly a tissue assimilates and for how long it maintains the signatures of ingested food. Turnover rate varies with body size (larger animals have slower turnover rates), and thus affects isotopic signatures (McIntyre and Flecker 2006). Therefore, large-sized and long-lived consumers have a slower turnover rate and show less variation in isotopic composition than their prey, which are generally smaller (Post 2002, McIntyre and Flecker 2006).

Mixing models

Over the course of the years many types of mixing models have been developed to estimate the proportional contribution of food sources to an animal’s tissues. The simplest mixing model allows one to estimate the fractional contribution of two different food sources knowing the isotopic signatures (for just one isotope) of the consumer’s tissue and of the two sources:

$$\delta X_{tissue} = p\ (\delta X_A + \Delta_A) + (1\text{-}p)\ (\delta X_B + \Delta_B)$$

where p is the fraction of food A, δX_A and δX_B are the isotopic compositions of food A and B, and Δ_A and Δ_B are the fractionation factors for food A and B (Karasov and Martinez del Rio 2007). If the isotopic composition of two elements is known, linear mixing models can calculate the contribution of 3 food sources to an animal's diet and so on (Ben-David and Schell 2001, Phillips 2001, Phillips and Gregg 2001). These linear mixing models, however, do not allow one to incorporate the variability of the source proportions, and are limited by the number of isotope employed, and also do not incorporate the high variation in sources and fractionation factors. Some of these problems were solved with the development of new models, some that incorporate sensitivity analyses and estimate the uncertainty in source proportions (Phillips and Gregg 2001), some that consider the concentration dependence of the food sources (their stoichiometry; Phillips and Koch 2002), and some that allowed one to calculate the proportion contribution to the diet of many sources (Isosource, Phillips and Gregg 2003). The most recent and innovative methods use Bayesian statistics and allow one to calculate the assimilation of many food sources, but most importantly to incorporate the variation in the sources and fractionation factors and also prior information (e.g. gut content) (Moore and Semmens 2008, Semmens et al. 2009, Parnell et al. 2010, Semmens et al. 2010)

SITE DESCRIPTION

I conducted my research in the streams of the Northern Range, located on the island of Trinidad (Trinidad and Tobago, West Indies). Trinidad is the southernmost island in the Antilles, and it is situated approximately 15 km off the coast of Venezuela. Trinidad has a total area of 4,768 km^2, and has an average length of 80 km and width of 59 km. Trinidad is characterized by a wet (June to December) and a dry season (January to May). In the Northern Range, the precipitation pattern ranges from 1800 mm in the west to 2400 mm in the east. Temperature does not vary greatly over the whole island; the lowest average temperature is experienced in January (~24°C) and the highest in May (~ 27°C). The Northern Range includes the highest mountains of the island, which reach a maximum elevation of 940 m (El Cerro del Aripo). The Northern Range is composed mainly of metamorphic rocks and alluvial soils.

The aquatic fauna of Trinidad is closely related to the fauna found on the mainland, because of its close proximity to Venezuela and its recent continental origin (Kenny 1995, Phillip 1998). However, in terms of ichthyofauna, the Northern Range represents a zoogeographical barrier which separates the southern slope, with typical South American assemblages, from the northern slope, with Antillean fauna (Phillip 1998). The only two species of fish, both Cyprinodontiformes, that are present in great abundance on both slopes are the guppy (*Poecilia reticulata*) and the Hart's killifish (*Rivulus hartii*) (Kenny 1995, Phillip 1998). The North and South

Slope of the Northern Range have shown striking differences in decapod diversity, with the South Slope showing a high depletion in number of species (Rostant 2005). The lack of amphidromous species, mainly shrimps, in the South Slope streams could likely be related to the strong human impact and consequent high water pollution occurring in the lower parts of the drainage (around the capital Port of Spain). On the other hand, the North Slope is still relatively pristine and does not have intense anthropogenic pressure, either in the coast or inland, because of its inaccessibility (Agard et al. 2005). Thus, its streams are more pristine and biotic communities are more intact (Rostant 2005). All these characteristics made the Northern range streams an ideal natural field laboratory for a comparative study to assess intraspecific differences in the trophic niches of both guppies and Hart's killifish.

Fauna

One of the main guppy competitors is the Hart's killifish (*Rivulus hartii*), which is native of Trinidad, Venezuela, and Eastern Colombia. In Trinidad, it is found basically in every stream, river, and riffle from the headwaters to the lowlands (Kenny 1995, Fraser et al. 2006). It is a small fish, reaching a maximum standard length (SL, from the tip of the snout to the beginning of the caudal bones) of 90 mm. It has good dispersal capability because it can stand periods of drought or dwell in small and temporal water bodies. It can jump out of the water and move on land, especially during wet weather (Seghers 1978). It is oviparous with external fertilization, and reproduction is not seasonal. Guppies and *Rivulus* are thought to compete for resources, and *Rivulus* may sometimes prey on small guppies (Mattingly and Butler 1994). When *Rivulus* is alone, its density can be up to 3 times higher than when it occurs with guppies or with other species, suggesting that adult guppies have a strong impact on *Rivulus* populations either directly or indirectly (Gilliam et al. 1993, Walsh and Reznick 2010).

The other fish species present in the Northern Range streams are shown in Table 1-1. Many families have been documented in the lower parts of the streams, as biodiversity is higher with increasing proximity to the ocean (Gilliam et al. 1993). Some of the most abundant species belong to the family Characidae and Cichlidae on the South slope and Gobiidae on the North slope. Among the most important guppy predators are the pike cichlid (*Crenicichla sp.*) and the wolfish (*Hoplias malabaricus*) on the South slope of the Northern Range and *Gobiomorus dormitur* on the North slope streams.

The decapod community is very diverse on the North and East slope of the Northern Range. The most common crustaceans on the North slope are the shrimp *Macrobrachium carcinus* and *M. crenulatum*, which are omnivores and predators, and the shrimp *Atya scabra*, that is a scraper and collector (Rostant 2005). On the South slope shrimp are only occasionally sighted due to the high pollution levels at the estuary of the Caroni river, which represents a barrier to the anadromous species of shrimp. The only common species of decapod on the South slope is a crab of the family Pseudothelphusidae (*Eudaniela garmani*).

The presence and abundance of other invertebrate species changes along abiotic and biotic gradients and riparian characteristics along the stream (Vannote et al. 1980). A list of macroinvertebrates present in the Northern Range streams is shown in Table 1-2.

Other potential guppy predators

Although there might be other non-aquatic organisms that prey on guppies, our knowledge of their identity and impact on guppy population is sparse and rarely considered (Magurran 2005). For instance, there are several species of birds in Trinidad that include fish in their diets, such as 6 species of kingfishers, 20 species of herons, egrets and bitterns, anhingas and cormorants (Ffrench 1992). However, other than the kingfishers, all the other species are very rarely observed in the smaller streams where I work (I have never observed any herons in our focal streams) and thus their impact on the guppies could be considered minimal. Some researchers investigated guppy reactions when exposed to a model bird predator and found that guppies from sites with high and low fish predation responded to the threat, but differently. High predation guppies reacted by hiding in shelters and low predation guppies froze on the bottom of the tank (in aquaria experiment; Templeton and Shriner 2004) or occupied the deeper parts of the streams (in natural habitats; Seghers 1974).

Other possible piscivore vertebrate and potential guppy predators are the fishing bat (*Noctilio leporinus*), the spectacled caiman (*Caiman crocodilus*), and snakes (Magurran 2005). The importance and frequency of predation of these vertebrates is very unclear, highly occasional and are unlikely to pose a major threat to guppy populations or to act as a relevant selective pressure.

NSF-FIBR (FRONTIERS IN INTEGRATIVE BIOLOGICAL RESEARCH) PROJECT IN TRINIDAD

Some of the data presented in this dissertation were collected as a collaborative effort of many people, myself included, who were working in the NSF-FIBR project entitled “From genes to ecosystems: How do ecological and evolutionary processes interact in nature?”. This project is interested in experimentally evaluating for the first time the feedback from adaptive evolution to ecosystem processes in a natural setting, which is the guppy system in the streams of Trinidad. The project develops around a main experimental manipulation, in which guppies from high predation localities are transplanted in sites where no guppies are present and the only fish species is the Hart’s killifish. The purpose is to document not only the evolution of guppies into a low predation phenotype, as has been done in the past (Reznick et al. 1990, Reznick et al. 1997), but also to evaluate the co-evolution of killifish, the impacts of both guppies and killifish on ecosystem structure and function, the role of resources in the fish evolution, and to develop eco-evolutionary theory. Around this main experiment, a lot of other projects, such as mesocosm experiments, electric exclosure manipulations, comparative analyses in replicate rivers throughout the island, whole-stream tracer additions, etc. are employed to develop predictions and refine experimental results. This project will generate a generalized conceptual framework that could be used in other ecosystems to evaluate, for instance, the anthopogenic effects in the environment, such as commercial exploitation of fish and its effect on the population or the introduction of exotic species.

Part of the work I conducted in the project is not included in this dissertation, but resulted in a publication in the Proceedings of the National Academy of Science of the United States of America (Bassar et al. 2010a) and several other manuscripts in preparation of which I am a co-author. In the Bassar et al. (2010a) study, I was responsible for the diet analysis of guppies, in which we assessed the effects of guppy phenotypes on ecosystem structure and function. We housed guppies for 28 days in artificial streams with different replicates that differed in guppy phenotype (guppies that had been exposed to high and low predation pressures, called in this dissertation high and low predation guppies) and guppy density (high or low). Diet analysis was essential to understand the effects of local adaptation of guppies on ecosystem properties. High predation guppies were found to feed more consistently on invertebrates than low predation guppies, which instead ate more detritus and algae.

These patterns in the diet were the same as I found in populations of wild-caught guppies during the dry season (Chapter 2).

I was also involved in a survey of 18 streams, where we collected numerous environmental variables to measure biomass standing stocks, plus fish, invertebrate and basal resource samples used for stable isotopes, stoichiometry, and diet analysis. Some of these data are reported in my dissertation, but are also included in many more publications in preparation, including a study on the stoichiometry and nutrient imbalance of guppies and Hart's killifish from different fish communities, as well as on the stoichiometry of epilithic algae.

I also participated in electric exclosure experiments, in which we excluded macroconsumers from an area of the stream and assessed their impact on ecosystem structure and function (e.g. leaf decomposition, algal accrual, invertebrate biomass).

OBJECTIVES

The general objective of my thesis was to investigate the intraspecific variation in the trophic ecology of Trinidadian guppies, an aspect of this species that has typically been overlooked. The main questions I addressed were:

1) Do guppies with different phenotypes show different trophic niches?
2) Are guppy diets correlated with life history traits?

3) Does resource availability affect guppy diet?
4) Are guppies selective in feeding?
5) Does guppy gut length correlate with diet quality?
6) Does seasonality affect guppy diet?
7) How repeatable are the differences in guppy diet across streams?
8) Are environmental characteristics similar within high and low predation environments?

In Chapter 2, I report differences in diet preferences, resource availability, and prey selectivity in guppies living in streams with high and low predation pressure. I compared guppy diets and prey selectivity with life history patterns and, most importantly I analyzed if prey selectivity was driven by food nutritional quality in terms of prey body stoichiometry. In this chapter I only analyzed guppies collected in the dry season, while in Chapter 3 I compared guppy diets between dry and wet season to assess a potential effect of seasonality on their feeding habits. I also examined one aspect of the digestive physiology of guppies examining the effect of diet quality on gut length. In Chapter 4 I tested whether the intraspecific variation in guppy trophic ecology was repeatable across high-low predation pairs from 6 replicate streams. I analyzed guppy trophic positions through the use of stable isotopes, which provided an estimate of food assimilated over a longer period of time. I also examined the existence of an ontogenetic shift in feeding choices. Finally, I characterized all the high and low predation sites sampled for biological and environmental variables, such as invertebrate, algae, fine and coarse organic matter biomass, stream morphometrics, and fish density. In general, in my dissertation I analyzed the role that guppies play in the food web and how this differed as an effect of predation intensity, with the goal of finding common, repeatable patterns that can help us understand how the evolution of this fish can impact differently the streams that it inhabits. My findings increase our knowledge of multiple aspects of the trophic ecology of the guppy, which in the past was simplistically considered just as an ‘opportunistic omnivore’.

Table 1-1. Fish species list for the streams of the Northern Range. Feeding habits and locations for each species are from Kenny (1995) and Phillip (1998).

Family	**Species**	**Common name in Trinidad**	**Feeding habit**	**Slope**
Gobioesocidae	*Gobiesox nudus*	Cling fish	Omnivorous, microphagous, grazing	North
Gobiidae	*Sicydium punctatum*	No common name	Omnivorous, microphagous, grazing bottom feeding	North
Gobiidae	*Awaous taiasica*	Sand fish	Omnivorous, microphagous, grazing bottom feeding	North
Eleotridae	*Eleotris pisonis*	Guabine	Carnivorous, macrophagous, ichthyophagous, bottom feeding	
Eleotridae	*Gobiomorus dormitor*	Guabine, Giant goby	Carnivorous, macrophagous, ichthyophagous, bottom feeding	North
Mugilidae	*Agonostomus monticola*	Mountain mullet	Omnivorous, surface, mid-water, bottom feeding	North
Synbranchidae	*Synbranchus marmoratus*	Swamp eel or Zange	Carnivorous, scavenging, bottom feeding	South
Cichlidae	*Cichlasoma taenia*	Brown Coscarob	Carnivorous – fish and invertebrates	South
Cichlidae	*Aequidens pulcher*	Blue Coscarob	Carnivorous – fish and invertebrates	South
Cichlidae	*Crenicichla alta*	Matawal or Millet	Carnivorous – fish and invertebrates	South
Erythrinidae	*Hoplias malabaricus*	Guabine	Carnivorous, macrophagous, ichthyophagous	South
Erythrinidae	*Hoplerythrinus unitaeniatus*	Yarrow	Carnivorous, macrophagous, ichthyophagous	
Curimatidae	*Steindachnerina argentea*	Stout sardine	Omnivorous, microphagous, deposit feeding	
Characidae	*Astyanax bimaculatus*	Sardine doree or two spotted sardine	Omnivorous, macrophagous, surface, mid-water or bottom feeding	South
Characidae	*Corynopoma riisei*	Sword-tail sardine	Omnivorous, macrophagous, surface, mid-water or bottom feeding	
Characidae	*Roeboides dayi*	Glass sardine or Hunch back sardine	Omnivorous, macrophagous, surface, mid-water or bottom feeding	South

Characidae	*Hemigrammus unilineatus*	Featherfin sardine	Omnivorous, macrophagous, surface, mid-water or bottom feeding	South
Characidae	*Hemibrycon taeniurus*	Guppy's sardine or Mountain stream sardine	Omnivorous, macrophagous, surface, mid-water or bottom feeding	South
Loricariidae	*Hypostomus robinii*	Teta	Omnivorous, microphagous, grazing	South
Loricariidae	*Ancistrus cirrhosus*	Jumbie teta	Omnivorous, microphagous, grazing	South
Pimelodidae	*Rhamdia quelen*	Catfish or Silver catfish	Carnivorous, scavenging, macrophagous, bottom feeding	South
Nandidae	*Polycentrus schomburgkii*	King Coscarob	?	
Anguillidae	*Anguilla rostrata*	Eel or Zange	Carnivorous, scavenging, macrophagous, ichthyophagous	
Callichthyidae	*Corydoras aeneus*	Pui-pui	Omnivorous, microphagous, select bottom deposit feeding	
Poeciliidae	*Poecilia reticulata*	Guppy or Millions	Omnivorous, surface, mid-water, bottom feeding	North/ South
Rivulidae	*Rivulus hartii*	Leaping guabine or Jumping guabine	Carnivorous, surface and bottom feeding	North/ South

Table 1-2. List of the most common aquatic invertebrates and their functional feeding group (from (Merritt et al. 2007) found in the streams of the Northern Range in Trinidad.

Family	**Functional Feeding Group (FFG)**
	Insects
Diptera	
Tipulidae	Predator
Chironomidae	Collector (non-Tanypodinae)/Predator (Tanypodinae)
Ceratopogonidae	Predator
Muscidae	Collector
Psychodidae	Collector
Simuliidae	Filtering collector
Ephemeroptera	
Baetidae	Scraper/Collector
Leptohyphidae	Collector
Leptophlebiidae	Collector
Euthyplocidae	Predator
Trichoptera	
Calamoceratidae	Shredder
Hydropsychidae	Filtering collector/Omnivore
Leptoceridae	Collector
Polycentropodidae	Predator
Glossosomatidae	Scraper
Philopotamidae	Filtering collector/Omnivore
Helicopsychidae	Scraper
Plecoptera	
Perlidae (Anacroneuria)	Predator

Odonata	
Odonata	
Zygoptera (Coenagrionidae, Calopterigidae)	Predator
Anysoptera (Libellulidae, Gomphidae)	Predator
Coleoptera	
Elmidae	Collector (larva), Predator (adult)
Psephenidae	Scraper
Lepidoptera	
Crambidae (Petrophila)	Scraper
Hemiptera	
Gerridae	Piercer predator
Veliidae	Piercer predator
Tricladida (Planaridae*)*	Collector
Oligochaete (Tubificidae)	Collector
Hydrachnida (Hydrachnidae)	Predator
Gastropoda (Thiaridae and other families)	Scraper

CHAPTER 2: Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies

ABSTRACT

Life histories evolve as a response to multiple agents of selection, such as age-specific mortality, resource availability or environmental fluctuations. Predators can affect life history evolution directly, by increasing the mortality of prey, and indirectly, by modifying prey density and resources available to the survivors. Increasing prey densities can intensify intraspecific competition and cause evolutionary changes in the prey selectivity, also affecting nutrient acquisition. Here we show how the evolution of different life history traits in guppies (*Poecilia reticulata*) is correlated with differences in resource consumption and prey selectivity. We examined differences in guppy diet among stream types with high and low predation pressure and how these were related to benthic invertebrate biomass. Fish and invertebrate samples were collected from two high and two low predation reaches of two distinct study rivers in Trinidad. Our results showed that guppies from high predation environments matured earlier, had higher fecundity and reproductive allotment, fed more consistently on higher quality food, and showed higher prey selectivity. Guppies from low predation sites displayed the opposite patterns in life history traits and had diets with prevalence of detritus and algae, which are a poorer

quality food. Low predation guppies fed on invertebrates according to their availability, while high predation guppies were selective toward those invertebrates with the lower C:N body ratio, and thus with higher nutritional value. Our study corroborates the important role of predators in shaping their prey’s life histories in concert with other traits, such as resource specialization and diet selectivity.

INTRODUCTION

Early models of life history evolution envisioned extrinsic, age-specific mortality as the dominant agent of selection in the evolution of life histories (reviewed in Charlesworth 1980, Roff 1992, Stearns 1992, Roff 2002). Those early theories were formulated in a density-independent context and predicted that the effect of increasing adult mortality could lead to the evolution of increased reproductive effort and earlier age of maturation (e.g. Gadgil and Bossert 1970, Law 1979). Conversely, increased mortality of young would favor individuals with lower reproductive effort and delayed maturity.

However, natural populations rarely experience complete density-independence and are likely to be limited at some point by biotic or abiotic factors (Cappuccino and Price 1995, Bassar et al. 2010b). Population growth can be limited by top-down forces (predation), in which case classical theories may be sufficient to explain life history responses to changes in mortality. This is not the case for populations regulated by bottom up forces (resource availability). Removal of predators from a system previously regulated through predation may increase the density of the prey and, in doing so, decrease the per capita resource availability (Wootton 1994). These changes may be dramatic enough to alter the system to one that is regulated by bottom-up forces.

Increased prey density may thus strengthen intraspecific competition for high quality resources, leading to a change in diet selectivity of individuals (e.g. Werner and Hall 1974). Under resource scarcity, it may be optimal to consume resources more indiscriminately and include a higher proportion of lower quality food in the diet (e.g. Gende et al. 2001). Such shifts in diet preference might be accommodated by concomitant changes in physiology (e.g. Olsson et al. 2007), morphology, and behavior (Svanback and Bolnick 2007), which may further act to affect these interactions (Werner and Peacor 2003).

Hence, variation in the mortality regime, density, and resource availability may all influence the evolution of life histories (Gadgil and Bossert 1970, Abrams and Rowe 1996, Reznick et al. 2002, Walsh and Reznick 2008, 2009). However, there is no consensus (Gadgil and Bossert 1970, Kozlowski and Wiegert 1987) and little experimental data (e.g. Walsh and Reznick 2008) on how resource availability influences life history evolution. Even less explored are the effects of diet and resource quality on the evolution of life history traits. Life histories can respond to the quantity but also the quality of resources consumed (Twombly et al. 1998, Jensen and Verschoor 2004). For instance, low quality food could affect individual fitness, alter reproductive allocation, or decrease growth rates (e.g. Sterner 1993, Jensen and Verschoor 2004). However, the responses of multiple life histories to low food quality are not predictable, likely due to differences in nutritional requirements for different functions and between organisms (Twombly et al. 1998, Urabe and Sterner 2001).

Here we examined how life histories of guppies (*Poecilia reticulata*), resource abundance, and prey selectivity co-vary in natural streams of Trinidad. Guppies are found in two types of environments, referred to as high predation (HP) and low predation (LP), in which they have evolved different life history traits (Reznick and Endler 1982, Reznick 1989). In HP sites, they experience strong predation pressure mostly by the wolfish (*Hoplias malabaricus*) and the pike cichlid (*Crenicichla sp*). In LP sites, guppies coexist with only one other fish species, the Hart’s killifish (*Rivulus hartii*), which may occasionally prey on juvenile guppies (Mattingly and Butler 1994).

Age-specific mortality has typically been considered the presumed agent of selection determining life history evolution in guppies (Reznick and Endler 1982, Reznick 1989). If guppies are mainly preyed upon as adults in HP sites and as juveniles in LP sites, density-independent life history theory predicts that HP guppies should mature earlier and have higher reproductive allotment than LP guppies (Reznick et al. 2002). These predicted differences in life histories have been observed in many replicates of natural populations (Reznick and Endler 1982, Reznick 1989, Reznick et al. 1996a, Reznick et al. 1996b) and confirmed by laboratory common garden experiments (Reznick 1982b, Reznick and Bryga 1996).

However, there are some inconsistencies between all available data about guppies and the theory of age-specific mortality as the sole agent of selection. For example, density-independent life history theory predicts that evolutionary changes can only occur when there are changes in juvenile relative to adult mortality (Gadgil and Bossert 1970, Law 1979, Charlesworth 1980). Mark-recapture experiments on wild guppy population have shown that, although predation was indeed higher in HP sites, the pattern of size-specific predation was similar in both environments (Reznick et al. 1996a). This suggests that other factors may be playing a role in guppy life-history evolution.

Resource availability, which can also shape life histories, varies considerably among natural populations of guppies (Grether et al. 2001). Resource levels, however, often co-vary with predation gradient, as HP sites are generally located downstream and are commonly wider streams with less canopy cover and higher primary productivity compared to LP sites (Reznick et al. 2001). Increased resource levels could be an indirect effect of increased predation (Wootton 1994). Guppy biomass is higher in LP communities, leading to lower resources per capita, and consequently increasing competition for food (Rodd and Reznick 1997, Grether et al. 2001). Food availability may therefore represent another selective factor leading to differences in guppy growth rates, as well as other life history traits such as size at maturity and reproductive allotment (Gadgil and Bossert 1970). However, even if resources are more abundant in HP sites, they might not be reflected in what guppies consume. Predators affect guppy habitat use by forcing them to occupy only the margins of pools (Fraser and Gilliam 1992), thus limiting available foraging areas. This may force HP guppies to feed on suboptimal resources (Werner et al. 1983).

Bassar et al. (2010a) found the opposite pattern in a recent study performed in artificial streams: guppies from HP sites ate more invertebrates and less detritus and algae than guppies from LP sites. This result suggests that guppies from high predation sites have evolved a preference for higher quality food items, which may promote the evolution of increased investment in growth and reproduction (Walsh and Reznick 2008). Nevertheless, differences among guppies from HP and LP localities in resource consumption have not yet been examined in natural populations.

Here we investigate how a suite of guppy life history traits co-varies with resource consumption and prey selectivity across sites in Trinidad that differ in their predation regime. We collected wild fish from HP and LP sites in two different drainages and examined their diets, life history traits, and invertebrate availability in the streams. We examined guppy prey selectivity for the two phenotypes and determined if it was correlated with the prey’s nutritional quality, expressed as their C:N content.

MATERIALS AND METHODS

We conducted this study in montane streams draining the Northern Range of Trinidad. Our study streams belonged to two different drainages, Aripo and Guanapo, which are both part of the Caroni River system on the south slope of the Northern Range. In each drainage, we sampled a low predation (LP) and a high predation (HP) site. The LP environments were generally found upstream of barrier waterfalls which prevented the dispersion of many fish species (Gilliam et al. 1993). The catfish *Rhamdia quelen* was also present in the LP Guanapo site. However, this species did not appear to represent a real threat to guppies (Gilliam et al. 1993, Zandonà unpubl. data). Fish community composition was based on observations and captures during multiple years. We measured stream width and canopy openness for 3 randomly chosen pools in each site. Canopy openness was measured with a hemispherical densiometer. Results are reported in Table 2-1.

We collected samples during 18-24 March 2007, which corresponded to the dry season in Trinidad. In each site, we collected guppies and benthic invertebrates from three pools and, within each pool, from locales with different stream velocity (low, medium, and high) to ensure sampling of most microhabitats found in the stream. We collected 56-136 individuals per pool (N = 1003 individuals across all sites), but this was always less than the total number present in the pool. All samples from a given site were collected on the same day. Fish were collected with hand nets and euthanized immediately with an overdose of the neutrally buffered MS-222.

Guppies were then measured for standard length with a digital caliper, weighed, and intact guts were removed for the diet analysis. Guts and guppies were preserved in individual containers in 5% formalin solution.

LIFE HISTORY MEASUREMENTS

We measured the following life history traits for all guppies collected: size at maturation in males and females, fecundity, offspring size and reproductive allotment as follows (but see Reznick and Endler 1982, Reznick 1989 for detailed description of the procedures). Males stop growing at maturity, so for mean male size at maturation we randomly sampled 25 mature males from each site collection and measured their standard length (to the closest 0.05 mm) and wet mass (to the closest 1 mg). For female reproductive traits, we separated females into 2 mm size classes and selected a minimum of 3 females from each size class from each pool (N = 16-48 individuals per pool). Females were measured and then dissected. We determined the number of embryos in each female and their stage of development according to Reznick and Endler (1982). Developing embryos and reproductive tissues were separated from the female and, along with the female soma, were dried overnight in an oven and weighed the following day. For the minimum female size at maturation, we determined the pregnancy status of all females and found the smallest reproductive female in each pool. Fecundity was determined by counting the number of offspring in pregnant females. Mean offspring size for each female was calculated as the litter dry mass divided by the number of offspring in the litter. Reproductive allotment was estimated as the summed dry mass of offspring and reproductive tissues. Only females carrying developing embryos were considered (Reznick and Endler 1982).

For all traits, we examined the effects of predation regime and drainage using a linear mixed model approach. Drainage and predation were modeled as fixed effects while pool was modeled as a random effect within predation x drainage to control for non-independence of individuals measured within the same pool. When there was a significant interaction between drainage and predation, we used tests of simple main effects to compare life history traits between predation regimes within each drainage (Winer 1971). Female dry mass was included as a covariate in analyses of fecundity and offspring size. Because offspring dry mass declines as development progresses (Reznick and Endler 1982), stage of development was also included as a covariate in the analysis of offspring size. To analyze reproductive allotment, we used the summed dry mass of offspring and reproductive tissues as the dependent variable and female dry mass as a covariate.

RESOURCE AVAILABILITY

We collected benthic invertebrates from 3 pools in each site. Within each pool, we sampled invertebrates from 3 benthic areas with different water velocity (high, medium, and low) (N=36). We collected benthic invertebrates using a PVC pipe sampler (12.1 cm diameter). Invertebrates were picked and identified to the lowest taxonomic level (Perez 1996, Merritt et al. 2007). We used mass-length regression equations (Benke et al. 1999) to calculate total invertebrate biomass, expressed in mg of dry mass per m^2. A two-way ANOVA tested for differences in benthic invertebrate biomass/m^2 between drainages and predation level.

DIET ANALYSIS

We analyzed 83 guppies for diet content. Only the anterior part of the guts (stomach and a small part of the foregut - at the point where the gut turns 180 degrees) was analyzed, because here food was not fully digested. Invertebrates were identified at the most inclusive taxonomic level, usually the family category, following Perez (1996) and Merritt et al. (2007). The selected portion of the gut was placed onto a gridded slide, where ten squares (out of 64) were randomly chosen for quantification of the gut content under a compound microscope. Invertebrates and detritus proportions were estimated for each square. Individual diatoms and filamentous algae were counted in each of the 10 squares. Diatoms and filamentous algae were counted because they are too small to estimate their proportion coverage in one square. An average size for diatoms and one for filamentous algae was subsequently assigned to calculate the area they occupied in the 10 squares. The area taken by each food category was calculated for the whole slide (64 squares). Plant matter, inorganic material, and other algae were not included in the analysis as their occurrence was very low.

We performed a multivariate analysis of covariance (MANCOVA) to test for differences in guppy diet across drainages and predation regimes. Proportions of invertebrates, detritus, and algae (diatoms and filamentous algae) were the dependent variables of our general linear model (GLM). We included drainage (Aripo and Guanapo) and predation level (HP, LP) and their interaction as fixed factors. We used fish standard length as a covariate. We also included pool number nested within the interaction between predation and drainage as a random effect to remove non-independent effects associated with sampling multiple individuals within a pool and thereby provide the proper error term for comparisons between drainage and predation regime. In addition to this multivariate approach, we also tested the proportion of each diet class in the guts using three univariate ANCOVAs. Proportion of each food item was included as a dependent variable and the independent variables were the same as they were for the MANOVA.

Finally, to assess if the benthic invertebrate abundance in the stream had an effect on the amount of invertebrates found in the fish guts, we ran a two-way ANCOVA. Our dependent variable was the proportion of invertebrates found in the fish guts, predation and drainage were fixed factors, and benthic invertebrate biomass (mg/m^2) was set as a covariate. Fish standard length was not included in the GLM, as it did not have a significant effect.

PREY SELECTIVITY

To investigate if guppies were selectively choosing to feed on specific invertebrate taxa, we calculated an index of prey selectivity as follows:

$$L_i = r_i - p_i$$

where L_i was the index of prey selectivity for the taxa i, r_i was the relative abundance of prey i in the fish gut, and p_i was the relative abundance of the prey i in the pool where we caught the fish (Strauss 1979, Palkovacs and Post 2008). The index L_i can have values from +1 to -1. If L_i is greater than 0 the fish is actively selecting prey i, if it is less than 0 the fish is avoiding prey i, if L_i is equal to 0 the fish is selecting prey i in proportion to its abundance. We chose 9 different invertebrate taxa, which were all the ones found in the guppy guts, as our prey items and for each of them we calculated guppies' selectivity index. The chosen taxa were: Ephemeroptera, Trichoptera (excluding Helicopsychidae and Glossosomatidae as they have never been found in guppy guts), Odonata, Chironomidae, other Diptera larvae (Ceratopogonidae, Tipulidae, Simuliidae), Elmidae (only larvae), Psephenidae, Ostracoda, Copepoda.

We created random diets with a Monte Carlo simulation for each of the pools (N=12) where we caught the fish. The simulated random diets were used to test the significance of the selectivity indices L_i for each of the 9 invertebrate taxa. The program first calculated a pooled diet for all fish from the same pool, with the purpose of comparing it to the invertebrate abundance in the environment. The simulation created random diets for each pool by randomly drawing invertebrate items based on the abundances from the same pool. The randomly simulated diets had the same number of prey items as in the actual diets. Benthic invertebrate taxa were drawn, with replacement, from each of the pools, creating 10,000 random diets for each pool. The program calculated a selectivity index from the simulated diets for each of the 9 prey taxa in all the 12 pools, thus creating a null distribution of L_i. If the selectivity index of the individual fish was within the 95% confidence interval of the null distribution, the fish was feeding according to availability. If it was significantly above or below the limits of the confidence interval, fish respectively selected or avoided that particular taxa. The Monte Carlo simulation was performed using MatLab 7.6.0.

We also ran a linear mixed-effects model to investigate if guppies were selecting invertebrate taxa according to their quality, referred to as their elemental composition (body stoichiometry). Typically, prey with a relatively high nitrogen content represent good quality food items, so we characterized the invertebrate taxa based on their C:N body composition. The C:N ratios indicate the balance between energy and nutrient (nitrogen) acquisition: high C:N values indicate low nitrogen content and thus low quality food, while low C:N values characterize high nitrogen content and high quality. We estimated the C:N body composition of compiled invertebrate samples collected during a stream site survey in Trinidad in 2007 and 2008. The C:N data were available for 7 of the 9 invertebrate taxa (not for Copepods and Ostracods). We excluded Psephenidae from the analysis due to their low occurrence in both diet (1%) and in the environment (2%). The other 6 taxa were the most abundantly found in guppy guts. In the model, our response variable was the selectivity index calculated by the Monte Carlo simulation, and the explanatory variables were invertebrates' C:N body compositions, predation levels, and the interaction between predation and C:N. The invertebrate taxa identity was set as a random effect to account for other random factors that may affect their selectivity (e.g. species-specific anti-predatory adaptations).

Dependent variables and covariates were either arcsin square root transformed (for proportions) or log transformed when appropriate. All statistical analyses, except the Monte Carlo simulation, were performed using SAS and PASWStatistics 18.0 and the levels of significance were accepted at 0.05.

RESULTS

LIFE HISTORY TRAITS

Guppies showed clear life history differences between HP and LP sites in both drainages. Male guppies were smaller at maturity in HP relative to LP sites ($F_{1,8}$ = 79.7, P < 0.001; Fig. 2-1a), and there was no significant effect of drainage ($F_{1,8}$ = 1.67, P = 0.23) or an interaction between predation and drainage ($F_{1,8}$ = 3.85, P = 0.08). The marginal interaction arose because the difference in size of mature males from high and low predation sites on the Guanapo River was larger than on the Aripo River. Females also matured at a smaller size in HP relative to LP sites ($F_{1,8}$ = 26.0, P < 0.001; Fig. 2-1b) and there were no differences between drainages ($F_{1,8}$ = 3.73, P = 0.08) nor any significant interaction between drainage and predation ($F_{1,8}$ = 1.31, P = 0.28). Female fecundity increased with female body size ($F_{1,150}$ = 194.0, P < 0.001) and was higher in HP than in LP sites ($F_{1,8}$ = 10.99, P = 0.01; Fig. 2-1c). Fecundity was greater in females from the Guanapo relative to the Aripo drainage ($F_{1,8} = 6.33$, $P = 0.04$), but there was no significant interaction between predation and drainage ($F_{1,8} = 4.55$, $P = 0.07$). The marginal interaction for fecundity occurred because the difference between high and low predation localities was larger in the Aripo than the Guanapo rivers. Offspring size increased with female body size ($F_{1,119} = 22.26$, $P < 0.01$), and decreased with stage of development ($F_{1,119} = 71.8$, $P < 0.01$). There was a significant effect of predation ($F_{1,8} = 15.86$, $P < 0.01$, Fig. 2-1d), but there was also a significant effect of drainage ($F_{1,8} = 6.62$, $P = 0.03$) as well as a significant interaction between predation and drainage ($F_{1,8} = 68.6$, $P < 0.01$). Offspring were larger in LP relative to HP sites in the Aripo drainage ($F_{1,8} = 83.8$, $P < 0.01$; Fig. 2-1d), but were smaller in LP relative to HP sites in the Guanapo drainage ($F_{1,8}=5.62$, $P=0.04$). Finally, reproductive allotment increased with female dry mass ($F_{1,119} = 270.7$, $P < 0.001$) and decreased with stage of development ($F_{1,119} = 16.18$, $P < 0.001$). Reproductive allotment was larger in HP relative to LP sites ($F_{1,8} = 5.38$, $P = 0.04$; Fig. 2-1e), and was not affected by drainage ($F_{1,8} = 1.57$, $P = 0.24$) or the interaction between predation and drainage ($F_{1,8} = 2.08$, $P = 0.19$).

RESOURCE AVAILABILITY

Predation ($F_{1,8} = 6.92$, $P = 0.03$), drainage ($F_{1,8} = 9.52$, $P = 0.015$), and the interaction between predation and density ($F_{1,8} = 10.1$, $P = 0.013$) all had a significant effect on benthic invertebrate biomass per area found in the streams. All three effects were caused by the much higher benthic invertebrate biomass in the Aripo HP site relative to the other three sites (Table 2-1). We then ran a one-way ANOVA with site identity as a fixed factor and LSD post-hoc analysis to assess the differences in benthic invertebrates between the 4 sites. We found that site of origin had a significant effect ($F_{3,8} = 8.85$, $P = 0.006$) and that the Aripo HP site had a significantly higher invertebrate biomass/m^2 than the other 3 sites (LSD: Aripo HP-Aripo LP $P = 0.003$; Aripo HP-Guanapo HP $P = 0.002$; Aripo HP-Guanapo LP $P = 0.004$; see Table 2-1). Invertebrate biomass was not significantly different between Aripo LP, Guanapo LP, and Guanapo HP (Table 2-1).

DIET ANALYSIS

Guppies from sites with different predation regimes had significantly different gut content composition. The MANCOVA indicated a significant effect of predation ($F_{3,7} = 6.05$, $P < 0.02$) and fish length ($F_{3,67} = 8.06$, $P < 0.001$) on the composition of guppy diets. There was no significant effect of drainage ($F_{3,7} = 0.52$, $P = 0.68$) nor of the interaction between drainage and predation ($F_{3,7} = 0.99$, $P = 0.45$). Univariate tests showed that HP guppies ate significantly more invertebrates than LP guppies ($F_{1,9} = 23.08$, $P = 0.001$), which instead fed significantly more on algae ($F_{1,9} = 10.74$, $P = 0.01$) and detritus ($F_{1,9} = 20.28$, $P = 0.001$) (Fig. 2-2 and Table 2-2).

When we incorporated the pool benthic invertebrate biomass as a covariate in the two-way ANCOVA, we found that it did not have a significant effect ($F_{1,79} = 0.16$, $P = 0.69$) on the proportion of invertebrates found in the guppies guts. Even with benthic invertebrate biomass as a covariate, the model still detected a significant effect of predation ($F_{1,79}$ = 17.09, P < 0.001), a marginally non significant effect of drainage ($F_{1,79}$ = 3.47, P = 0.066), and no significant effect of the interaction between drainage and predation ($F_{1,79}$ = 1.18, P = 0.28) (Fig. 2-3).

PREY SELECTIVITY

In general guppies from HP sites were more selective when feeding on invertebrates than guppies from LP sites, which instead fed on what was available in the environment (Fig. 2-4). Overall, Chironomidae represented 40% of the invertebrate portion of guppy diet. Trichoptera were 14%, other Diptera 13%, and Ephemeroptera 8%. Even though Ephemeroptera was the most abundant taxon found in the environment (34%), the Monte Carlo simulation showed that HP guppies tended to avoid them (Fig. 2-4). Elmidae and Ostracoda also had relatively high abundance in the environment, with 11% and 19% respectively, but they were uncommon in the guts (0.5% and 2% respectively). Some invertebrate taxa commonly found in the benthic samples were never found in the guppy guts. These taxa were Trichoptera with rocky cases such as Helicopsychidae and Glossosomatidae, Oligochaete (Tubificidae), and Gastropoda (Thiaridae).

The linear mixed-effects model showed selectivity was on average stronger in guppies in HP sites (t_{15}=-3.37; P=0.004). Moreover, while selectivity was unrelated to C:N ratio in guppies in LP sites (t_3=0.40; P=0.71), selectivity of guppies in HP sites was strongly related to C:N ratio (interaction predation x C:N; t_{15}=3.48; P=0.003). The shape of this relationship for guppies in HP sites was quadratic (t_{15}=-3.58;

P=0.003), showing stronger preference for invertebrates with a C:N ratio lower than 5 and avoidance for lower quality invertebrates (with high C:N ratios; Fig. 2-5). Guppies in LP sites instead showed no selectivity, and fed according to what was available in the stream.

DISCUSSION

Guppies adapted to different levels of predation displayed marked differences in diet and prey selectivity. Fish from high predation environments fed on food of higher quality (invertebrates as opposed to detritus or algae) and selected those invertebrate taxa that had higher relative nitrogen content (Fig. 2-5). Low predation guppies were more opportunistic in their feeding behavior. They mostly ate detritus and algae, which have low nitrogen content, and did not show preferences for specific invertebrate taxa; they instead fed according to what was available in the stream (Figs. 2-4 and 2-5).

The life history differences that we observed between HP and LP guppies overall confirmed the results commonly found in other studies on wild-caught guppies in Trinidad (Reznick and Endler 1982, Reznick 1989). HP guppies matured at a smaller size (which has served as an indicator of earlier maturity in prior studies – Reznick and Endler 1982; Reznick 1982; Reznick et al. 1996a,b), and had higher fecundity and reproductive allotment than their LP counterparts. HP fish also typically produce smaller offspring (Reznick and Endler 1982; Reznick 1982; Reznick et al. 1996 a.b), but in the Guanapo drainage we did not find a significant difference in this trait between HP and LP guppies (we discuss about this anomaly further below).

The patterns we found on resource (invertebrate) levels across predation regimes were complex. We expected higher invertebrate biomass in HP sites than in LP sites because HP sites were generally bigger streams with more primary productivity (Reznick et al. 2001), which should sustain higher levels of secondary production (Hill et al. 2001). There were instead no consistent differences between HP and LP sites (Table 2-1). The Aripo HP site had almost 9 times the invertebrate biomass/m^2 of the other three sites and it was also significantly wider. The other 3 sites did not significantly differ between each other for these two measurements (Table 2-1). Nevertheless, there were consistent differences in guppies from HP and LP sites for all variables in this study.

A missing link in our assessment is guppy population density and hence per capita food availability. Previous studies (Rodd and Reznick 1997, Reznick et al. 2001) reported that the guppy size distribution is smaller in HP sites due to the higher death and birth rates, resulting in ¼ of the guppy biomass per area found in LP sites. If our sites replicated these guppy biomass differences, then the per capita invertebrate availability would be lower in LP sites.

There were correlated disparities in diet that were consistent with what we would predict if resources were less abundant in LP sites. Optimal diet theory predicts that a predator should have a broader diet in unproductive environments and become more of a specialist on high quality food when prey density is high (MacArthur and Pianka 1966). For instance, Gende et al. (2001) found that brown and black bears became more specialized in energy-rich salmon (females that had not spawned) when their availability was high. In another study, Werner and Hall (1974) found that bluegill sunfish were more selective towards bigger size prey when prey density was high, but the fish were more opportunistic when prey density decreased. In a study on cadmium-intolerant *Drosophila*, Bolnick (2001) found that when cadmium-free food abundance was low and competition for it was high, *Drosophila* evolved tolerance for cadmium-rich food. In HP streams, with low guppy biomass, there are more high quality resources (invertebrates) available to each guppy, thus the fish can be more selective in what they feed on. HP guppies indeed showed higher invertebrate content in their diet and higher selectivity for certain taxa, especially the higher quality ones (with intermediate-low C:N values). HP guppies can afford to drop less profitable items from their diet, and they can specialize on higher quality prey, because food is not limiting.

In LP sites, where guppy biomass is typically higher and individual growth rates are lower (Reznick et al. 2001), guppies might be under stronger intraspecific competition for the fewer high quality resources available. Therefore, it would become advantageous for them to be more generalists and to feed on a broader variety of food items, even those of lower nutritional quality. Indeed, we found that LP guppies had a more herbivorous diet, with high proportions of low quality food (detritus), and invertebrates consumed according to availability.

Bassar et al. (2010a) found the same patterns; HP guppies preferred to eat invertebrates while LP guppies had a higher proportion of detritus and algae in their diet. Bassar et al. (2010a) worked with fish from the same localities as in the current study, but diet was assessed after fish were kept for 28 days in artificial streams that had been uniformly stocked with invertebrates collected from natural streams. Seeing such a pattern in the absence of any difference in resource availability or population density suggests that guppies have specialized diets that might have evolved as a response to different resource levels in their natural environment.

Increased resource availability, when modeled as an indirect effect of predation, represents a factor that can select for the evolution of early age/size at maturity and higher reproductive effort according to some models (Gadgil and Bossert 1970, Abrams and Rowe 1996). On the other hand, chronically low food levels have been linked to the evolution of slower growth rates, as a strategy to minimize the costs of growth (Sinervo and Adolph 1994, Arendt and Reznick 2005). In a series of studies conducted on the Trinidadian killifish *Rivulus hartii*, Walsh and Reznick (2008, 2009) found that high resource availability was associated with the evolution of earlier maturity, increased reproductive allotment and the production of more, smaller eggs. In our study system, high resource levels were likewise correlated with smaller size at maturation, higher reproductive allotment and fecundity, while low food levels led to the opposite patterns. For this reason, resource availability, which is indirectly affected by the presence/absence of predators, has to be considered an important agent of selection in the life history evolution of guppies.

Offspring size anomaly

In the Aripo River, HP fish produced smaller offspring size than LP fish, as in earlier studies. Offspring size instead was not significantly different between HP and LP guppies in the Guanapo. Reznick (1982a) and Torres-Dowdall (in prep) found that there is a genetic basis for these offspring size differences in both rivers. Most life history theory predicts how overall reproductive allocation evolves (e.g. Gadgil and Bossert 1970, Charlesworth 1980) but does not address how this allocation is provisioned to offspring (e.g., many small versus few large). The evolution of offspring size is often represented by independent theory (e.g, Smith and Fretwell 1974, Lloyd 1987). It is possible that offspring size might evolve independently of other components of the life history. The fitness consequences of offspring size depend strongly on the competitive environment. Being larger is a big advantage when food is scarce and competition is intense, but of little advantage when food is abundant and competition is lax (Bashey 2008).

If our HP streams had similar guppy biomass, then the Aripo HP site, with its very high benthic invertebrate biomass per unit area (Table 2-1), could have more resources available per individual newborn guppy than the Guanapo HP site. The Aripo HP site was indeed the one with the smallest offspring size. The Guanapo HP site has lower invertebrate biomass and bigger offspring size than the Aripo HP, so resource availability to newborn guppies may have played a role in shaping this life history trait. More unusual was the difference in offspring size between our two LP streams. The Guanapo LP site had bigger offspring size than the Aripo LP. If we hypothesize that this difference evolved in response to chronic differences in resource availability, then the Guanapo LP site should have greater per capita invertebrates available. We found that the Guanapo LP fish had a higher proportion of invertebrates in their diet than the Aripo LP fish (~40% vs. ~10%), which supported the hypothesis that the Guanapo LP fish had more resources available. Also, the relative difference in the proportion of invertebrates in the diet between HP and LP fish was much greater in the Aripo river than in the Guanapo river, which corresponded to the greater difference in offspring size as well. Higher relative resources in the Guanapo LP site than in the Aripo LP site could be the cause of more invertebrates in the diet and smaller offspring size.

Eco-evolutionary perspective

Predators can drive community divergence in prey and these changes can feedback to mold the evolution of predators' traits in contemporary time (e.g. trophic morphology; Palkovacs and Post 2008). For instance, natural populations of anadromous and landlocked alewives (*Alosa pseudoharengus*) differently modified the structure and composition of the zooplankton community (Brooks and Dodson 1965). Consequently, the effect of the alewives on the zooplankton community feeds back, affecting the alewives' trophic morphology and favoring those traits that are more adapted to the modified zooplankton community (Palkovacs and Post 2008, 2009). Possibly, eco-evolutionary feedback could have caused divergence between HP and LP guppies. The population densities of the guppies may dictate the nature of resource availability. In LP sites guppies are at higher population densities due to the lack of predators (Rodd and Reznick 1997) and they may actively depress the abundance of invertebrates. If such conditions persist, then there could follow selection in favor of those individuals that are less selective in their choice of prey. If such diet preferences do indeed evolve, then they could conceivably be accompanied by selection for differences in the digestion and absorption of nutrients from the diet (e.g. modified gut length and intestinal micro structure) and other changes to the metabolism, changes to external trophic morphology (e.g. gill raker spacing, gape width), and lastly, life history traits. We have yet to explore these other possibilities.

Conclusions

Predation can have direct and indirect effects on the evolution of life history traits of prey (Gadgil and Bossert 1970, Abrams and Rowe 1996, Walsh and Reznick 2008, 2009). Predators directly affect mortality rates and population size structure (Rodd and Reznick 1997), and indirectly influence the amount of per capita resources available to surviving prey (Wootton 1994). In this study, we showed an association between evolved life history traits in guppies and their diet preferences. Guppies that lived in streams with predators display smaller size at reproduction and higher reproductive allotment. They also had more resources available per capita, which was reflected in their feeding habits: they were more specialized on invertebrates and had higher prey selectivity. Guppies from predator-free streams showed the opposite pattern in life history traits and had fewer resources available in the environment. They also had lower individual growth rates (Reznick et al. 2001). They fed more consistently on lower quality food (detritus), most likely as an effect of the more intense intraspecific competition that guppies experience. Our results suggest that patterns of resource availability and diet selectivity may be linked to the evolution of life history traits. We suggest that resource-based life-history theory should incorporate knowledge of the dietary responses to predation and resource availability. Such a framework would provide a link between foraging and life-history theory. Understanding the coevolution of diet and life-histories can have profound implications for understanding the effects of evolution on communities and ecosystems.

Table 2-1. Stream characteristics for the 4 study sites in Trinidad. Values of the environmental variables have been averaged across the 3 pools. Values with the same uppercase letter are not significantly different. Fish species reported are the ones that have been observed and caught in the sites during multiple years. Differences in average % canopy and stream width were calculated with a Kruskal-Wallis test. Values indicate means (±1 SE).

Stream characteristics	**Aripo HP**	**Guanapo HP**	**Aripo LP**	**Guanapo LP**
Invertebrate biomass (mg/m^2)	177 (37.5) $^{\mathbf{a}}$	20.9 (11.6) $^{\mathbf{b}}$	19.7 (7.0) $^{\mathbf{b}}$	19.2 (4.1) $^{\mathbf{b}}$
Invertebrate abundance (# of individuals)	122 (33.3) $^{\mathbf{a}}$	29 (7.4) $^{\mathbf{b}}$	29.7 (9.9) $^{\mathbf{b}}$	42.3 (18.2) $^{\mathbf{b}}$
Total number of invertebrate families	17	14	14	10
Fish community	*Poecilia reticulata, Rivulus hartii, Hoplias malabaricus, Crenicichla sp,* Characidae (many species), *Rhamdia quelen, Aquidens pulcher, Ancistrus cirrosus, Synbranchus marmoratus*	*Poecilia reticulata, Rivulus hartii, Hoplias malabaricus,* Characidae (many species), *Rhamdia quelen, Aquidens pulcher, Ancistrus cirrosus*	*Poecilia reticulata, Rivulus hartii*	*Poecilia reticulata, Rivulus hartii, Rhamdia quelen*
Canopy Openness (%)	31.5 (8.4)^{a}	12.4 (1.9)^{a}	30.4 (12.7)^{a}	16.3 (3.7)^{a}
Stream Width (cm)	917 (159)^{a}	653 (52.7)^{a,b}	387 (30.3)^{b}	403 (103.9)^{b}

Table 2-2. Univariate ANOVAs for the three food categories found in guppy diets in the streams of Trinidad.

Univariate ANOVAs						
Effect	**Error type**	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Inverts						
Fish length	error	1	0.25	0.25	1.96	0.17
Drainage	Pool (Drainage x Predation)	1	0.20	0.20	0.97	0.35
Predation	Pool (Drainage x Predation)	1	4.72	4.72	23.08	0.001
Drainage*Predation	Pool (Drainage x Predation)	1	0.29	0.29	1.44	0.26
Pool (Drainage*Predation)	error	9	1.84	0.20	1.62	0.13
Error		69	8.70	0.13		
Detritus						
Fish length	error	1	0.75	0.75	8.15	0.006
Drainage	Pool (Drainage x Predation)	1	0.08	0.08	0.66	0.44
Predation	Pool (Drainage x Predation)	1	2.34	2.34	20.28	0.001
Drainage*Predation	Pool (Drainage x Predation)	1	0.34	0.34	2.91	0.12
Pool (Drainage*Predation)	Error	9	1.04	0.11	1.25	0.28
Error		69	6.34	0.09		
Algae						
Fish length	Error	1	0.15	0.15	7.15	0.009
Drainage	Pool (Drainage x Predation)	1	0.07	0.07	1.62	0.23
Predation	Pool (Drainage x Predation)	1	0.48	0.48	10.74	0.01
Drainage*Predation	Pool (Drainage x Predation)	1	0.00	0.00	0.03	0.87
Pool (Drainage*Predation)	error	9	0.40	0.04	2.17	0.03
error		69	1.42	0.02		

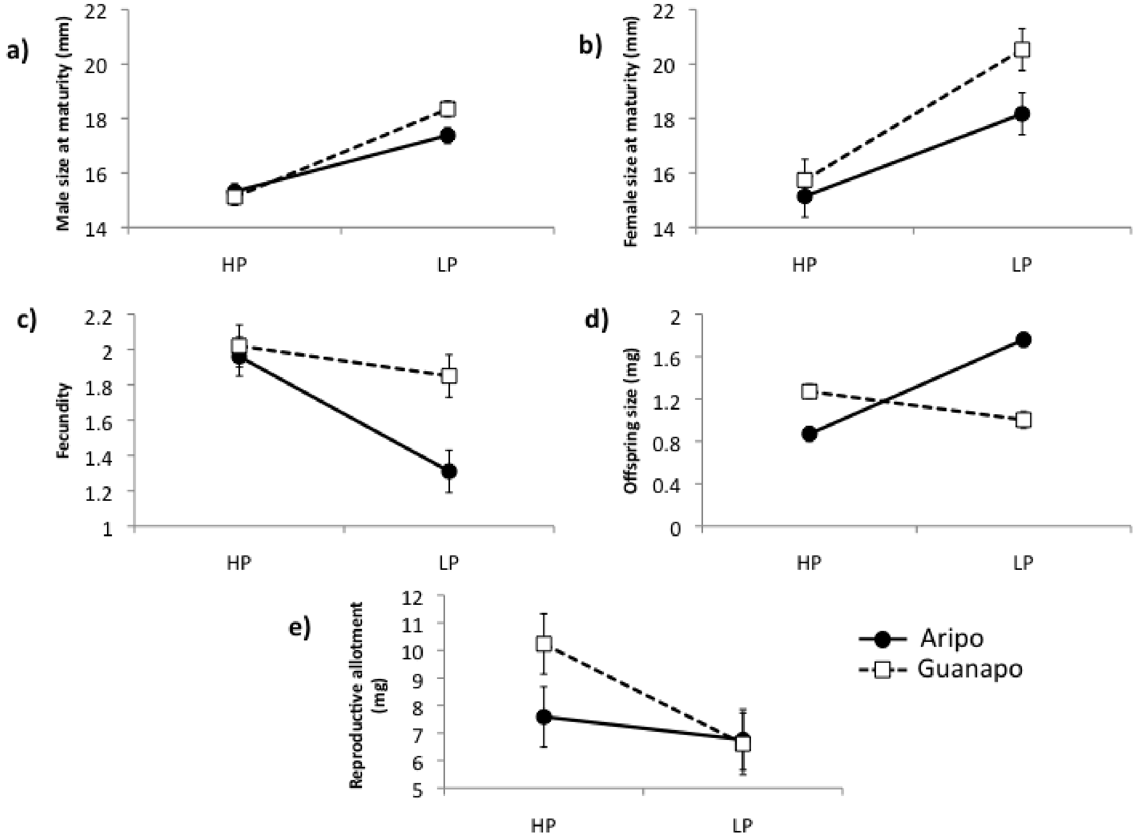


Figure 2-1. Differences in life history traits between high (HP) and low (LP) predation guppies from the Aripo (closed symbols) and Guanapo (open symbols) rivers in Trinidad. Values represent the estimated marginal means calculated by the general linear model (GLM) as explained in the text. Bars represent ±1 S.E.

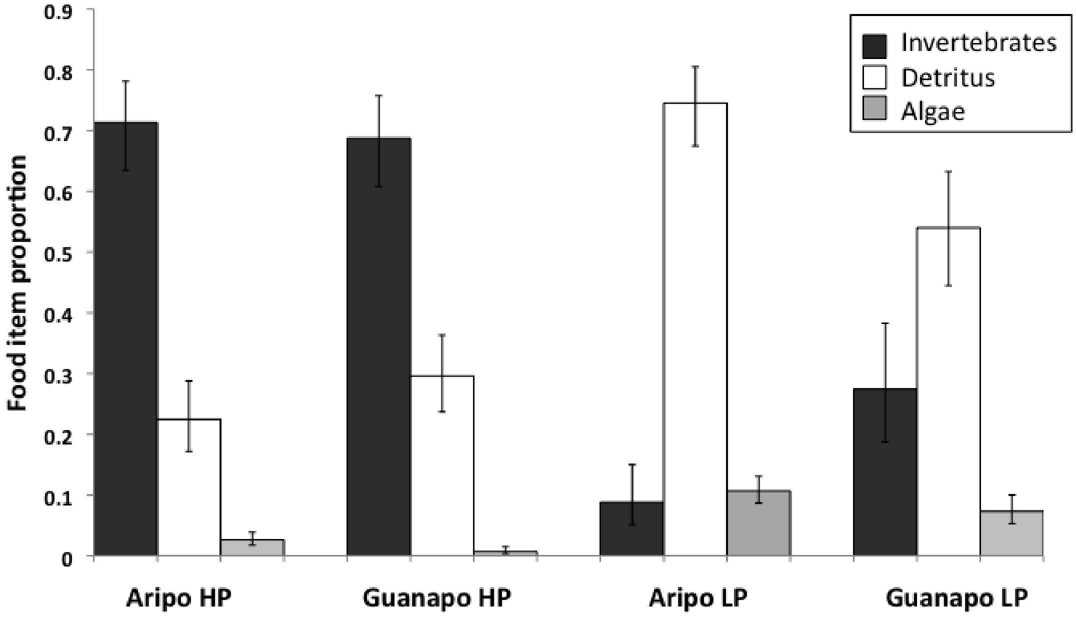


Figure 2-2. Proportional diet composition of HP (high predation) and LP (low predation) guppies from the two study drainages, Aripo and Guanapo, in Trinidad. Data showed here represent the estimated marginal means calculated by the general linear model (GLM) on arcsin transformed data. Estimated marginal means and standard errors have been back-transformed for the graphical representation. Food categories analyzed are invertebrates, in dark grey; amorphous detritus, in white; and algae (filamentous and diatoms) in light grey. Bars represent ±1 S.E.

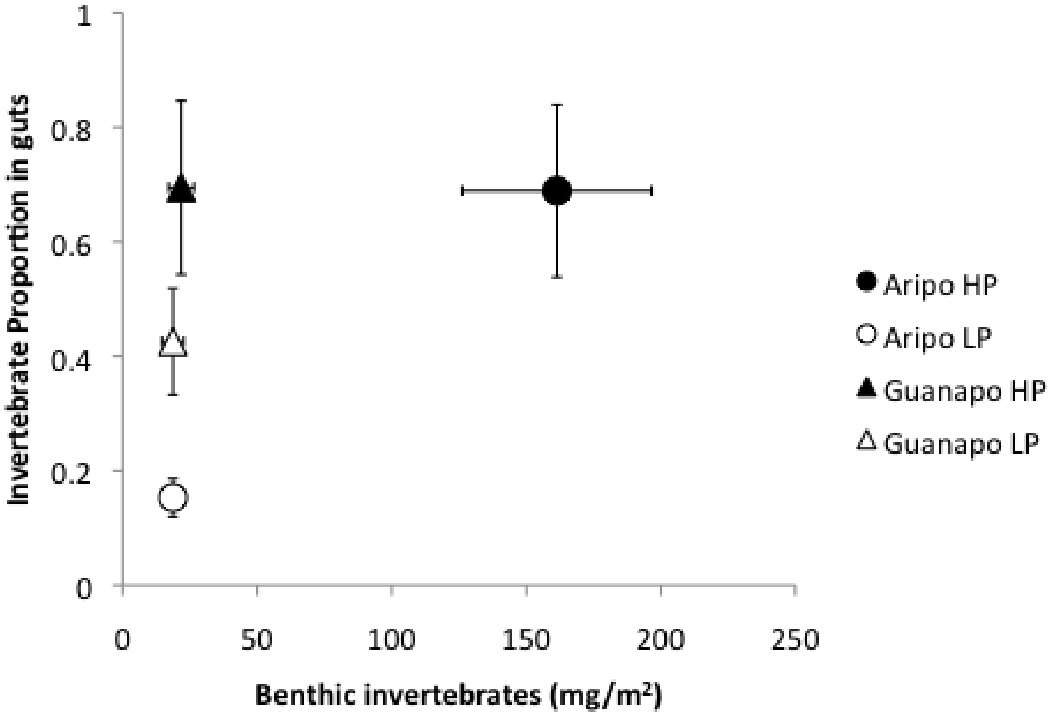


Figure 2-3. Relationship between benthic invertebrate density in the environment and proportion of invertebrates found in the fish guts. Aripo fish are indicated with circles and Guanapo with triangles; high predation (HP) fish are closed symbols, while low predation (LP) are open. Bars represent ±1 S.E.

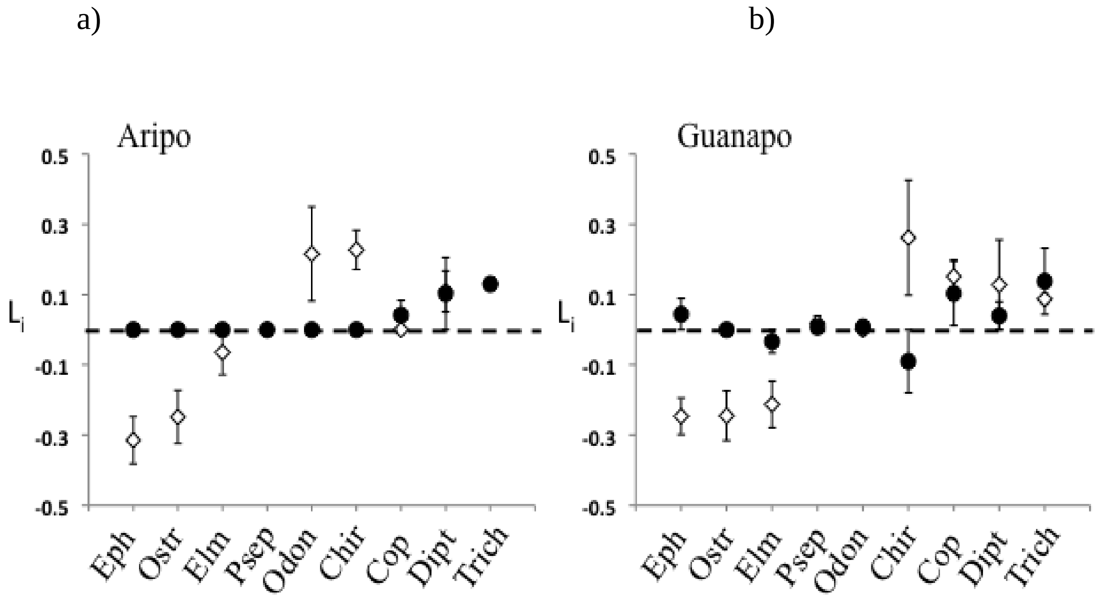


Figure 2-4. Selectivity indexes (L_i) for the 9 invertebrate taxa found in the guppy guts for a) Aripo HP and LP and b) Guanapo HP and LP. Positive values indicate positive selectivity, while negative values indicate avoidance. Error bars represent standard errors calculated across the means of three pools for each site. (Eph = Ephemeroptera; Ostr = Ostracoda; Elm = Elmidae; Psep = Psephenidae; Odon = Odonata; Chir = Chironomidae; Cop = Copepoda; Dipt = Diptera excluding Chironomidae; Trich = Trichoptera).

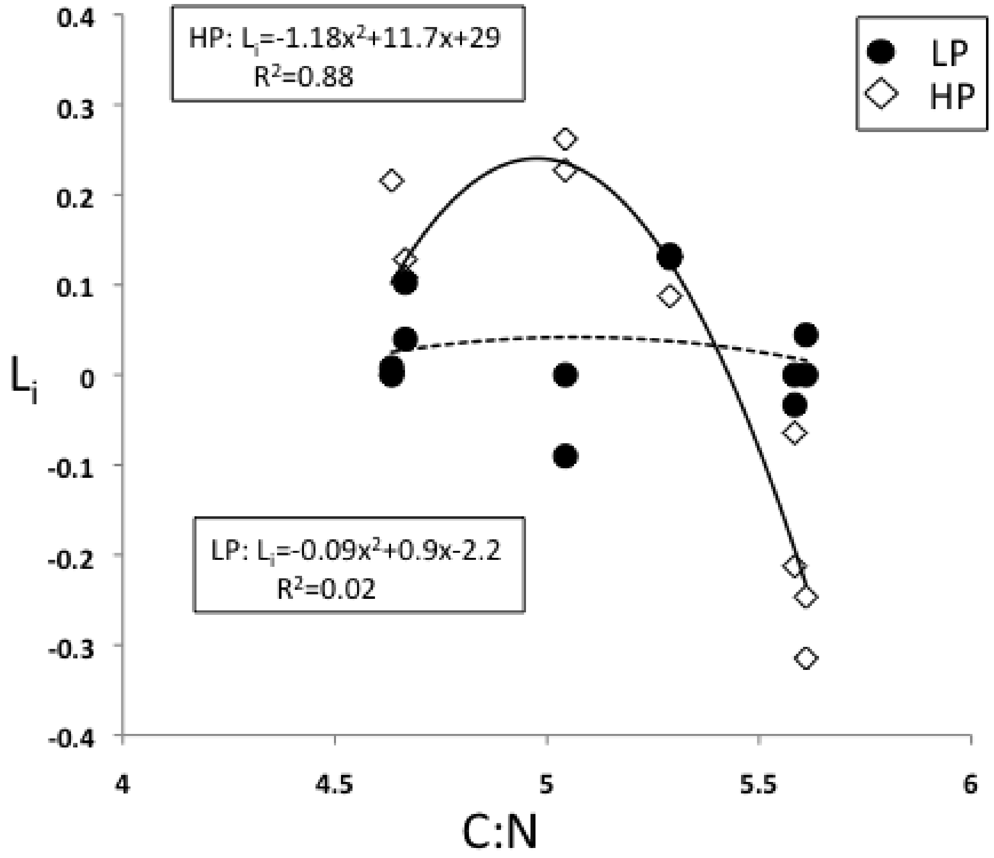


Figure 2-5. Relationship between C:N (molar) invertebrate body composition and selectivity index for HP and LP fish. Guanapo and Aripo have been lumped together in a single graph because the patterns for the two drainages were very similar. Open diamonds indicate HP and closed circles LP. Regression equation and R^2 values are shown in graph.

CHAPTER 3: Seasonal variation in diet and gut length in guppies (*Poecilia reticulata*) from sites with different predation regimes

ABSTRACT

Phenotypic plasticity is an advantageous trait for organisms that live in variable environments. The digestive system is often plastic, changing its size and morphology in response to changes in diet. This occurs because digestive tissues are highly expensive to maintain, thus gut length is the result of a trade-off between maximum nutrient absorption and minimum cost for its maintenance. Here we assessed the variation in gut length of Trinidadian guppies (*Poecilia reticulata*) as a function of diet, season, and ontogeny. We sampled fish from sites that differed in their predation regime (high predation –HP; low predation – LP), in which guppies have evolved different life history traits and have different diets. Guppies from HP and LP sites differ in their diet, with guppies in HP sites feeding mostly on invertebrates, while guppies in LP sites feeding mainly on detritus. We collected fish during both the dry and wet season, assessing their diet and gut length. During the wet season the differences in diet between guppies from HP and LP sites disappeared, with guppies in HP sites decreasing the invertebrate proportion in their diet and guppies in LP sites increasing it. Gut length was negatively correlated with the

proportion of invertebrates in diet, supporting the hypothesis that in guppies digestive systems adapt in size to changes in diet. Our study showed that even in omnivorous fish gut length adapted to different diets, being more evident when the magnitude of difference between animal and plant material in diet was very large.

INTRODUCTION

Some organisms that live in a variable environment show the ability to generate a range of phenotypes, each better suited to certain conditions. These ranges of phenotypes are environmentally induced and thus are not the expression of genetic variation. However, this ability to alter phenotypes, referred to as phenotypic plasticity, is inherited (West-Eberhard 2003, Travis 2009). When individuals that show phenotypic plasticity have an increased fitness advantage compared to those that can only express one phenotype, this is indicated as adaptive plasticity and it can be a target of natural selection (Pigliucci 2001). Adaptive plasticity is advantageous when selection favors different phenotypes for different environmental conditions and if there is not a single phenotype that is best adapted to all circumstances (Ghalambor et al. 2007).

There are many examples of phenotypic plasticity, which can be expressed through behavioral, morphological, and physiological changes. The expression of a different phenotype is triggered by an environmental cue that is unmistakably perceived by the organism. Phenotypic plasticity can be reversible, which typically occurs when environmental changes take place throughout an individual's lifetime, during which the organism can switch from one phenotype to another (Travis 2009). Or it can be irreversible, more common in organisms living in environments that fluctuate less frequently (Travis 2009). A typical example of reversible phenotypic plasticity is the capability of the digestive tissues to adjust according to changes in diet or in the organism's energy demand (Starck 2003). Digestive tissues are one of the most expensive to maintain and it is therefore very important to adjust them to an optimal energy intake/maintenance balance (Sibly 1981). The digestive tract is highly flexible and can adjust in size and morphology to changes in diet quantity and quality, as shown in many vertebrate species (Piersma and Lindstrom 1997, Starck 1999, Naya et al. 2007, Olsson et al. 2007). An animal cannot maintain a unique digestive system that is well adapted for every type of diet, because different food is absorbed through different biochemical pathways or has different processing times (Karasov and Martinez del Rio 2007). For instance, an animal needs a longer gut to digest low quality food (e.g. fiber-rich) than more easily digestible food (e.g. protein-rich) (German and Horn 2006, Olsson et al. 2007, Wagner et al. 2009); longer guts have higher surface area and allow a longer retention time of the food, consequently enhancing nutrient absorption (Sibly 1981). Of course, organisms that have relatively fixed diets (e.g. strict carnivores) do not need to show gut plasticity, because their digestive system is already adapted to optimal efficiency (Buddington et al. 1991). However, gut flexibility becomes of paramount importance for those animals that feed on a wide array of food types or that live in environments with high levels of variation in resources (Karasov and Martinez del Rio 2007). Such animals, for instance omnivorous or diet switchers, display high plasticity in their digestive systems because they often shift to different types of food that have different digestive requirements (e.g. animal vs. plant food) (Piersma and Lindstrom 1997). Also organisms that undergo long periods without eating (e.g. hibernating or migrating animals) require flexible digestive systems, so that they can minimize the cost of maintaining an active organ by reducing its size and cell proliferation (reviewed in Starck 2003 and German et al. 2010).

Here we investigated how differences in diets were reflected in differences in gut length of an omnivorous tropical fish, the Trinidadian guppy (*Poecilia reticulata*). We also assessed how seasonality affected guppy diets and how it was reflected in the gut morphology. Guppies are an ideal model organism because they show two distinct phenotypes that differ in their life history traits (Reznick and Endler 1982, Reznick 1989) and diets (Bassar et al. 2010a) (Zandonà *in prep* – Chapter 2). The two phenotypes have evolved in streams that differ in the degree of predation experienced by guppies. When predation is high, guppies show higher growth rates, smaller size at maturation, and higher reproductive allotment, producing more and smaller offspring. In streams where predation is low or absent, guppies show the opposite patterns: they have slower growth rates, bigger size at maturation, they invest less in reproduction, and have larger and fewer offspring. These different life history trait patterns might have different energetic requirements, which could be met through different feeding choices. Guppies are omnivores (Dussault and Kramer 1981), feeding on aquatic invertebrates, algae, and detritus, yet the two phenotypes show differences in the relative proportion of these food items in their diets. In a mesocosm study, where guppies from high and low predation sites were kept in artificial streams under the same conditions for 28 days, it has been shown that high predation guppies feed prevalently on invertebrates, while guppies from low predation sites have higher proportions of detritus in their diet (Bassar et al. 2010a). Detritus is a low quality food compared to invertebrates and might thus require longer guts to optimize the absorption of nutrients. Here we tested if guppies exhibited phenotypic plasticity in their digestive systems, and if this was related to their different diet preferences. We hypothesized that guppies from low predation sites had longer guts than guppies from high predation sites, being adapted to a lower quality diet.

Seasonality

Organisms living in aquatic ecosystems often change their diets as an effect of ontogeny (Werner and Gilliam 1984) or seasonal variation in the availability of food (Winemiller 1990). For instance, tropical streams often undergo rapid shifts in the quantity and quality of resources and suitable habitats available due to changes in precipitation patterns. It is advantageous for organisms that live in such variable environments to respond to these sudden changes by modifying their diet and their digestive system. The streams in Trinidad experience variation in resource availability due to the presence of distinct dry and wet seasons, which are characterized by changes in precipitation patterns. During the wet season, flooding and associated scouring events increase in frequency, reducing benthic invertebrate and algal biomass (Pringle and Hamazaki 1997), but increasing allochthonous inputs into the stream (Owens 2010). Benthic invertebrate communities generally decrease in abundance and species composition, and their spatial distributions, stoichiometric and nutritional content can be altered. Fish populations are also affected by precipitation patterns: in the wet season densities decline and the competitive or predatory interactions might weaken (Winemiller and Jepsen 1998).

Guppies also respond to the environmental changes occurring during the wet season by decreasing population size and modifying their life history patterns (Reznick 1989). In the wet season guppies mature at a larger size, their fecundity decreases, and they invest less energy into reproduction as compared to the dry season (Reznick 1989). Differences in life history traits between high and low predation localities persist, but they change, mostly decreasing, in magnitude. The changes in life history traits with season can be the result of changes in resources available to guppies, which most likely decrease in the wet season. We expected that guppies would respond to these changes in the type and amount of resources available by modifying their diet and gut morphology. To test this, we collected guppies from both the dry and the wet season and analyzed their diet and gut length. We hypothesized that guppies would change diet with season and that their digestive system morphology would reflect this variation in diet.

MATERIALS AND METHODS

We conducted this study in the Aripo river, which is part of the Caroni drainage system, located on the South slope of the Northern Range of Trinidad. Guppies were collected from two sites indicated as low predation (LP) and high predation (HP), which were characterized by different fish communities and different guppy phenotypes. In the HP environments guppies co-occured with a large suite of fish species, including the pike cichlid (*Crenicichla sp.*) and the wolfish (*Hoplias malabaricus*), which are piscivorous fish that prey upon guppies. In the LP sites, guppies co-occured with only one other fish species, the Hart's killifish (*Rivulus hartii*), which competes with guppies and can occasionally prey on them. The LP environments were generally found upstream of barrier waterfalls, which prevent the upstream movement of many fish species. The HP sites are generally bigger streams, with less canopy cover, and higher productivity, while LP sites are smaller, with more canopy cover, and fewer resources (Reznick et al. 2001).

We collected samples in July 2006 (wet season) and March 2007 (dry season) on individual dates for each site. In each site, we collected guppies from three pools and within each pool, from areas with different stream velocity (low, medium, and high) to ensure sampling of most of the microhabitats guppies use in the stream. Fish were collected with hand nets and euthanized immediately with an overdose of the anesthetic MS-222. Guppies were then measured for standard length with digital calipers, weighed, and intact guts were removed. Guts and fish were preserved in a 5% formalin solution.

DIET ANALYSIS

A total of 41 dry season (21 for HP and 20 for LP) and 54 wet season guppies (26 in HP and 28 in LP) were analyzed for diet content. Using a dissecting microscope, the stomach and a small part of the foregut were separated from the rest of the gut, at the point where the gut turns 180 degrees. The rest of the gut (hindgut) was not used for the analysis, because food was too digested to be recognized. Stomach and foregut were placed onto a gridded slide where their contents were taken out and the gut wall removed. Invertebrates were identified to the lowest possible taxonomic level, usually down to family (Perez 1996, Merritt et al. 2007), and when possible, length and head width were measured. After the invertebrate identification, the material on the gridded slide was distributed evenly with a probe and covered with a cover slip. Ten squares (out of 64) of the slide were chosen randomly for quantification of the gut content under a compound microscope. Proportions of invertebrates and detritus were estimated for each square, and individual diatoms and filamentous algae were counted because they were too small to estimate their proportion coverage in one square. An average size for diatoms and one for filamentous algae was subsequently assigned to calculate the area they occupied in the 10 squares. The area taken by each food category was calculated for the whole slide (64 squares). Plant matter, inorganic material, and other algae were not included in the analysis as their occurrence was very low.

We used a multivariate analysis of covariance (MANCOVA) to test for predation and seasonal effects on the proportion of food items (invertebrates and detritus) in the guppy diet. We used fish length as a covariate, because fish often switch their diet with age/size. We did not include the proportion of algae as a dependent variable into the MANCOVA because it violated the assumptions of equality of variances and normal distribution of the residuals.

GUT LENGTH MEASUREMENT

We measured gut length of 80 guppies from the dry season (39 for HP, 41 for LP) and 44 guppies from the wet season (23 for HP, 21 for LP). All guppies analyzed for gut length measurement were different individuals from the ones used for dietary analyses. We placed individual guts in a petri dish and cut them into 2-3 parts, as the intestines can be very convoluted. In this way we could measure every part without stretching the guts, which would bias the total length measurement. We took pictures of the individual guts with a camera connected to a Leica dissecting microscope and to a computer. We measured each gut picture using the software J-Image. We took 3 different measurements for each gut and averaged them to get the final gut length.

Because of the allometric relationship between fish length and gut length, we employed a two-way ANCOVA, with fish standard length as a covariate, to test for differences in gut length between groups. In the model, predation level, season, their interaction, and the interaction between fish length and predation were the main effects.

In order to gather more insights into gut length morphology, we also measured the gut length of guppies caught in the dry season from HP and LP localities of another river, the Guanapo. In a companion paper (Chapter 2), we analyzed the diet content of guppies from these same Guanapo localities and hence we wanted to correlate the diet result with gut lengths. The diet results for the Guanapo river are reported in Table 2-2. We performed a one-way ANCOVA, with fish length as our covariate, to test for differences in gut length between HP and LP fish. Because there was no significant interaction between predation and fish length, we removed this effect from the model and left only predation as a fixed factor.

Finally, we ran a linear regression analysis to examine the relationship between relative gut length and proportion of invertebrates in the diet. We included in the regression analysis only fish between 15 and 20 mm standard length, so we could minimize the effects of the allometric relationship between gut length and fish length (see also Kramer and Bryant 1995a, b). Guppies’ length ranges from approximately 8 to 25 mm and they are generally sexually mature above 15 mm. We also chose this size range because we had the highest sample size across all sites and seasons. The relative gut length was calculated as the gut length divided by the fish length. The use of this metric can be controversial when used to compare individuals of different sizes (Kramer and Bryant 1995a, b), thus we minimized this problem by using fish of the same size class across all sites. Because we did not have both gut length and diet measurements for each individual fish, an average value was assigned for the proportion of invertebrates for each site and season (Aripo HP and LP for both dry and wet season; Guanapo HP and LP for only the dry season), which was the estimated marginal mean obtained from the diet analysis.

Gut length, fish length, and relative gut length measurements were log transformed while proportion of food items values were arcsin square root transformed to meet the assumption of normal distribution of data. All levels of significance were accepted at 0.05 and statistical analyses were performed using PASWStatistics 18.0 (SPSS inc.).

RESULTS

SEASONALITY IN DIET

Guppies from the Aripo river changed their diet with season. In the dry season there were significant differences between diets of guppies in HP and LP sites, but in the wet season those differences disappeared and guppies in the HP and LP sites had essentially the same diet (Fig. 3-1). The MANCOVA showed a significant effect of predation ($F_{2,88} = 7.543$, $P = 0.001$), season ($F_{2,88} = 10.168$, $P < 0.001$), and of the interaction between season and predation ($F_{2,88} = 12.294$, $P < 0.001$) on the guppy diet. Guppy length also had a significant effect on diet ($F_{2,88} = 8.085$, $P = 0.001$), as well as did the interaction between guppy length and season ($F_{2,88} = 7.572$, $P = 0.001$).

This heterogeneity of slopes was generated by the different correlations between proportion of invertebrates in the diet and guppy length between dry and wet season (univariate ANOVA: $F_{1,89} = 7.146$, $P = 0.009$) (Fig. 3-2). However, there was no heterogeneity of slopes for the proportion of detritus in the diet and guppy length between seasons (univariate ANOVA: $F_{1,89} = 2.344$, $P = 0.129$) (Table 3-1 and Fig. 3-2). We ran two regression analyses to look at the relation between the proportion of invertebrates in the diet and guppy length for each season. In the wet season, there was a significant ($F_{1,53} = 9.464$, $P = 0.03$; $y = -0.045x + 1.33$, $r^2 = 0.15$) negative relationship between proportion of invertebrates in the diet and guppy length (Fig. 2). In the dry season instead there was no significant relationship ($F_{1,40} = 0.100$, $P = 0.753$; $y = 0.007x + 0.57$, $r^2 = 0.03$) (Fig. 3-2).

GUT LENGTH

The two-way ANCOVA confirmed the allometric relationship between gut length and fish length (fish length effect: $F_{1,118}=423.510$, $P<0.001$). It also showed a significant effect of predation ($F_{1,118}=8.654$, $P=0.004$) and a marginally significant effect of season ($F_{1,118}=3.792$, $P=0.054$) on gut length in guppies. However, there was also a significant effect of the interaction between predation and season ($F_{1,118} =12.919$, $P<0.001$), and of the predation by guppy length interaction ($F_{1,118}=11.565$, $P=0.001$). Because of the heterogeneity of slopes due to the significant interaction between predation and guppy length, we need to be careful interpreting the results for the main effects (predation, season, and their interaction). For this reason, we also ran regression analyses for each site and each season to examine in more detail the relationship between gut length and guppy length (Fig. 3-3). The slopes of the linear regressions were steeper for low predation guppies (dry: $F_{1,39}$= 278.32, $P < 0.001$, $y = 1.76x – 0.81$, $r^2 = 0.88$; wet: $F_{1,19}$= 74.31, $P < 0.001$, $y = 1.9x – 1$, $r^2 = 0.80$) than for high predation guppies (dry: $F_{1,37}$= 91.68, $P < 0.001$, $y = 1.29x – 0.38$, $r^2 = 0.71$; wet: $F_{1,21}$= 14.15, $P = 0.01$, $y = 1.14x – 0.13$, $r^2 = 0.40$) both in the dry and wet season. This indicated that low predation guppy guts increased in size with fish length faster than for high predation guppies. The wet season samples were more limited in size range, with high predation guppies being mostly small individuals and low predation big individuals, thus reducing the overlap in size between the two sites. This limited overlap made the patterns in gut length more difficult to interpret. For instance, the regression analysis for the low predation site was highly affected by the only small fish data point (size: 11.66 mm). When this individual was removed from the analysis, the equation and r^2 value change substantially ($F_{1,18}$= 32.20, $P < 0.001$; $y = 1.57x – 0.6$; $r^2 = 0.64$), reducing the difference in slope between high and low predation guppies. Overall, in the dry season there was an obvious difference in gut length patterns between high and low predation fish, with low predation guppies having overall longer guts in particular when fish were above a certain size. In the wet season instead the patterns were not so clear primarily due to the more limited size range of our samples (Fig. 3-3).

Analysis on the Guanapo river showed that predation did not have a significant effect on gut length ($F_{1,77}$=5.571, P=0.073). The regression equations for high and low predation guppies are very similar (HP: $F_{1,39}$= 160.42, $P < 0.001$; $y = 1.515x - 0.56$, $r^2 = 0.80$; LP: $F_{1,37}$= 211.30, $P < 0.001$; $y = 1.75x - 0.88$, $r^2 = 0.85$), confirming the patterns found in the ANCOVA. The diet differences between high and low predation guppies in the Guanapo (Table 3-2) did not have the same magnitude as in the Aripo (Fig. 3-1a). In particular, the Guanapo LP guppies had very similar proportions of detritus and invertebrates in their diet, thus still maintaining a relatively high proportion of invertebrates in their diet, while in the Aripo river guppies in the LP site had very low amounts of invertebrates in their guts.

The allometric equations for all 6 data sets fitted well the relationship between gut length and guppy length, with r^2 values that ranged from 0.40-0.88 (average = 0.74). The slope of the allometric equations ranged from 1.14-1.9, indicating that guppy intestine length always increased faster than body length (Figs. 3-3 and 4-4).

There was a significant negative relationship between the proportion of invertebrates in the diet and relative gut length across all our guppy samples ($F_{1,117}$ = 31.79, $P < 0.001$; $y = -0.19x + 0.135$; $r^2 = 0.21$). In other words, as the proportion of invertebrates in the diet decreased (indicating a herbivory increase and thus an overall lower quality diet), there was an increase in the relative gut length (Fig. 3-5)

DISCUSSION

In vertebrates, the gastrointestinal tract responds to changes in diets, optimizing nutrient absorption and minimizing energy expenditure (Karasov and Martinez del Rio 2007). In our study gut morphology in guppies was a plastic trait influenced by temporal and spatial changes in diet. Guppies from high and low predation sites had differences in their diets, which were correlated with differences in gut length. However, gastrointestinal external morphology responded to diet differences mainly when the magnitude of change was big. Our study also confirmed the allometric relationship between fish length and intestine length in guppies, with slope values within the range found in other studies (Kramer and Bryant 1995a).

Seasonality in diets

In the dry season we found the biggest differences in diet between high and low predation guppies (Fig 3-1. and Table 3-2). In particular, low predation guppies fed more consistently on low quality food – detritus – and showed relatively longer guts compared to high predation fish, which instead had a higher quality diet (mostly based on invertebrates) and had comparatively shorter guts. In the wet season, guppies from both localities switched their diet: in low predation sites they increased the proportion of invertebrates, while in high predation sites they decreased it and ate more detritus. As a result, the differences in diet between guppies in high and low predation sites became negligible in the wet season.

There are several possible explanations for why guppies switch their diet in this fashion between seasons. During the wet season the density of guppies decreases due to both the increased volume of water in the streams and the reduction in population size caused by high frequencies of washouts. We might thus expect that in the wet season the per capita resources available increase and intraspecific competition decreases. As a response, guppies could increase the amount of higher quality (invertebrates) food in their diet, especially in those streams where intraspecific competition was higher and the access to high quality resources more limited. This would explain the larger amount of invertebrates in the diet of low predation guppies, which live in sites with higher guppy density and thus stronger intraspecific competition.

Alternatively, in the wet season, especially after heavy rain episodes, the amount of resources, such as periphyton and benthic invertebrates, drops off (Maharaj 1994, Pringle and Hamazaki 1997, Ramirez et al. 2006), thus reducing food sources available to the guppies. High predation sites are generally located more downstream and are generally wider, while low predation sites are more upstream, narrower and have higher canopy cover (Reznick et al. 2001), a distinction that also characterizes our two Aripo sites. Upstream sites might recover much faster from heavy rain events than downstream, wider streams, which instead receive the water from all tributaries, thus increasing their own discharge many fold. The high predation population might be more heavily affected by the increasing precipitation during the wet season and might have more problems finding suitable refugia and food. This can explain why the guppies in high predation sites decreased the proportion of invertebrates in their diet from dry to wet season.

Reznick (1989) showed that guppy life history differences between high and low predation phenotypes persist during the wet season but decrease in magnitude. For instance, during the wet season the size at maturity increases for both phenotypes, but in low predation sites increases more than in high predation. In laboratory studies, decreased food availability leads to smaller sizes at reproduction (Walsh and Reznick 2008). Our data showed that low predation fish increased the amount of invertebrates in their diet – perhaps due to the higher per capita availability in the stream -, which might be linked to the observed increase in size at maturation. High predation guppies decreased the proportion of invertebrates in their diet in the wet season compared to dry season, but the proportion was still high (~40%). This might explain why their size at maturation did not change as much. Fecundity and reproductive allotment decreased in the wet season, especially for high predation guppies (Reznick 1989). A decrease in these traits is consistent with the response to a decrease in food availability (Reznick and Yang 1993, Walsh and Reznick 2008). The greater decrease for these traits in guppies in high predation than low predation sites may indicate a larger magnitude decrease in high quality resources (invertebrates) for high than for low predation sites. This was confirmed by our diet analysis, which showed a decline in invertebrates in the high predation guppies diet, but smaller changes in the low predation guppies.

Gut length

We expected gut lengths to mirror the patterns found in the diet, so that nutrient absorption could be optimized. That is, shorter guts would correspond to more carnivorous diets (more invertebrates) and, vice versa, longer guts would correspond to more herbivorous diets (more detritus and algae). We found such a correlation between the high and low predation sites in the Aripo river during the dry season. In this time, we found the biggest differences in diet between the two populations (Fig. 3-1a) and also the biggest difference in gut length (Fig. 3-3a). In the Guanapo sites, we did not find such a clear relationship between diet and gut length (Fig. 3-4). However, in this stream the differences in diet between high and low predation were not as big as in the Aripo. For instance, in both drainages the percentage of invertebrates in the diet for guppies in high predation sites was around 70%. In the Aripo river the percentage of invertebrates in the diet for guppies in low predation sites was much lower (~10%), but it was up to ~40% for guppies in the Guanapo low predation site. A similar but opposite pattern was found for the detritus.

In a study on 21 species of fish from Panama, Kramer and Bryant (1995b) showed that the allometric equations relating fish body length to intestine length were different and with decreasing values between herbivorous, omnivorous, and carnivorous fish. However, within omnivores, they did not find differences in gut length between species consuming different proportion of plant material. Our data on the Aripo dry season did not agree with Kramer and Bryant's (1995b) results, but our Guanapo data did. Perhaps the differences in invertebrate and detritus proportions between the diet of high and low predation guppies in the Aripo river were big enough to lead to correspondent differences in their gut length, but they were not so in the Guanapo river. Further work is required to clear this up.

There was an allometric relationship between guppy length and gut length, with slopes greater than 1, indicating that gut length increased faster than guppy length. This result suggests that guppies might switch their diets with age. However, we found only limited indications of an ontogenetic shift in diet in our gut content analysis. Only in the wet season was there a significant relationship between the proportion of invertebrates in diet and guppy length in both high and low predation sites, indicating that small fish were more carnivorous than bigger ones, which has been shown in several species of fish. Nevertheless, considering that in the dry season we did not find indications of an ontogenetic diet switch and that in the wet season it was a limited phenomenon (the regression equation only explained 15% of the variation), it was more likely that the allometry of gut length in guppies was explained by the necessity of maintaining the surface-to-volume ratio with increasing size (Kramer and Bryant 1995a).

Conclusions

While our findings confirmed that guppies are omnivores (Dussault and Kramer 1981), they also indicated that guppies have a broad range of variation in the proportion of invertebrates and detritus in their diet, which changed temporally with season and spatially with the presence/absence of predators. These variations in diet preferences were often correlated with the gastrointestinal tract length. Guppies that showed higher levels of carnivory also had the shortest guts, and vice versa, higher levels of herbivory were correlated with longer gut lengths. Gut length in guppies appeared to respond to different diets, but it could not be used alone as an indication of the herbivory or carnivory level. The plasticity of the digestive system is an important attribute for guppies because it offers the possibility of responding favorably to changes in food sources, maximizing nutrient absorption and energy extraction from different food types.

Our study shows that even within omnivores, gut length can change to relative differences in the proportion of animal and plant food sources. If differences between these two food categories are not very high, the gastrointestinal morphology may not change substantially in length. The cost of adapting to small changes in diet could perhaps be higher than the actual benefit of changing the morphology of the guts.

Table 3-1. Univariate ANCOVA results on the effects of predation and season on the proportion of invertebrates and detritus in the diets of guppies from Trinidad. SS = sums of squares; df = degrees of freedom; MS = mean squares. All F-ratios are based on type-III sums of squares.

Univariate ANCOVA					
Source	**SS**	**df**	**MS**	**F-ratio**	**P-value**
Invertebrates					
Predation	1.93	1	1.93	15.26	<0.001
Season	0.79	1	0.80	6.26	0.01
Guppy Length	0.40	1	0.40	3.15	0.08
Predation*Season	3.01	1	3.01	23.88	<0.001
Season*Guppy Length	0.90	1	0.90	7.15	0.01
Error	11.23	89	0.13		
Detritus					
Predation	1.16	1	1.16	13.45	<0.001
Season	0.10	1	0.10	1.11	0.29
Guppy Length	0.73	1	0.73	8.49	0.005
Predation*Season	2.10	1	2.10	24.31	<0.001
Season*Guppy Length	0.20	1	0.20	2.34	0.13
Error	7.71	89	0.09		

Table 3-2. Diet composition of Guanapo guppies from high predation (HP; N = 21) and low predation (LP; N = 21) sites (data from Chapter 2). Proportions of the 3 food items are estimated marginal means (±SE) calculated by a MANCOVA (predation as the fixed effect and fish length as a covariate); data reported have been back-transformed. All F-ratios are based on type-III sums of squares. Num df = numerator degrees of freedom; Den df = denominator degrees of freedom.

Predation	**Food item**	**Proportion**
HP	Invertebrate	0.70 (0.08)
HP	Detritus	0.27 (0.06)
HP	Algae	0.01 (0)
LP	Invertebrate	0.42 (0.08)
LP	Detritus	0.47 (0.07)
LP	Algae	0.04 (0.01)

MANCOVA

Source	**Statistics**	**Value**	**F-value**	**Num df**	**Den df**	**P-value**
Predation	Wilks' Lambda	0.78	3.38	3	37	0.03
Guppy length	Wilks' Lambda	0.83	2.55	3	37	0.07

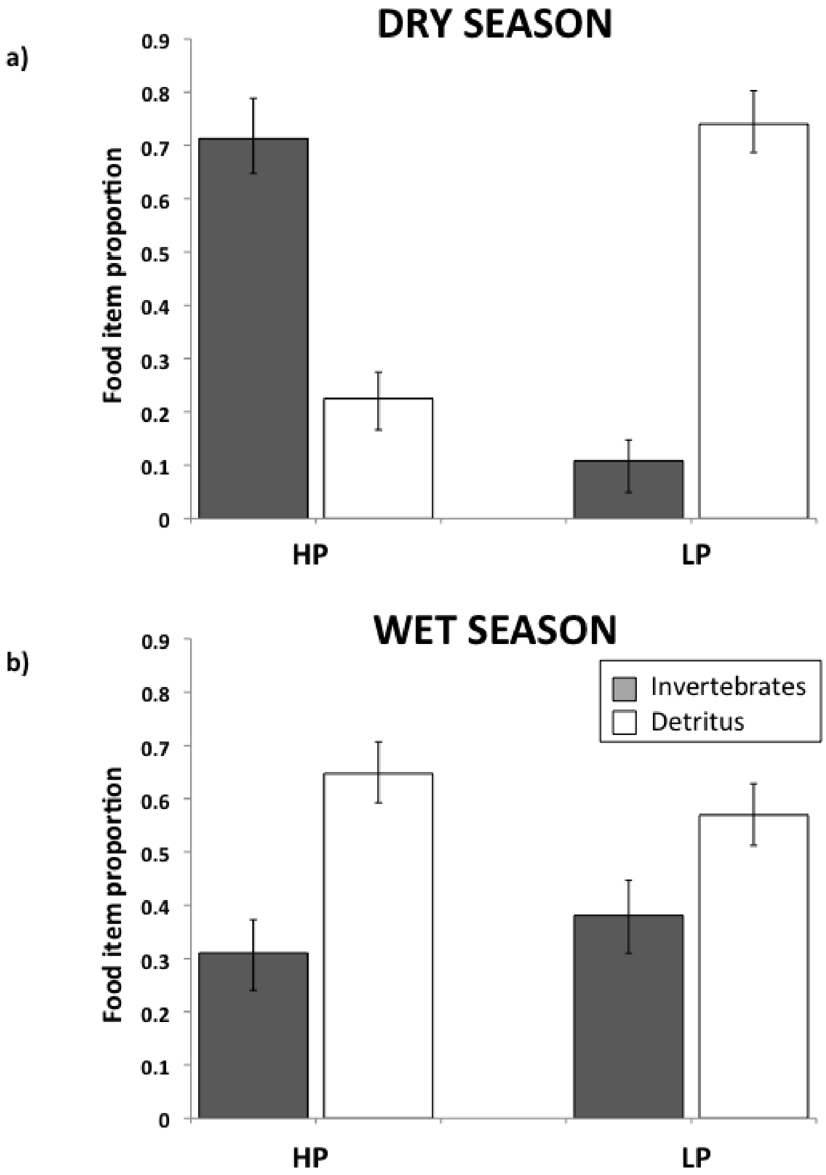


Figure 3-1. Seasonal effect on the diet of guppies from HP (high predation) and LP (low predation) guppies from the Aripo river. Percent diet composition is shown for the dry (a) and the wet (b) seasons. Data showed here represent the estimated marginal means calculated by the MANCOVA on arcsin transformed data. Estimated marginal means and standard errors have been back-transformed for the graphical representation. Food categories analyzed are invertebrates, in dark grey and amorphous detritus in white. Bars represent ±1 SE.

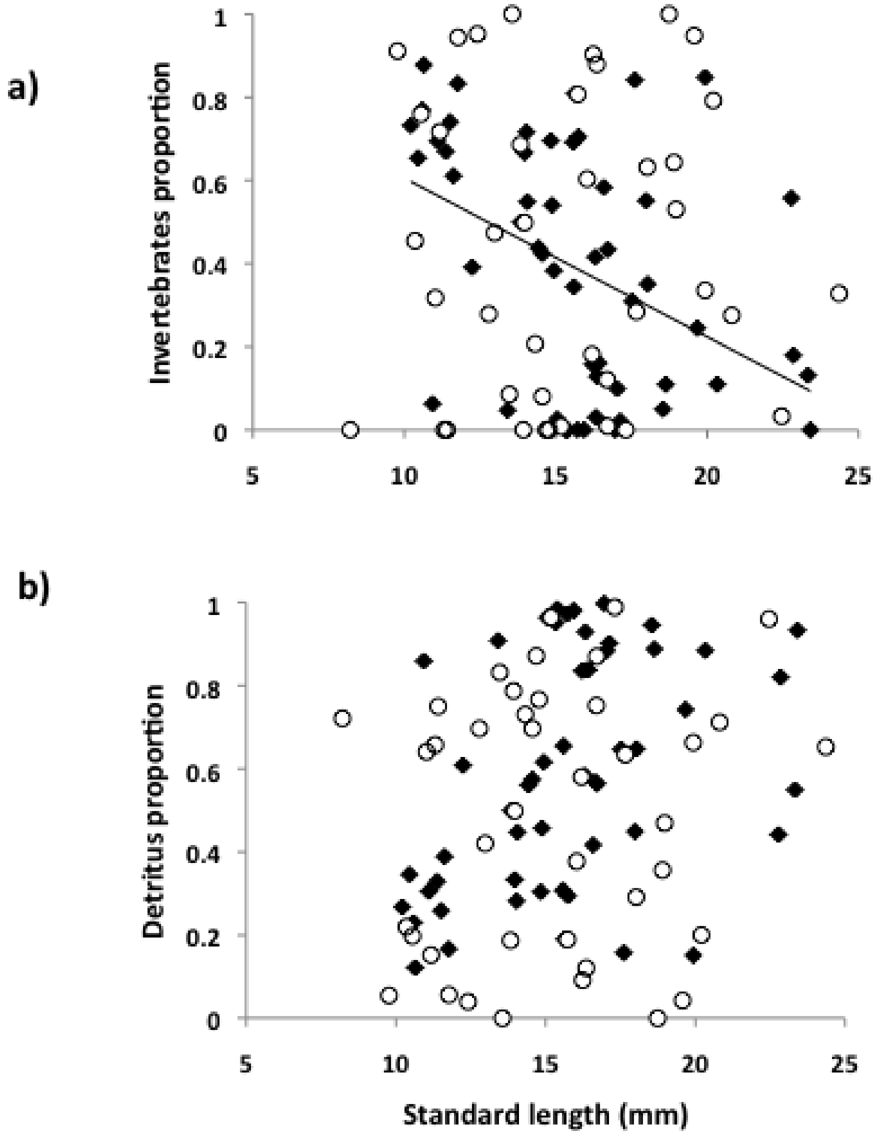


Figure 3-2. Correlation between guppy standard length and a) proportion of invertebrates and b) proportion of detritus in guppies diet. Wet season (filled diamonds) and dry season (open circles) fish are shown. Regression line shows the only significant relationship, which is between proportion of invertebrates in diet and guppy length during the wet season.

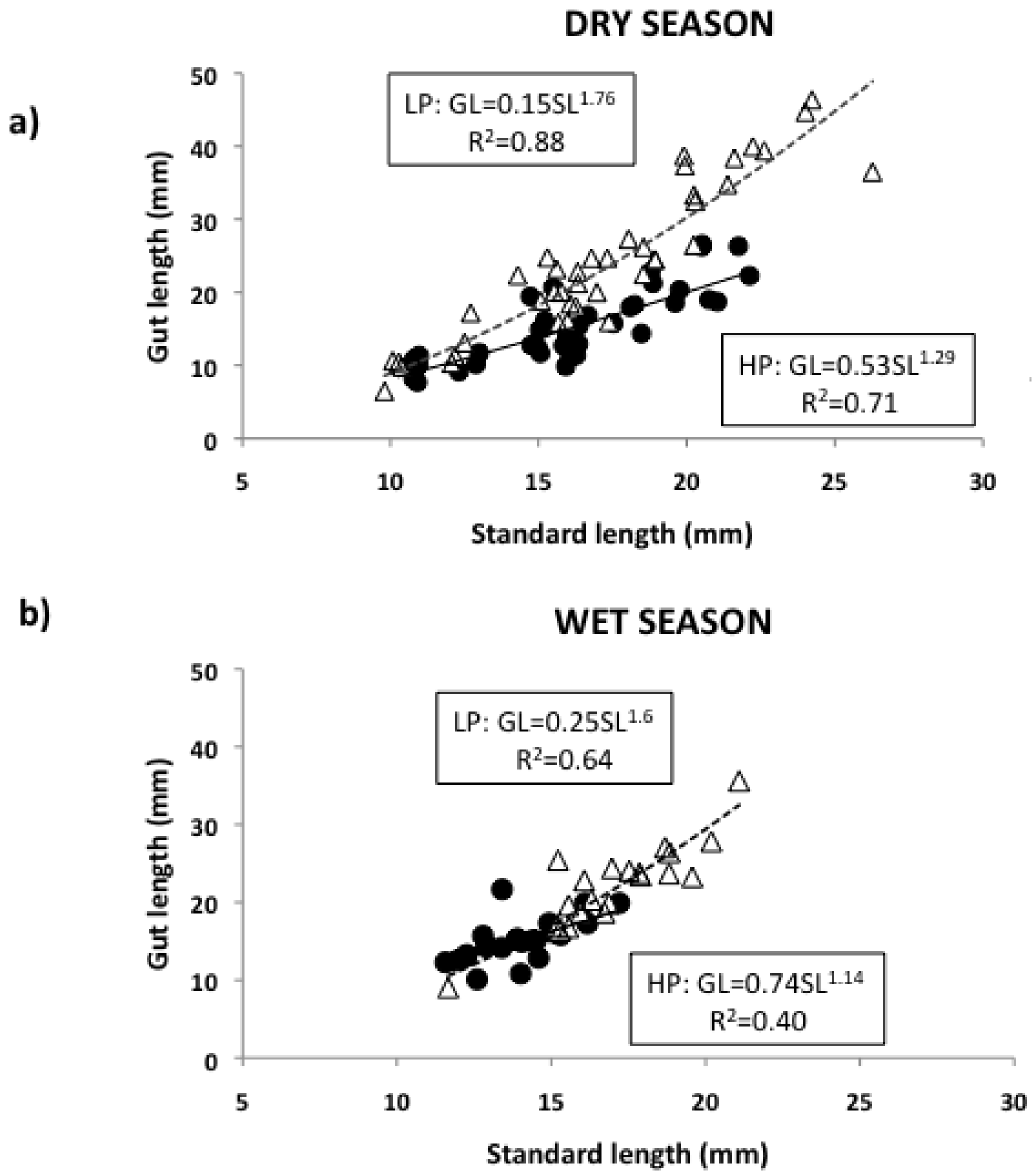


Figure 3-3. Correlation between fish standard length and gut length in fish from HP (filled circles) and LP (open triangles) streams in the dry (a) and wet (b) season. Data are not transformed and equations and R^2 values are calculated on non-transformed data. Trend lines are exponential.

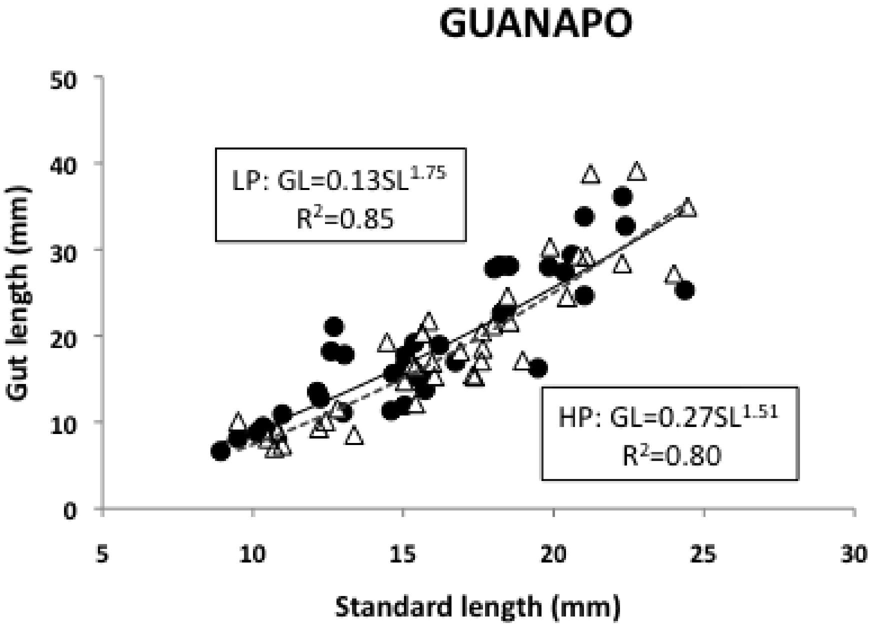


Figure 3-4. Correlation between fish standard length and gut length in fish from HP (filled circles) and LP (open triangles) sites in the Guanapo river. Data are not transformed and equations and R^2 values are calculated on non-transformed data. Trend lines are exponential.

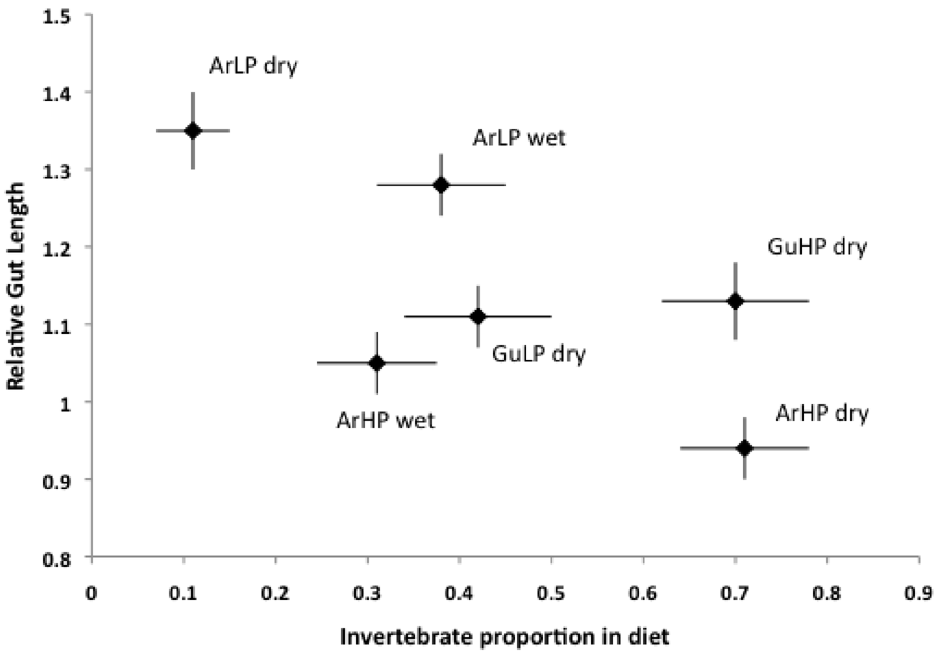


Figure 3-5. Mean proportion of invertebrates in diets vs. mean relative gut length for all guppies between 15 and 20 mm. Each data point represents one site (Aripo HP and LP for both dry and wet season, and Guanapo HP and LP from the dry season). Relative intestine length was calculated as the gut length divided by fish length. An average value was assigned for the proportion of invertebrates for each site, which was the estimated marginal mean obtained from the diet analysis.

CHAPTER 4: Intraspecific variation in trophic niches of the Trinidadian guppies (*Poecilia reticulata*)

ABSTRACT

Intraspecific difference in trophic niches is a widespread characteristic of many vertebrates that live in diverse habitats or with different competition and predation regimes. Guppies have evolved different life history traits as an effect of different predation pressure, where populations that experience high predation (HP sites) grow faster, reproduce earlier, produce more and smaller offspring than populations that live in low predation sites (LP). In this study, we assessed the existence and repeatability of intraspecific differences in trophic niches of guppies from a series of 6 replicate HP-LP population pairs. We examined direct diet (through gut analysis), trophic position and sources contribution to diet (with $\delta^{13}C$ and $\delta^{15}N$ stable isotopes) of guppies collected in 12 streams during the wet season. We also measured the environmental characteristics (invertebrate, algae, fine and coarse organic matter biomasses, and stream morphometrics) of each site and investigated the existence of common patterns within HP and LP sites. We found that in the wet season guppies in LP sites generally had higher trophic positions and had higher invertebrate proportion in their diets than guppies in HP sites. This result was in

contrast with what we previously observed in guppies in the dry season. The river of origin had an equally important effect as predation in shaping guppy trophic positions and diets. The causes of this pattern require further exploration. There was no indication of a shift in resource consumption (in diet and trophic position) as a function of guppy size. Sites with the same predation regimes showed many similarities in their environmental characteristics: HP sites generally had higher invertebrate biomass, less canopy cover, lower algae biomass, lower coarse and fine benthic organic matter biomass, while LP sites showed the opposite patterns. We discuss the patterns found in guppy trophic niches and site characteristics as an effect of intraspecific competition, top-down forces, and abiotic components due to seasonality.

INTRODUCTION

Intraspecific niche differentiation is widespread among many species of vertebrates, such as birds (Smith 1987), amphibians (Martin and Pfennig 2010), and fish (Robinson et al. 1993, Robinson and Wilson 1994, Svanback and Persson 2004), and can be manifested through morphological, behavioral, physiological, and life history adaptations (Smith and Skulason 1996). Niche differentiation and trophic polymorphism are often observed when populations adapt to a number of different habitats and resource abundances (Smith 1987, 1990).

Aquatic habitats offer a great number of examples of niche differentiation, because they are often heterogeneous and can present temporal and spatial variation in environmental characteristics. For instance, the quantity and quality of resources available can be altered as an effect of biotic (e.g. presence of predators, competitors, etc.) and abiotic (e.g. precipitation patterns, light regime, water velocity, turbidity, etc.) factors. Typical examples of such spatial variations in habitat characteristics are found in lakes between littoral (near shore) and pelagic (off-shore) areas. Species that inhabit each of these habitats show characteristic and different morphological adaptations, diet selectivity, feeding behavior, and so on (e.g. perch: Svanback and Persson 2004; stickleback: Svanback and Bolnick 2007; pumpkinseed: Wainwright et al. 1991, Robinson et al. 1993). Similar intraspecific niche differences are also found in alewives, between landlocked and anadromous populations (Palkovacs and Post 2008, Post et al. 2008).

Intraspecific diversification can also be manifested as an ontogenetic shift in niche use between young and adult individuals. Fish often show ontogenetic niche shifts, which can be expressed as differences in use of food resources or as habitat switches (see references in Werner and Gilliam 1984). It is not uncommon to find species that shift from feeding on zooplankton when young to feeding on fish when adults (Olson 1996), or from insectivory to frugivory (Drewe et al. 2004), or from carnivory to herbivory (German and Horn 2006). There can also be less drastic changes, where fish feed on progressively larger prey with increasing size, prey that they could not get when they were smaller due to gape limitation. These changes in diet with age help to partition resources along an age gradient between individuals of the same population, reducing competition for food (Werner and Gilliam 1984).

Decreased resource availability and intraspecific competition, resulting from increased conspecific density, can facilitate both niche differentiation and resource polymorphism (Svanback and Bolnick 2007, Martin and Pfennig 2010). However, other factors, such as the presence of predators, can contribute to the development of niche diversification within the same species (Eklov and Svanback 2006). For instance, predators can affect habitat use of prey, limiting their foraging areas and activities, and forcing them to occupy suboptimal microhabitats that they would not normally use (Werner et al. 1983). The presence of predators might thus force prey to change their feeding behavior and eat other types of food. Prey might thus be forced to specialize on different food sources, such as habitat-specific resources, or otherwise become more opportunistic. These changes in diet selectivity can then affect morphological adaptations, in particular of trophic traits (e.g. jaw size, gill rakers, gut length, etc.). Predators can thus affect the density, distribution and also the phenotypic occurrence of prey.

Here we examined the existence and extent of intraspecific niche diversification in the guppy (*Poecilia reticulata*), a species of freshwater fish inhabiting the streams of Trinidad, which has evolved phenotypic divergence in its life history traits. Guppies are found in streams that span a gradient in predation risk and environmental variables. At one extreme of this gradient, referred to as high predation (HP), guppies experience strong predation pressure mostly by the wolfish

(*Hoplias malabaricus*) and the pike cichlid (*Crenicichla sp.*). At the other extreme, referred to as low predation (LP) environments, guppies coexist with only one other fish species, the Hart's killifish (*Rivulus hartii*), a guppy competitor which may occasionally prey on juvenile guppies (Liley and Seghers 1975, Mattingly and Butler 1994). Guppies from HP and LP sites evolved different phenotypes with characteristic life history traits. For instance, guppies in HP sites mature at a smaller size, produce more and smaller offspring, allocate more energy into reproduction (Reznick and Endler 1982, Reznick 1989) and have faster growth rates (Arendt and Reznick 2005) compared to guppies in LP sites. In a previous study carried out in the dry season (Chapter 2), we showed that diet quality and prey selectivity correlated with the life history patterns, with guppies in HP sites fed more consistently on invertebrates, preferring those with higher nutritional quality. These results were also confirmed by a controlled mesocosm study (Bassar et al. 2010a), in which high and low predation guppies were kept in artificial streams that had been seeded with equal amount of resources (invertebrates, algae, and benthic organic matter). The guppies used for this mesocosm experiment were from the same 2 rivers, the Aripo and Guanapo, of the study in Chapter 2. At the end of the 28-day long experiment, guppies still showed differences in diet choice, with the same patterns as found in the wild. This suggests that HP guppies have evolved to feed on diets rich in invertebrates.

Here we tested if the differences in trophic niches were widespread and repeatable among many high-low predation population pair replicates. We performed a site survey across the Northern Range of Trinidad and collected guppies from 6 different rivers, including the Aripo and Guanapo. We used gut contents to infer direct diet, and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to provide information on what the animals were actually assimilating from what they eat. With stable isotopes, we inferred trophic position and sources contributing to the guppies' diets, how they differed between sites with different predation pressures and whether they change with guppy size (a proxy for age). We hypothesized that guppies from HP sites would occupy a higher trophic position than guppies from LP sites, due to their higher reliance on invertebrates. Furthermore, we characterized the resource availability of the different fish communities and assessed if there were similarities between sites with the same predation regime. We measured a suite of environmental characteristics, including benthic invertebrates, algae, fine benthic organic matter standing stocks and stream morphometrics.

Our objectives were thus 1) to describe and compare guppies' trophic niches across many replicates of HP and LP sites; 2) to assess the existence of an ontogenetic niche shift in guppies; 3) to characterize a series of HP and LP sites to identify common patterns in resource abundance and environmental characteristics; 4) to assess if environmental differences were correlated with the patterns found in guppies' trophic niches.

MATERIALS AND METHODS

We collected samples during the wet season from 6 different rivers in the Northern Range of the Caribbean island of Trinidad. Three of our focal rivers (Arima, Aripo, Guanapo) were located on the South slope of the range and were part of the Caroni drainage, two of them (Quare and Turure) were on the East slope belonging to the Oropuche drainage, and one of them (Marianne) was found on the North slope. In each river we sampled a pair of streams: one high predation (HP) and one low predation (LP) site. The HP and LP sites were generally located along an altitudinal gradient, with HP being downstream of LP locales. All of the HP sites in our study had a large diversity of fish, including some of the most common guppy predators such as the pike cichlid (*Crenicichla sp.*) and the wolfish (*Hoplias malabaricus*). The Marianne, being located in the North slope, had a different ichthyofauna than the other rivers, and the main predators of guppies here were the Spinycheek sleeper (*Eleotris pisonis*) and the Bigmouth sleeper (*Gobiomorus dormitor*).

We conducted this study during the wet season: 3 rivers (Aripo, Guanapo, Marianne) were sampled between 6 July and 14 August 2007, and the other 3 (Arima, Quare, Turure) on 12-26 July 2008. We collected all samples in one or occasionally two subsequent sampling dates. At each site we sampled a stream reach of approximately 100-200 m of length, which comprised at least 3 separate pools and 3 riffles.

ENVIRONMENTAL VARIABLES

In each of the three pools and riffles we sampled environmental characteristics, including algal biomass (as a proxy of habitat productivity), invertebrate standing stocks, fine benthic organic matter (FBOM) biomass, and coarse particulate organic matter (CPOM) standing stock. We also measured the stream morphometrics, percent open canopy, and discharge.

Algal standing stocks were sampled by scraping 5 rocks from 3 pools and 3 riffles at each site. We assessed algal biomass by measuring the ash-free dry mass (AFDM) of a predetermined subsample of the scraped slurry that was filtered on a 25 mm pre-combusted and weighed glass fiber filter (at 450^{o}C for 2 hours). The filter was then dried at 105^{o}C until it reached a constant mass, it was then weighed and oxidized in a muffle furnace at 500^{o}C and reweighed. The AFDM mass was equal to the mass of the dried material plus the filter, minus the filter mass, minus the mass of the remaining ashes after the oxidation. The AFDM weight was then divided by the subsampled area to obtain the AFDM/m^{2}. AFDM represents a proxy for algal biomass, but it does not distinguish between algal material and other organic matter, such as fungi and bacteria; furthermore it also includes the biomass of senescent material (Steinman et al. 2007).

Benthic invertebrates were sampled in 3 pools and 3 riffle habitats in each site using a Hess sampler (Hess 1941). Samples were then immediately stored in 95% ethanol solution (which was generally diluted to 70% due to the small amount of water in the sample). Back in the laboratory, samples were stained with rose bengal for at least 24 hours and then separated using 2 sieves (1mm and 250 μm). Only the > 1mm and between 1 mm and 250 μm fractions were picked and identified at the lowest taxonomic level (generally Family or Genus) following Perez (1996) and Merritt et al. (2007). The <250 μm fraction was not used for this analysis, as we were not interested in estimating meiofauna. Samples that had more than 100-150 invertebrates were subsampled using a plankton splitter. Invertebrates were counted and identified using a dissecting scope. Invertebrate biomass was calculated using length-mass regression equations from the literature (Benke et al. 1999) and from estimates of Trinidadian specimens (T. Heatherly unpubl. data)

Fine benthic organic matter (FBOM) was collected in 3 pools and 3 riffles in 2007 and 2 pools and 2 riffles in 2008 in each site. We collected FBOM using a sampling corer and graduated bucket following the procedure described in Wallace et al. (2007). All the material collected was passed through nested sieves of 63 μm, 250 μm, and 1000 μm and everything that was retained by the sieves, including what passed through the smaller sieve, was stored in bags. Back in the lab, the material collected by each sieve was then dried at 50^{o}C until it reached a constant mass and then ashed at 500^{o}C. The AFDM/m^2 for each size fraction was calculated following the formulas in Wallace et al. (2007) and then summed up to obtain the total FBOM biomass of the whole sample.

Coarse particulate organic matter (CPOM) is mostly composed of allochthonous material (mainly leaves). The CPOM biomass was assessed by randomly tossing a pvc frame (1.25 m^2 area) in the stream and collecting all material within the frame by hand. The material was then dried at 50^{o}C for at least 48 hours or until completely dry, and subsequently weighed. We collected CPOM in 3 pools and 3 riffles in 2007 and 3 random pools or riffles in 2008.

Percent open canopy was measured using a hemispherical densiometer. Pool width and depth were measured at transects across the pool, then averaged. Pool widths measurements were all wetted width. Discharge was measured using the midsection method (Gore 2007). All stream characteristics and environmental variables are reported in Table 1.

STABLE ISOTOPE ANALYSIS (SIA)

We used stable isotopes to estimate the source proportion contribution to guppy diets from the different sites and to calculate their trophic position. We collected a minimum of 17 and a maximum of 48 guppies (mean and median=26) of all size classes per site. We collected them with hand nets and immediately put them on ice in a cooler and transported them back to the laboratory. Viscera were subsequently removed and the fish were put in a drying oven until completely dry. Guppies' guts were stored in a 5% formalin solution until diet analysis was performed, although those from the 2007 collection were not well preserved and thus not suitable for diet analysis.

We collected the most abundant invertebrate taxa with hand nets. Upon collection, they were left in separate containers with water for approximately 6-8 hours, to allow them to clear their guts. In order to reach enough mass for SIA, we put several individuals of the same taxon in one sample and then dried.

Epilithon from pools and riffles, benthic organic matter, seston, and leaves were collected for stable isotope analysis. However, we only used epilithon from pools for this study, because it represented what guppies ingested, being a mixture of algae and fine detritus. Epilithon was collected from rocks using a loeb sampler. The collected slurry was then filtered on a 25 mm pre-combusted GFF (at 450°C for 2 hours) until it clogged and the filter was subsequently dried. Once dried, the material was scraped off the filter and used for SIA.

All samples were dried at 50-60°C until completely dry and then ground to fine powder with a mortar and pestle. Approximately 1 mg of each sample (~3 mg for epilithon samples) was weighed and put in 4 x 6 mm tin capsules (5 x 9 for epilithon samples) and sent to the University of Georgia (Athens, Georgia, USA) for SIA. Samples were analyzed using a Finnigan Delta C mass spectrometer connected to a Carlo Erba 1500 CHN analyzer. Isotopic ratios (heavy isotope/light isotope) were expressed in the typical δ notation (Minagawa and Wada 1984, Peterson and Fry 1987, Fry 2006) as parts per thousand deviation from international standards, which is atmospheric nitrogen for $\delta^{15}N$ and Pee Dee belemnite limestone for $\delta^{13}C$.

We calculated guppy trophic position using the formula proposed by Vander Zanden et al. (1997) and Anderson and Cabana (2005):

$$TP = [(\delta^{15}N_{gup} - \delta^{15}N_{base})/\Delta] + \lambda$$

Where Δ is the fractionation factor (equal to 3.2‰, from Minagawa and Wada 1984), and λ (=1) is the trophic position of the baseline, $\delta^{15}N_{base}$ is the signature of the baseline, which is a mixture of two invertebrate primary consumers. The $\delta^{15}N$ of basal resources varies temporally and spatially, making it necessary to choose a primary consumer that can function as a proxy of the base of the food web (Vander Zanden et al. 1997, Post 2002, Anderson and Cabana 2005). We chose as our baseline an average value between *Psephenus sp.*, a grazing water penny, and *Phylloicus sp.*, a shredding caddisfly. These two taxa were among the most common primary consumers, being found in almost all of our sites, and overall had among the lowest $\delta^{15}N$ signatures. We chose to use an average between a shredder and a grazer, because they represented the baselines for the allochthonous and autochthonous pathways respectively. Other studies conducted in streams propose to only use grazers, but suggest to use a shredder as a baseline when the stream is detrital-based (Anderson and Cabana 2005). Our streams most likely had an important detrital-based component, which was the reason why we chose to use both. In addition, we did not have both taxa for all our sites, so we calculated an average difference between *Psephenus* and *Phylloicus* and used it for those sites in which we only had one of the two taxa.

In order to estimate the proportional contribution of each food item to the guppy diet we employed the SIAR mixing model, which is a software in R that utilizes Bayesian statistics (Parnell et al. 2010). SIAR is a novel methodology that incorporates multiple dietary sources and generates solutions as probability distributions of the different sources. We found that SIAR was the most appropriate model to use, as it allows one to incorporate the variability of the sources, of the end members (consumers), and of the fractionation factors. It is very important to be able to incorporate variability into a mixing model, especially for fractionation factors that are difficult to assess and are a problematic issue (Gannes et al. 1997, Vander Zanden and Rasmussen 2001, McCutchan et al. 2003, Vanderklift and Ponsard 2003, Kilham et al. 2008, Martinez del Rio et al. 2009). For the guppy fractionation values we chose to use 3.2 ±0.5 ‰ for $\delta^{15}N$ and 0 ±0.5 ‰ for $\delta^{13}C$ (Minagawa and Wada 1984). SIAR allowed us to use many dietary sources, however, like other mixing model (e.g IsoSource; Phillips and Gregg 2003), its performance decreased with increasing number of sources. Thus, we tried to reduce the number of sources including only the most abundant taxa found in the guppy diet and clumping them together into functional feeding groups. We assumed that invertebrates belonging to the same functional feeding group would have similar isotopic signatures, as they feed on similar food sources. In this way we should be able to have a better definition of the sources contributing to guppies' diet. The sources we chose for the SIAR were: epilithon from pools (from now on indicated as EPI), collector/grazers, filterers, shredders, omnivores, and predators.

DIET ANALYSIS

Guppies from the Quare and Arima rivers were analyzed for gut contents. We only used the stomach and a small part of the foregut - at the point where the gut turns 180 degrees, because here food is not completely digested. Invertebrates were identified at the most inclusive taxonomic level, usually the family category, following Perez (1996) and Merritt et al. (2007). The gut content was emptied onto a gridded slide, where ten squares (out of 64) were randomly chosen for the quantification of the different food items using a compound microscope. The areas occupied by invertebrates and detritus were estimated for each square. Individual diatoms and filamentous algae were counted in each of the 10 square, because they are too small to estimate their proportion coverage in one square. An average size for diatoms and one for filamentous algae was subsequently assigned to calculate the area they occupied in the 10 squares. The area taken by each food category was calculated for the whole slide (64 squares). Plant matter, inorganic material, and other algae have not been included in the analysis as their occurrence was very low.

SITE ANOMALIES

Two of the 12 surveyed sites presented some anomalies. The Arima HP site was found to be contaminated with manure, which altered the $\delta^{15}N$ signatures of several organisms of the food web, including guppies. Stable isotope data from this site must thus be carefully interpreted.

The Turure LP site had a very uncommon morphology, being composed entirely of very large travertine pools separated by high waterfalls. Its morphology prevented us from collecting several of the biomass compartments standing stocks. Moreover, guppies did not naturally occur in this site, but they were introduced 50 years ago by Haskins from the Guanapo river (Magurran 2005). These factors make both the site and the guppies living in it an anomaly and must thus be carefully considered.

STATISTICAL ANALYSIS

Differences in trophic position of guppies between predation regimes were assessed using a Mann-Whitney test, while differences in trophic position between rivers were estimated with a Kruskal-Wallis test. Even if the data were normally distributed, we used a non-parametric test because the variances across groups were not equal. We also ran separate Univariate ANOVAs for each river, because when we kept rivers separated the variances became equal. For the Quare river we ran a Univariate ANCOVA, with fish standard length set as a covariate, because only in this river was there a significant effect on the trophic position. In the Quare river the Levene’s test for homogeneity of variances was significant, however, because the p value was not very low ($P = 0.029$) we considered the ANCOVA a test powerful enough for our purposes. The Arima HP site was contaminated with manure, as indicated by the unusually high $\delta^{15}N$ signatures of many organisms from the stream (Aravena et al. 1993), in particular those relying on autochthonous food sources. For this reason, we did not consider the trophic position results from the Arima HP site comparable with those from other sites and, as such, we did not include it in the analysis.

We performed a multivariate analysis of covariance (MANCOVA) to test for differences in guppy diet across rivers and predation regimes. Proportions of invertebrates, detritus, and algae (diatoms and filamentous algae) were the dependent variables of our general linear model (GLM). We included river (Arima and Quare) and predation level (HP, LP) and their interaction as fixed factors. We used fish standard length as a covariate. Because the interaction between river and fish length was significant, we included it in the model. In addition to this multivariate approach, we also tested the proportion of each diet class in the guts using three univariate ANCOVAs. Proportion of each food item was included as a dependent variable and the independent variables were the same as for the MANOVA. For these two rivers, we also examined the linear relationship between guppies' trophic position and proportion of invertebrates in the guts using a linear regression.

We assessed the existence of ontogenetic shift in guppies by using both the stable isotope and gut content data. Linear relationships between trophic position and fish standard length were examined in separate regressions for high and low predation fish from each site. For the Arima and Quare rivers, we also ran linear regressions to look at the relationship between proportion of food items (invertebrates and algae) and fish standard length.

We performed Univariate ANOVAs to assess differences in resources biomass between predation regimes and rivers (our fixed factors). For each ANOVA, the dependent variables we used were benthic invertebrate biomass/m^2, epilithon AFDM/m^2, CPOM/m^2, and FBOM/m^2 (only the 63-250 μm size fraction). In the model we also included the interaction between predation and river. The Turure river was included only in the epilithon analysis. The Guanapo river was included in all analyses, but the epilithon, due to missing samples.

Data were transformed to meet normal distribution assumptions when necessary and levels of significance were set at $P<0.05$. All statistical analyses were carried out using PASWStatistics 18.0.

RESULTS

TROPHIC POSITION

We found that the trophic position of guppies differed between HP and LP sites (Mann-Whitney test, rejected null hypothesis) and also between streams (Kruskall-Wallis, rejected null hypothesis) (Fig. 4-1). When we individually analyzed the 6 rivers sampled, we found that in the Aripo river ($F_{1,33} = 25.52$; $P < 0.001$), Turure river ($F_{1,50} = 127.58$; $P < 0.001$), and Quare river ($F_{1,49} = 19.29$, $P < 0.001$) LP guppies had a significantly higher trophic position than HP ones, while in the Marianne river ($F_{1,48} = 3.38$; $P = 0.072$) there was no significant difference between sites. Only in the Guanapo river we found that HP guppies had a marginally significantly higher trophic position than LP guppies ($F_{1,66} = 3.71$, $P = 0.058$). In the Quare river (the only river where we ran an ANCOVA), we found that fish standard length also had a significant effect ($F_{1,49} = 4.52$, $P = 0.039$).

Overall, we did not find a significant relationship between trophic position and fish standard length in any of the sites, with the only exceptions being the Quare HP and the Turure LP. In the Quare HP, guppies significantly ($F_{1,24} = 5.43$, $P = 0.029$; $r^2=0.184$) increased their trophic position with increasing size (Fig.4-2), while in the Turure LP they significantly decreased their trophic position with increasing size ($F_{1,24} = 4.51$, $P = 0.044$; $r^2=0.158$).

DIET

Guppies from different rivers, different predation regimes, and of different size had different diets preferences. The most predominant food item in guppy diets was detritus, followed by invertebrates. Algae composed a very small part of their diet (Fig. 4-3). The MANCOVA showed a significant effect of predation ($F_{3,46} = 3.91$; $P = 0.014$), river ($F_{3,46} = 5.96$; $P = 0.002$), body length ($F_{3,46} = 11.87$; $P < 0.001$), while the interaction between river and predation was not statistically significant ($F_{3,46} = 0.98$; $P = 0.41$). The interaction between river and body length was significant ($F_{3,46} = 8.51$; $P < 0.001$) for all food items (see Table 4-2). This indicates a heterogeneity of slopes between rivers, suggesting a need for careful interpretation of the river main effect. We further investigated the causes of this heterogeneity of slopes by running linear regressions between the proportion of the 3 food items and fish length for each river. We found that in the Arima there was no significant relationship between fish length and any of the food items (invertebrates: r^2=0.071, $F_{1,28}$ = 2.13, P = 0.155; detritus: r^2=0.086, $F_{1,28}$ = 2.63, P = 0.116; algae: r^2=0.015, $F_{1,28}$ = 0.42, P = 0. 521; Fig. 4-4). In the Quare river guppies significantly decreased the amount of invertebrates in their diet with increasing size (r^2=0.614, $F_{1,22}$ = 34.96, $P < 0.001$), while they significantly increased the proportion of detritus (r^2=0.491, $F_{1,22}$ = 21.25, $P < 0.001$) and algae (r^2=0.428, $F_{1,22}$ = 16.48, P = 0.001) (Fig. 4-4). These changes in diet with guppy size showed the same patterns between HP and LP fish.

Even if we found a significant effect of predation on guppy diet, the magnitude of differences between high and low predation sites changed between the two rivers examined. For instance, in the Quare, HP guppies had a lower proportion of invertebrates and higher proportion of detritus and algae than the LP fish (Fig. 4-3). Instead, in the Arima, HP and LP guppies had more similar diets (Fig. 4-3).

Opposite to what we expected, there was no significant relationship between proportion of invertebrates in the guts of guppies and the trophic position of the guppies in any of the 4 sites (Arima HP: $F_{1,8}$ = 2.09, P = 0.19; r^2=0.23; Arima LP: $F_{1,13}$ = 2.96, P = 0.11, r^2=0.20; Quare HP: $F_{1,11}$ = 4.01, P = 0.73, r^2=0.29; Quare LP: $F_{1,11}$ = 0.50, P = 0.50, r^2=0.05) (Fig. 4-5).

ISOTOPIC SOURCE PROPORTIONS

From the mixing models, it emerged that guppies were indeed omnivorous but that invertebrates were a much more important food source than basal resources (pool epilithon, which is a mixture of algae and fine detritus). Indeed, in all sites the proportion of the 4 invertebrate categories contributed the great majority of food assimilated in the guppies' diet (Fig. 4-6). Guppies from the 12 sites showed considerable variation in the proportions of the 5 food sources (epi, collectors-grazers, shredders, omnivores, predators) assimilated in their diet, which made it difficult to identify common patterns. We found that LP guppies had a tendency towards higher assimilation of EPI than HP guppies in 3 rivers (Guanapo, Marianne, Quare), while the proportion of EPI was very similar between guppies in HP and LP sites in the Arima and Aripo rivers (Fig. 4-6). The Turure river was the only river in which we found a higher contribution of EPI to the guppy isotopic signature in HP than in LP sites. However, the results from the Turure LP site were not very clear or informative, which might be due to the fact that the guppies' signatures were outside the isotopic mixing polygon delineated by the food sources. When this happens, SIAR still tries to fit a model and thus it calculates a solution, but this violates the assumptions of the mixing model (Parnell et al. 2010). Guppies' isotopic signatures were outside the isotopic mixing polygon most likely because one food source was not sampled and thus not included in the analysis. We collected all the most abundant invertebrates found in the stream, but we must have missed some unknown, perhaps rare, food source, which was necessary to define the isotopic mixing polygon and which likely prevented SIAR from calculating a more precise solution.

STREAM CHARACTERISTICS

Overall, streams with comparable fish communities (HP vs. LP) showed similarity across rivers, with some exceptions. The HP sites generally had higher invertebrate biomass, lower epilithon ash-free dry mass, lower leaf litter, and a non-significant tendency toward lower benthic organic matter. River of origin was also an equally important factor determining differences between sites, especially for the benthic invertebrate, fine detritus, and algal biomasses.

Univariate ANOVA showed that predation ($F_{1,48} = 41.11$; $P < 0.001$), river of origin ($F_{4,48} = 19.66$; $P < 0.001$), and the interaction of these two factors ($F_{4,48} = 5.85$; $P = 0.001$) all had significant effects on the benthic invertebrate biomass found in the stream. Benthic invertebrate biomass was higher in HP sites than LP sites in all rivers but the Guanapo, which showed the opposite pattern (Table 4-1, Fig. 4-7). There was considerable variation between rivers and post-hoc tests (LSD) showed that the 3 rivers sampled in 2007 (Guanapo, Marianne, Aripo) did not differ between each other in the benthic invertebrate biomass, while the Arima and Quare had significantly less biomass than the 2007 rivers. The Quare river also had significantly lower biomass than the Arima river, exhibiting the lowest invertebrate biomass.

The amount of BOM AFDM/m^2 in the stream was not affected by predation regime ($F_{1,36} = 2.55$; $P = 0.119$), but differed between rivers ($F_{4,36} = 4.78$; $P = 0.003$).

The interaction between predation and river was also not significant ($F_{4,36} = 0.36$; P = 0.832). The river with the lowest amount of BOM was the Quare, while the one with the highest amount was the Aripo (Fig. 4-7).

The amount of leaf litter per area in the streams was significantly higher in LP than in HP sites ($F_{1,33} = 4.32$; P = 0.045). We did not find a significant effect of river ($F_{4,33} = 2.12$; P = 0.100) or of the interaction between predation and river ($F_{4,33} = 0.12$; P = 0.974). The Marianne river had the highest CPOM/m^2 and was significantly different from the Aripo and Guanapo, which were the rivers with the lowest values (Fig. 4-7).

In all the five rivers examined, LP sites had higher epilithon AFDM/m^2 than HP sites (Univariate ANOVA, predation: $F_{1,47} = 19.20$; $P < 0.001$) (Fig. 4-7). River of origin also had a significant effect on the epilithon AFDM/m^2 ($F_{4,47} = 5.21$; P = 0.001), but there was no interaction between predation regime and river ($F_{4,47} = 1.33$; P = 0.272). The Quare river had the highest epilithon AFDM/m^2 and post-hoc tests showed that it was significantly different from all the other 5 rivers. The Arima river, with its lowest epilithon AFDM/m^2, was significantly different from Quare and Turure, but not from the Aripo and Marianne (Fig. 4-7).

DISCUSSION

Our survey of Trinidadian streams during the wet season showed that both predation and river of origin had an equally important effect on the intraspecific differences in guppies' trophic niches. Guppy size was not an important factor in determining trophic niches, as guppies did not show an ontogenetic shift in trophic position and there was only limited data on its effect on diet. Guppies showed considerable variation in the proportion of prey assimilated, with no clear HP or LP patterns, even if we found a weak tendency towards guppies in LP sites to assimilate more basal resources (algae and detritus) than guppies in HP sites. In the guts, detritus was the most abundant food component, but stable isotopes indicated that guppies assimilated this only in limited amounts (Fig. 4-6). Invertebrates were, in contrast, less abundant than detritus in the guts but were highly assimilated into guppies' tissues. These results might indicate that detritus is accidentally ingested while foraging for invertebrates, which are a more nutritious food source. The amount of detritus found in guppies' guts might therefore also depend on the substrate they forage on. For instance, if guppies prevalently forage on benthic surfaces with great fine organic matter accumulation, they might incidentally ingest more detritus, which is then found in their guts. A prevalence of drift-feeding would alternatively decrease the amount of detritus ingested and found in their intestines.

Predation and competition

The observed predation effect on diet and trophic position was the opposite of what we previously reported for guppies collected in the dry season. Here we showed that wet season guppies from low predation (LP) sites generally had higher trophic position than guppies that lived with predators (HP), although the difference was not very high (~1/4 trophic position; Fig. 4-1). Gut content analyses on two of the 6 rivers surveyed, confirmed that predation regime had a significant effect on diet, with guppies in LP sites having overall higher proportion of invertebrates in their diet compared to guppies in HP sites. The river of origin also affected diet, and we found a greater difference in diet between HP and LP sites in the Quare river than in the Arima river, where guppies in HP and LP sites had a more similar diet (Fig.4-3).

In the dry season (Chapter 2), we found the opposite pattern: guppies in HP sites fed mostly on invertebrates, while guppies in LP sites ate more detritus and algae. These contrasting results suggest that seasonality might affect guppy’s diets and that its effect might be different in HP and LP sites. In Chapter 3 we showed some evidence of a change in diet between seasons, as we found that in the Aripo river LP guppies increased the proportion of invertebrates in their diet in the wet season, while HP guppies decreased it. The overall result was that the diets of guppies in HP and LP sites were very similar in the wet season, while in the dry season HP guppies were more insectivorous than LP guppies.

Palkovacs and colleagues (unpubl. data) found that guppies in LP sites were more efficient and morphologically better adapted at capturing invertebrates than guppies in HP sites. In LP sites guppies are released from predation pressure and their density is much higher than in HP sites (Table 4-1); the per capita high quality resources (invertebrates) available therefore might decrease, leading to increased intraspecific competition for these resources. Selective pressures might thus favor those individuals that are more efficient at finding and capturing invertebrates. On the other hand, guppies in HP sites live in very productive environments and their density is low. As a consequence, guppies in HP sites should not be resource-limited, thus selection towards anti-predator adaptations (e.g. body shape; Langerhans and DeWitt 2004) over feeding efficiency might be favored.

The patterns in resource availability and guppy density described above are typical of the dry season, but they can change during the wet season (Fig. 4-8). For instance, guppy density decreases substantially in the wet season, due to the frequent floods (Lopez-Sepulcre pers. comm.). In LP sites, the surviving guppies, which are very efficient at capturing invertebrates (Palkovacs et al. unpubl.data), are released from high intraspecific competition and could increase the amount of high quality food (invertebrates) intake (Fig. 4-8a). During the wet season, resources might decrease in abundance and HP guppies could begin to suffer low resource availability, driving them to become less selective. Abiotic disturbance, such as frequent floods and high spates, together with lower resources and the presence of predators, could affect and limit feeding activity of guppies in HP sites more than in LP sites. These changes could result in an overall decrease in invertebrates in the diet and reduced prey selectivity in guppies from HP sites (Fig. 4-8b).

In conclusion, guppies' diets could change cyclically according to changes in population density, which could alter the per capita resource availability and for instance the intraspecific competition strength. Possibly, in the wet season there could be a relaxation of some selective pressures (e.g. competition, predation), while abiotic disturbance could become a more important component in shaping guppy diets. It thus becomes imperative to look at the guppy diet and resource availability along a time series, especially focusing on the differences between dry and wet season and how guppies in HP and LP sites are differently affected.

Food web perspective

We found many environmental similarities between sites with the same predation intensity on guppies. With some exceptions, HP sites all tended to have higher invertebrate biomass, lower leaf litter (CPOM/m^2), lower algae biomass (epilithic AFDM/m^2), and a tendency towards lower FBOM (AFDM/m^2) compared to LP sites (Table 4-1, Fig. 4-7). The HP sites were also bigger rivers, with lower canopy cover and higher discharge (Table 4-1). In a subset of the surveyed streams, guppy density was measured (Table 4-1., E. Palkovacs unpubl. data) and it was always higher in LP sites. These correlations between environmental characteristics and predation regime make it difficult to understand if the intraspecific differences we found in guppies' trophic niches were an effect of the predation regime, of the resource availability in the streams, or of both. For instance, predator release certainly was the cause of an increased guppy density in LP sites. Predator release can thus alter the top-down effects in the food web. The patterns in our streams can be analyzed with a simple trophic cascade perspective (Fig. 4-9). For instance, in HP sites the presence of predators keeps guppy density low, which allows invertebrate biomass to increase and basal resources to decrease (Fig. 4-9). In LP sites, guppy density is higher due to the lack of predators. High guppy density keeps invertebrate abundance low, relaxing the pressure on basal resources that can increase in biomass (Fig. 4-9). These patterns on the trophic compartments relative abundance are confirmed by our data (Table 1, Fig. 4-7). In this top-down scenario, guppies are resource-limited in LP sites and could become more omnivorous. HP guppies instead have a lot of invertebrates available and could become more selective (more insectivorous). These patterns in guppies diet preferences reflect what we found in the dry season, but not what we observed in the present study. However, this is a simplified scenario, as these streams have high levels of omnivory and HP sites have other species of fish that can influence the ecosystem structure and function.

The differences observed in the biological characteristics between HP and LP sites were also consistent with what is observed along altitudinal gradients. The River Continuum Concept (RCC) states that biological and physical characteristics of streams change in a continuum with increasing stream order (Vannote et al. 1980). Upstream low-order streams (such as our LP sites) are small, with high canopy cover and elevated CPOM input and terrestrial through fall, with low light levels and low primary production. These attributes progressively shift moving downstream: stream size increase coincides with a decrease in canopy cover and terrestrial subsidy, along with an increase in light levels and primary productivity (Vannote et al. 1980). With the exception of algal biomass that we found to be higher in LP vs. HP sites, all of the other environmental characteristics described for upstream-downstream reaches in the RCC correspond to the patterns we found between our HP-LP stream pairs (Table 4-1, Fig. 4-7). This suggests that physical patterns or bottom-up effects could also be responsible in defining the biological characteristics of our surveyed sites.

Ontogenetic niche shift

Guppies change their microhabitat use with age: baby guppies stay at the surface, while bigger juveniles and adults mostly foraging in the water column and on the bottom (Croft et al. 2003). This change in habitat use with age suggests that guppies might also be changing their foraging behavior and diet. Our stable isotope data did not support the existence of an ontogenetic diet shift in guppies in the wet season. Gut content data were consistent with the stable isotope results for the Arima river, but showed a strong effect of fish size on diet for the Quare guppies. In this river, guppies decreased the proportion of invertebrates in their diet with increasing size (Fig. 4-4), hence we should have expected their trophic position to decrease with size as well. We instead found a positive relationship between size and trophic position in the Quare river, even if size explained only 22% of the variation in trophic position (Fig. 4-2). Nevertheless, the Quare river most likely represented an exception, as in the great majority of sites we did not find indication of a shift in trophic position with changing size. This finding indicates that there are sometimes differences between what is found in an organism’s gut and what is preferentially assimilated.

The overall lack of an ontogenetic shift could be related to the omnivorous nature of guppies’ diets and the small increase in size from birth to adulthood (guppies are around 8mm at birth and females can reach a maximum of ~25 mm). Fish species that change diet during their lifetime have bigger size differences between juvenile and adult stages. Alternatively, guppies of different size classes might avoid intraspecific competition by using different microhabitats without changing their diet preferences or what food they assimilate. Finally, guppies can elude competition with conspecifics by showing high levels of individual-based specialization that are not size-related.

Conclusions

Intraspecific niche diversifications are important to identify because they could initiate speciation processes (Skulason and Smith 1995, Schluter 2001). Guppies showed intraspecific differences in their trophic position and diet preferences as an effect of predation regime and river of origin. Guppy diets, in particular, exhibited high variation across sites (Fig. 4-6), but also within populations from the same site, as shown by the big range encompassed by their isotopic signatures (Fig. 4-10). Different degrees of individual-level diversification in resource use within a population can be promoted by different levels of competition (Svanback and Persson

2004, Svanback and Bolnick 2005, 2007). Future studies should address this potentially important aspect of guppies' trophic ecology.

Moreover, the present study suggests that seasonality could play an important role in determining guppy trophic niches by relaxing some of the selective pressures that act during the dry season, altering the system to one controlled by abiotic forces. We suggest that there is a need to monitor how guppy population dynamics, resource availability, and diet preferences change along a time series.

Table 4-1. Characteristics of the 12 surveyed sites in 6 streams in the Northern Range of Trinidad. R: *Rivulus*; G: guppy; Chrenic: *Crenicichla*. (Next page)

Site	**Date**	**Fish community**	**Guppy density (#/m^2)**	**% open canopy**	**Discharge Q (L/s)**	**CPOM (gDM/m^2)**	**Reach width (m)**	**algae AFDM POOL (mg/m^2)**	**algae AFDM RIFFLE (mg/m^2)**	**BOM - AFDM/ m^2**	**Inverts abun-dance (#)**	**Inverts biomass /m^2**
Arima HP	Jul-08	R, G, Chrenic, *Rhamdia*	1.01	38.4	32.0	20.5 (8.9)	32.6	3.59 (2.5)	2.84 (2.0)	9.92 (8.9)	156 (78.1)	971 (861)
Arima LP	Jul-08	R, G	3.06	14.8	15.8	195 (215)	5.40	7.58 (0.5)	1.85 (0.3)	17.2 (15.7)	72.8 (45.4)	208 (266)
Aripo HP	Jul-07	R, G, Chrenic, *Rhamdia*, *Aquidens*, *Hypostomus/ Ancistrus*, *Hoplias*, Characidae, *Sinbranchus*	0.27	28.4	52.7	31.0 (54.2)	17.4	3.51 (1.1)	2.45 (1.0)	15.3 (15.0)	250 (114)	5517 (1867)
Aripo LP	Jul-07	R, G	19.4	10.8	41.1	39.7 (22.2)	1.63	9.81 (6.0)	7.53 (4.3)	57.9 (83.3)	110 (69.1)	700 (629)
Guanapo HP	Jul-07	R, G, *Rhamdia*, *Aquidens*, *Hypostomus/ Ancistrus*, *Hoplias*, Characidae	2.20	18.3		18.6 (21.2)		3.36 (1.5)	2.14 (0.7)	9.95 (13.0)	306 (131)	1400 (755)
Guanapo LP	Jul-07	R, G	5.02	11.0	32.6	40.7 (38.2)	2.37			21.7 (22.3)	98.3 (32.2)	2431 (1799)

Site	**Date**	**Fish community**	**Guppy density (#/m^2)**	**% open canopy**	**Discharge Q (L/s)**	**CPOM (gDM/m^2)**	**Reach width (m)**	**algae AFDM POOL (mg/m^2)**	**algae AFDM RIFFLE (mg/m^2)**	**BOM - AFDM/ m^2**	**Inverts abun-dance (#)**	**Inverts biomass /m^2**
Marianne HP	Jul-07	R, G, *Sicydium, Awaous, Gobiesox, Agonostomus*	5.37	23.4	1323	112 (136)	6.61	5.39 (2.1)	2.40 (0.7)	16.6 (16.4)	206 (62.9)	21645 (1307)
Marianne LP	Jul-07	R, G		12.5	1478	242 (240)	3.55	8.21 (5.3)	5.13 (1.4)	19.1 (22.5)	75.5 (33.4)	816 (476)
Quare HP	Jul-08	R, G, Chrenic, *Rhamdia, Aquidens, Hypostomus/ Ancistrus, Hoplias,* Characidae		48.2	57.5	17.0(10.0)		9.89 (6.5)	7.48 (3.4)	8.67 (9.8)	93.5 (29.6)	415 (233)
Quare LP	Jul-08	R, G		11.4	11.9	43.1(29.3)	2.37	11.2 (4.7)	8.83 (3.1)	10.0 (9.8)	10.0 (5.7)	38.3 (47.4)
Turure HP	Jul-08	R, G, *Chrenic, Rhamdia,* Characidae, *Synbranchus*		22.2	157.4	53.5 (55.2)		5.13 (2.0)	2.00 (0.4)	10.3 (8.5)	22.2 (20.7)	89.1 (151)
Turure LP	Jul-08	R, G		14.9				8.41 (2.2)	8.96 (4.0)			

Table 4-2. Univariate ANCOVA results for the effects of predation and river on the proportion of invertebrates, detritus, and algae in the diet of guppies from Trinidad. SS=type-III sums of squares; df=degrees of freedom; MS=mean squares.

Univariate ANCOVA					
Source	**SS**	**df**	**MS**	**F-ratio**	**P-value**
Invertebrates					
Predation	0.45	1	0.45	8.01	0.007
River	0.49	1	0.49	8.80	0.005
Guppy Length	1.54	1	1.54	27.41	<0.001
Predation*River	0.04	1	0.04	0.75	0.390
River*Guppy Length	0.65	1	0.65	11.63	0.001
Error	2.69	48	0.056		
Detritus					
Predation	0.48	1	0.48	9.36	0.004
River	0.21	1	0.21	4.15	0.047
Guppy Length	0.95	1	0.95	18.38	<0.001
Predation*River	0.03	1	0.03	0.68	0.414
River*Guppy Length	0.27	1	0.27	5.22	0.027
Error	2.49	48	0.052		
Algae					
Predation	0.00	1	0.00	0.04	0.842
River	0.12	1	0.12	11.48	0.001
Guppy Length	0.16	1	0.16	15.05	<0.001
Predation*River	0.01	1	0.01	0.82	0.369
River*Guppy Length	0.19	1	0.19	17.66	<0.001
Error	0.52	48	0.01		

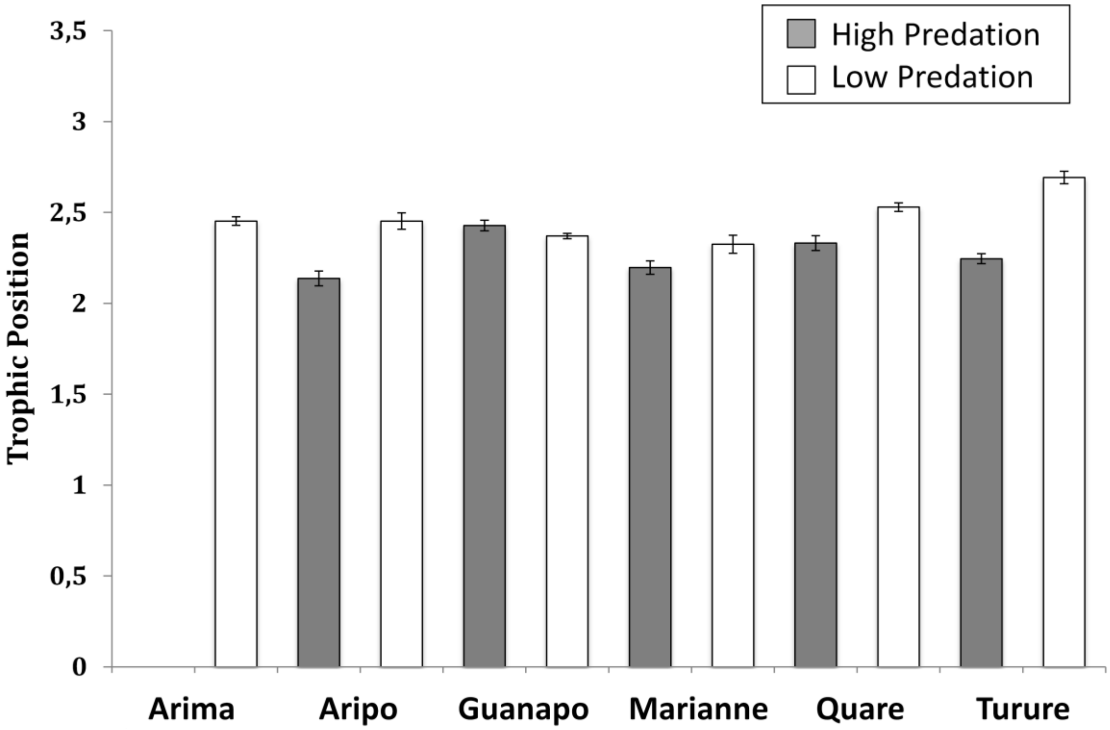


Figure 4-1. Guppy trophic position across HP and LP sites in the 6 study rivers in Trinidad. High predation sites are in grey, while low predation sites are in white. Trophic position of guppies from the Arima HP site is not included due to a manure contamination that altered the organisms' $\delta^{15}N$ signature. Error bars are ± 1 S.E.

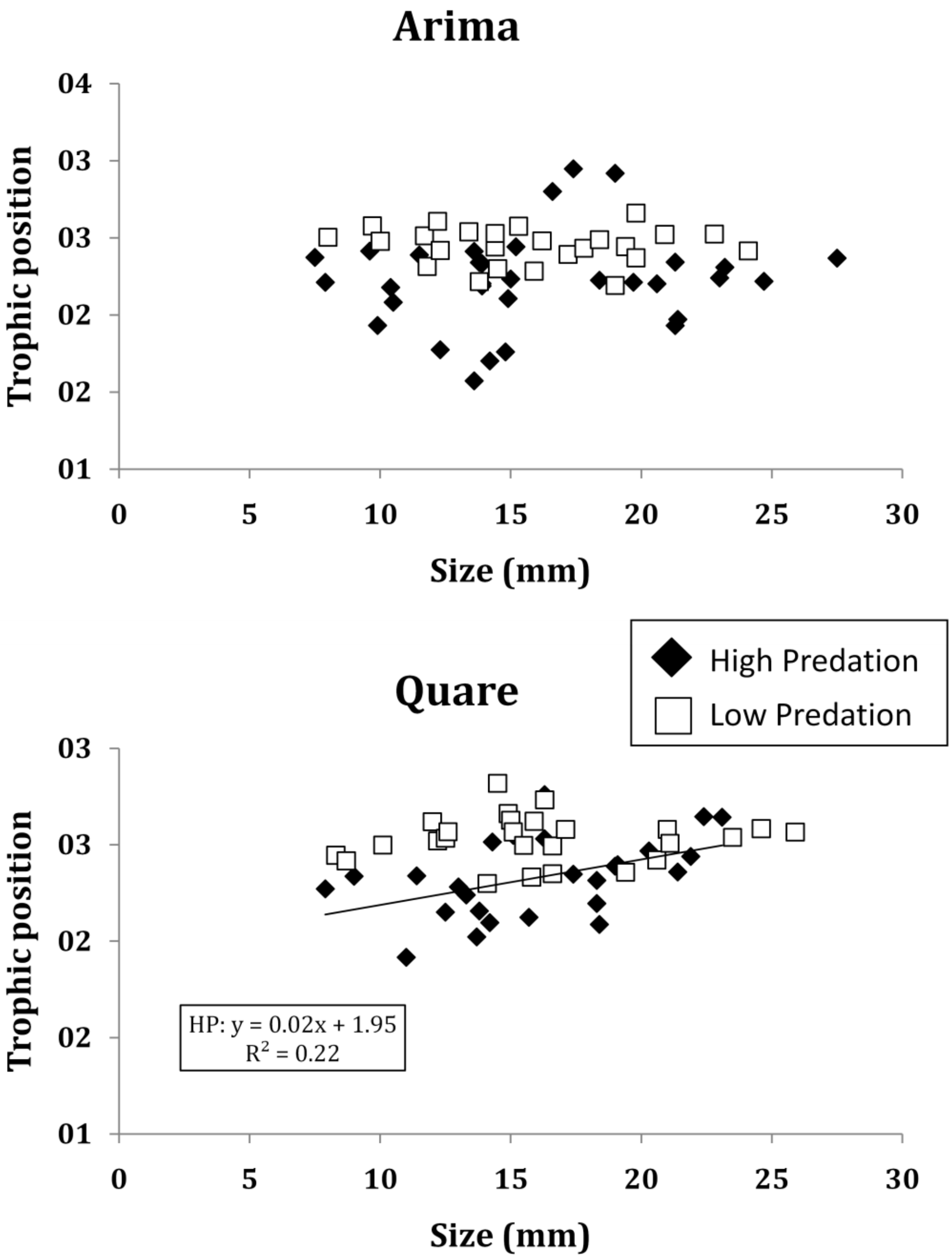


Figure 4-2. Relationship between guppy standard length and trophic position in the Arima (on top) and the Quare (on the bottom) rivers. The only significant relationship (in the Quare HP site) is indicated with a solid line.

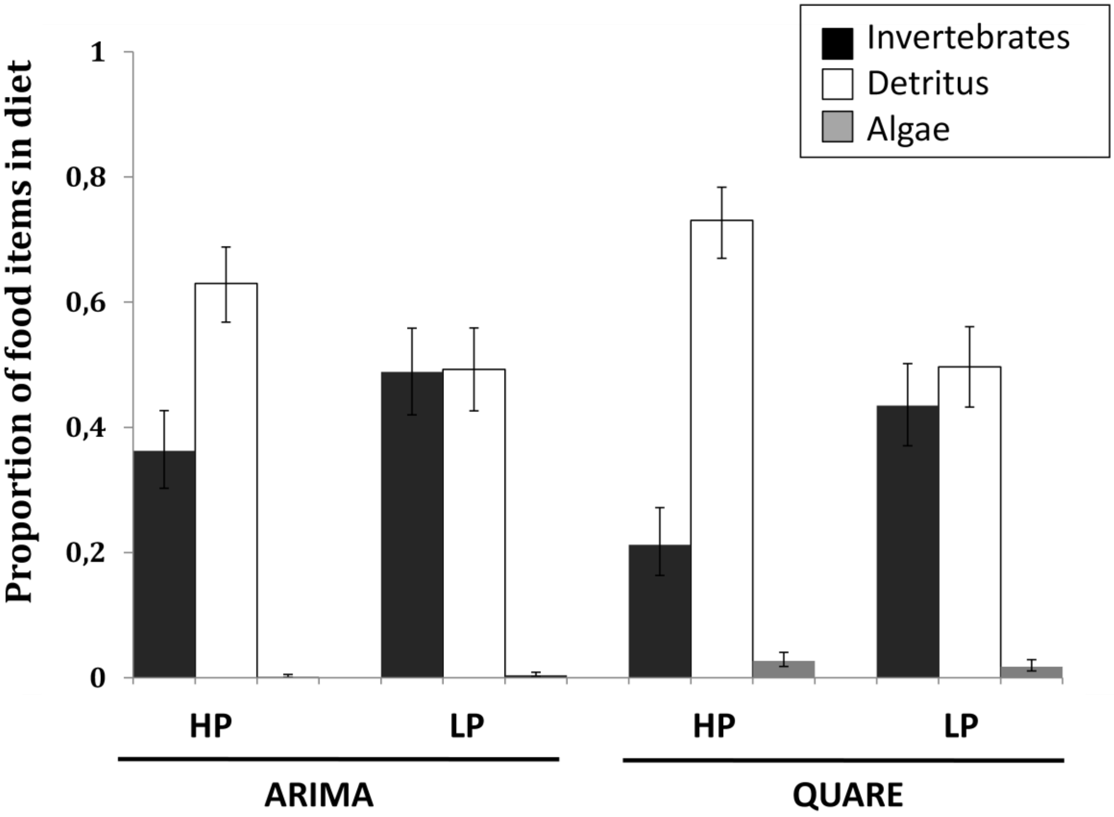


Figure 4-3. Proportional diet composition of HP (high predation) and LP (low predation) guppies from two study drainages, Arima and Quare. Data shown here represent the estimated marginal means calculated by the general linear model (GLM) on arcsin transformed data. Estimated marginal means and standard errors have been back-transformed for the graphical representation. Food categories analyzed are invertebrates, in dark grey; amorphous detritus, in white; and algae (filamentous and diatoms) in light grey. Error bars are ± 1 SE.

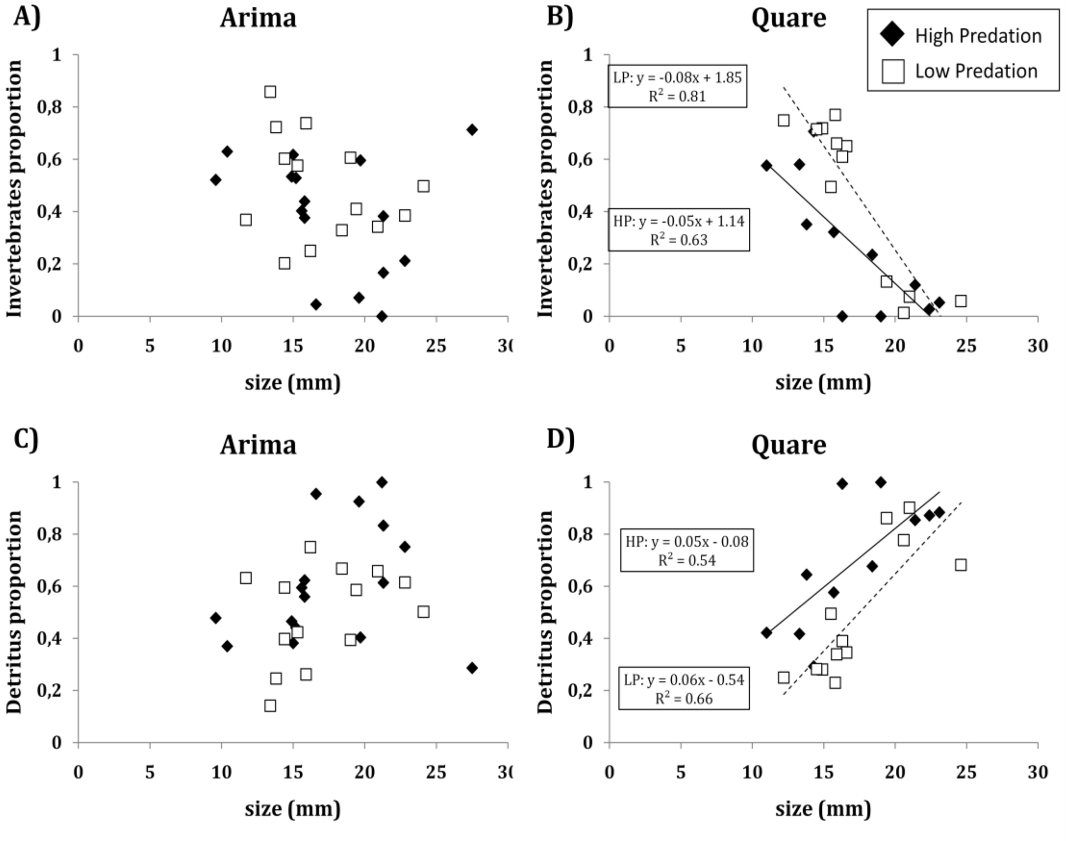


Figure 4-4. Correlation between fish size and (A-B) proportion of invertebrates and (C-D) proportion of detritus in diet in the Arima (A, C) and in the Quare (B, D) river. High predation (filled symbols) and low predation (open symbols) guppies are shown. Regression lines are shown for both high (solid line) and low predation (dashed line) sites when the relationship was significant. The relationship is significant for both invertebrates and detritus in both high and low predation in the Quare river only. The relationship in the Arima river are not significant.

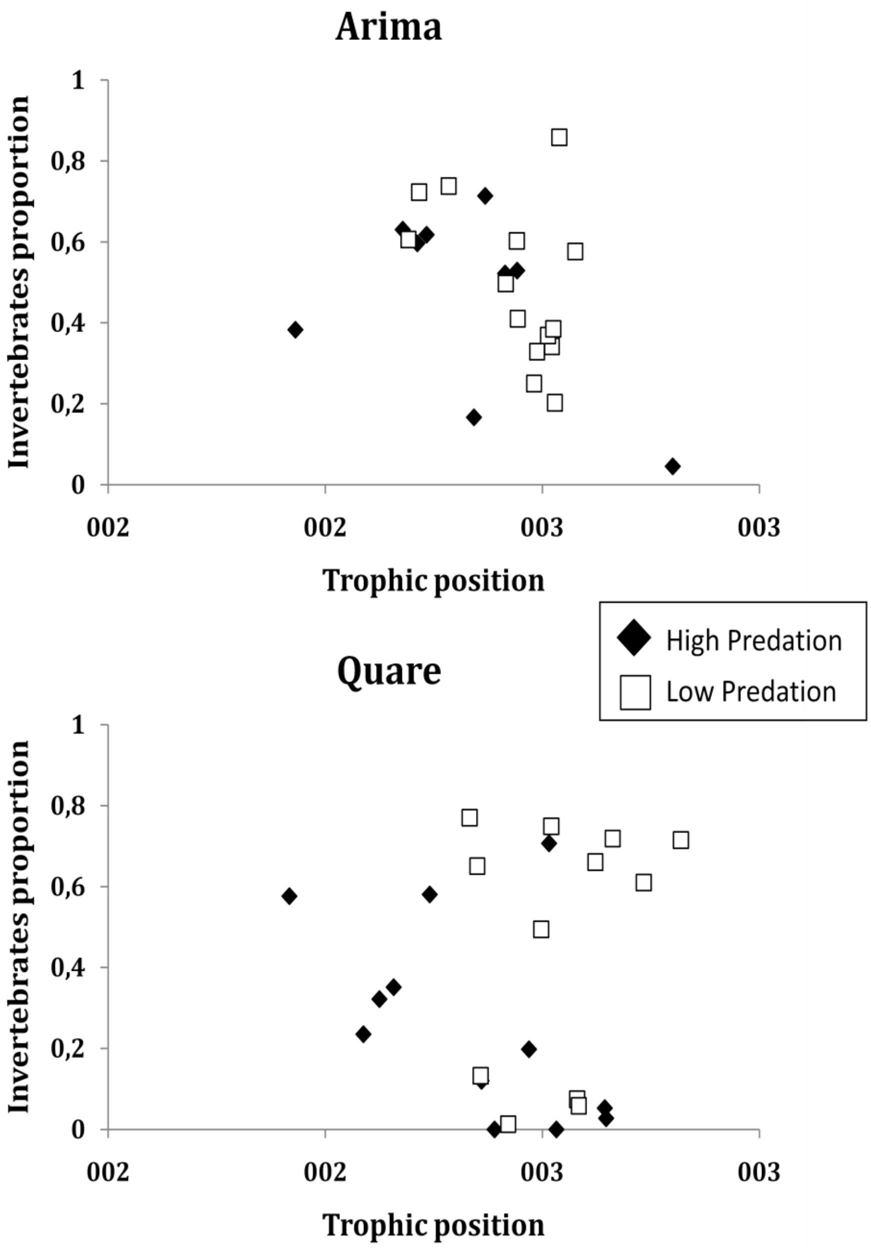


Figure 4-5. Relationship between trophic position and proportion of invertebrates in the Arima and Quare rivers. High predation (closed symbols) and low predation (open symbols) guppies are shown. No significant relationship was found.

Figure 4-6. Posterior probability intervals for prey source contribution, calculated by the SIAR model for each of the 12 sites sampled. 95th (light gray), 75th (grey), 50th (dark grey) percentiles are shown. Prey sources are different items found in guppy's diet: omnivore invertebrates (omniv.), predatory invertebrates (predat.), shredder invertebrates (shred.), collector-grazer invertebrates (coll/graz), epilithon (EPI, a mixture of algae and detritus). a) Sites sampled in 2007; b) sites sampled in 2008. (Next pages)

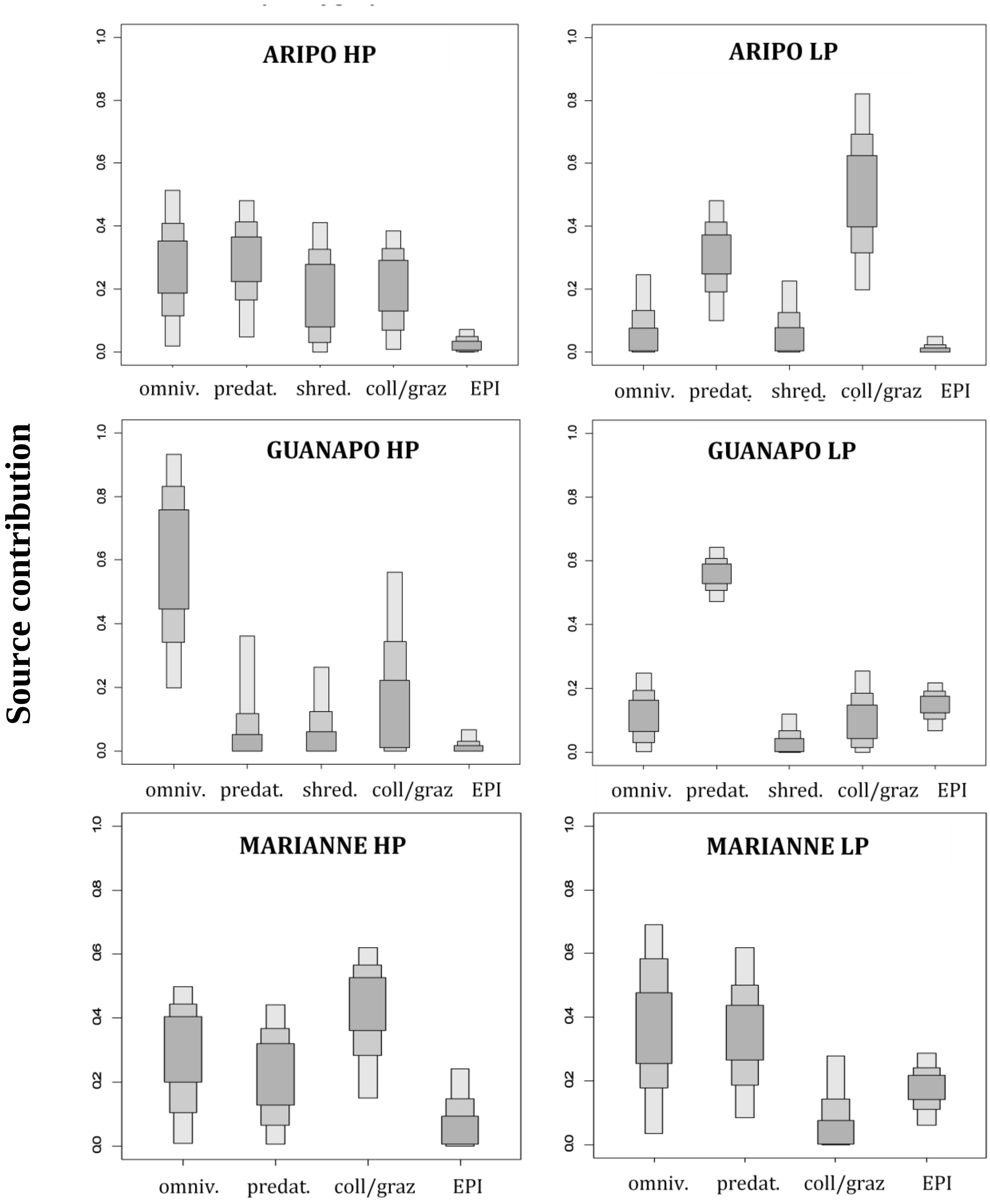

Source contribution
ARIPO HP
ARIPO LP
GUANAPO HP
GUANAPO LP
MARIANNE HP
MARIANNE LP
0.0
0.2
0.4
0.6
0.8
1.0
omniv.
predat.
shred.
coll/graz
EPI

Fig. 4-6 a)

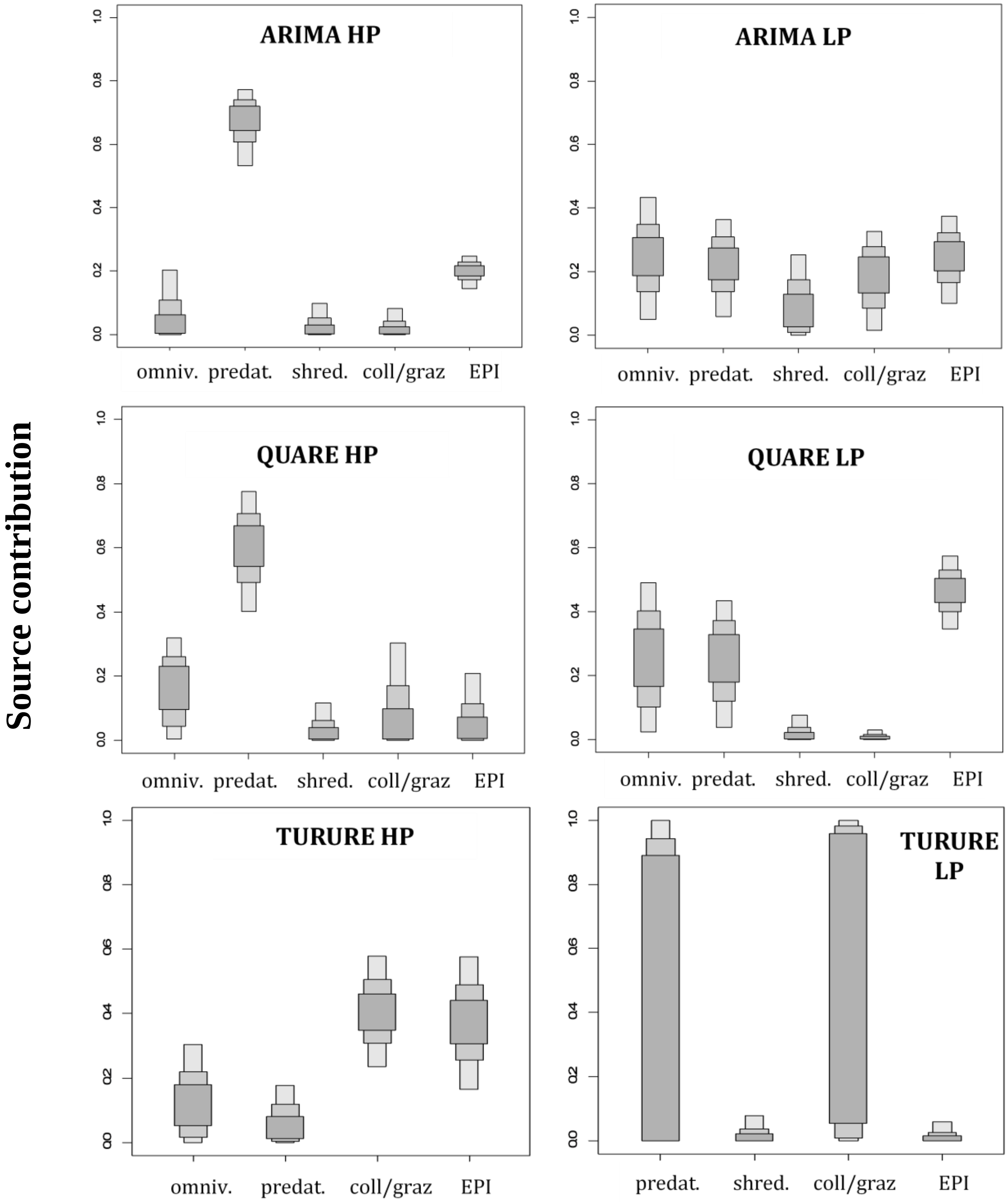
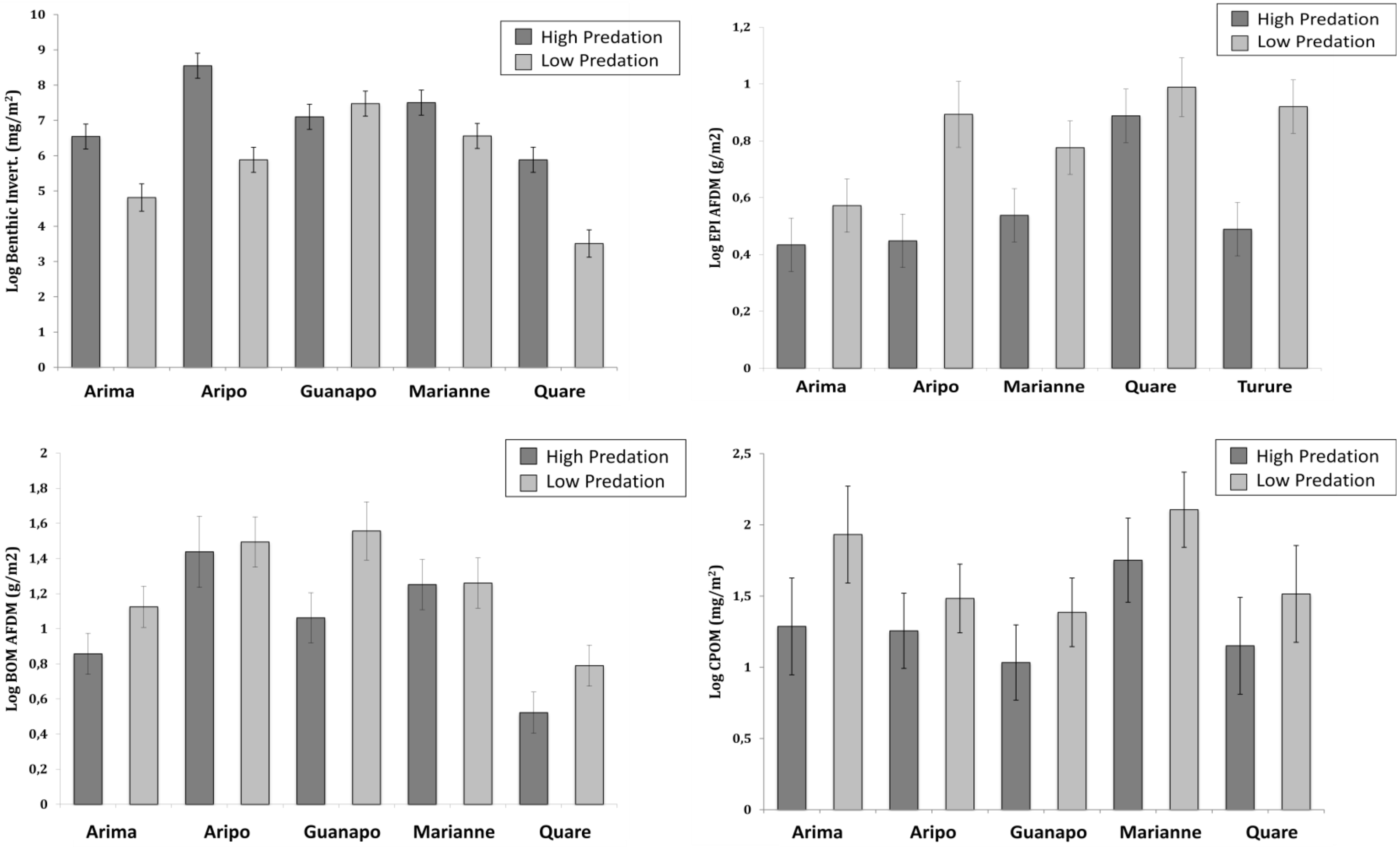

Source contribution
ARIMA HP
ARIMA LP
QUARE HP
QUARE LP
TURURE HP
TURURE LP
0.0
0.2
0.4
0.6
0.8
1.0
omniv.
predat.
shred.
coll/graz
EPI

Fig. 4-6 b)

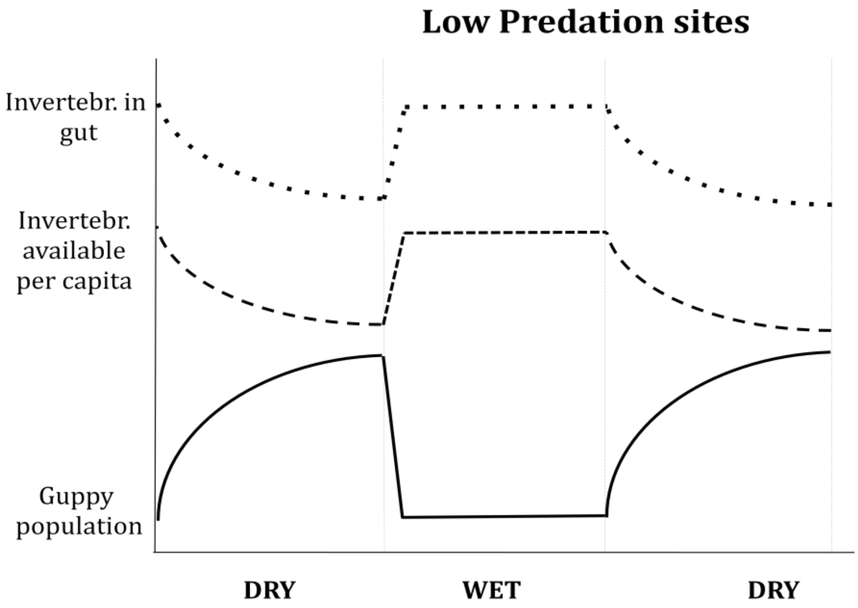
Figure 4-7. Biological characteristics of the 12 sites sampled in Trinidad. Top left: logarithm of the benthic invertebrate biomass (mg/m^2). Top right: logarithm of the epilithon (EPI) ash-free dry mass (AFDM) per meter square (g/m^2). Bottom left: logarithm of the benthic organic matter (BOM) ash-free dry mass (AFDM) per meter square (g/m^2). Bottom right: logarithm of the coarse particulate organic matter (CPOM or leaf litter) (mg/m^2). The Turure river was not included in the invertebrates, BOM, and CPOM analyses, as we could not sample its low predation site. The Guanapo river was not included in the EPI analysis as the samples were lost. High predation sites are in dark grey and low predation sites in light grey. Values are estimated marginal means calculated by the Univariate ANOVA. Error bars are ± 1 SE. (Next page)

Figure 4-7.



Log Benthic Invert. (mg/m²)
0
1
2
3
4
5
6
7
8
9
10
High Predation
Low Predation
Arima
Aripo
Guanapo
Marianne
Quare
Log EPI AFDM (g/m2)
0
0,2
0,4
0,6
0,8
1
1,2
High Predation
Low Predation
Arima
Aripo
Marianne
Quare
Turure
Log BOM AFDM (g/m2)
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
1,8
2
High Predation
Low Predation
Arima
Aripo
Guanapo
Marianne
Quare
Log CPOM (mg/m²)
0
0,5
1
1,5
2
2,5
High Predation
Low Predation
Arima
Aripo
Guanapo
Marianne
Quare

A)



B)

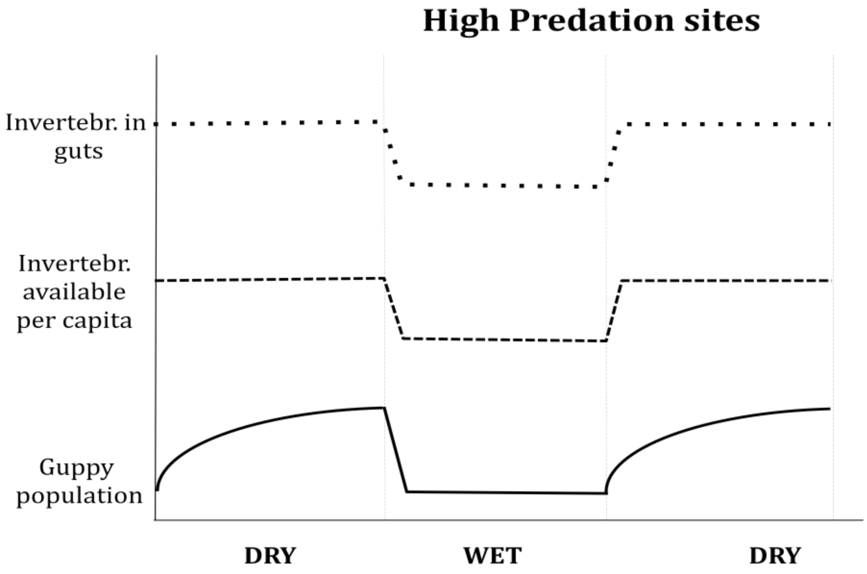


Figure 4-8. Model of the seasonal patterns of guppy population density (solid line), benthic invertebrate availability per capita (dashed line), and invertebrate abundance in diet (dotted line) in A) low predation sites and B) high predation sites.

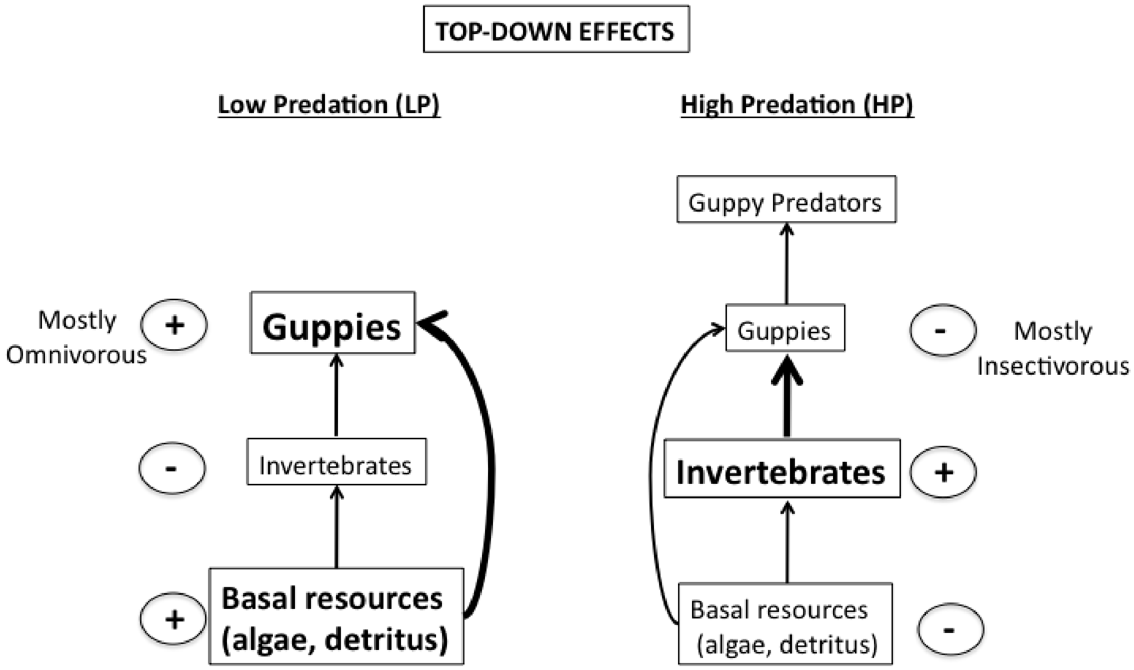


Figure 4-9. Model of the top-down effects in the food webs of high predation (HP) and low predation (LP) sites in streams of Trinidad.

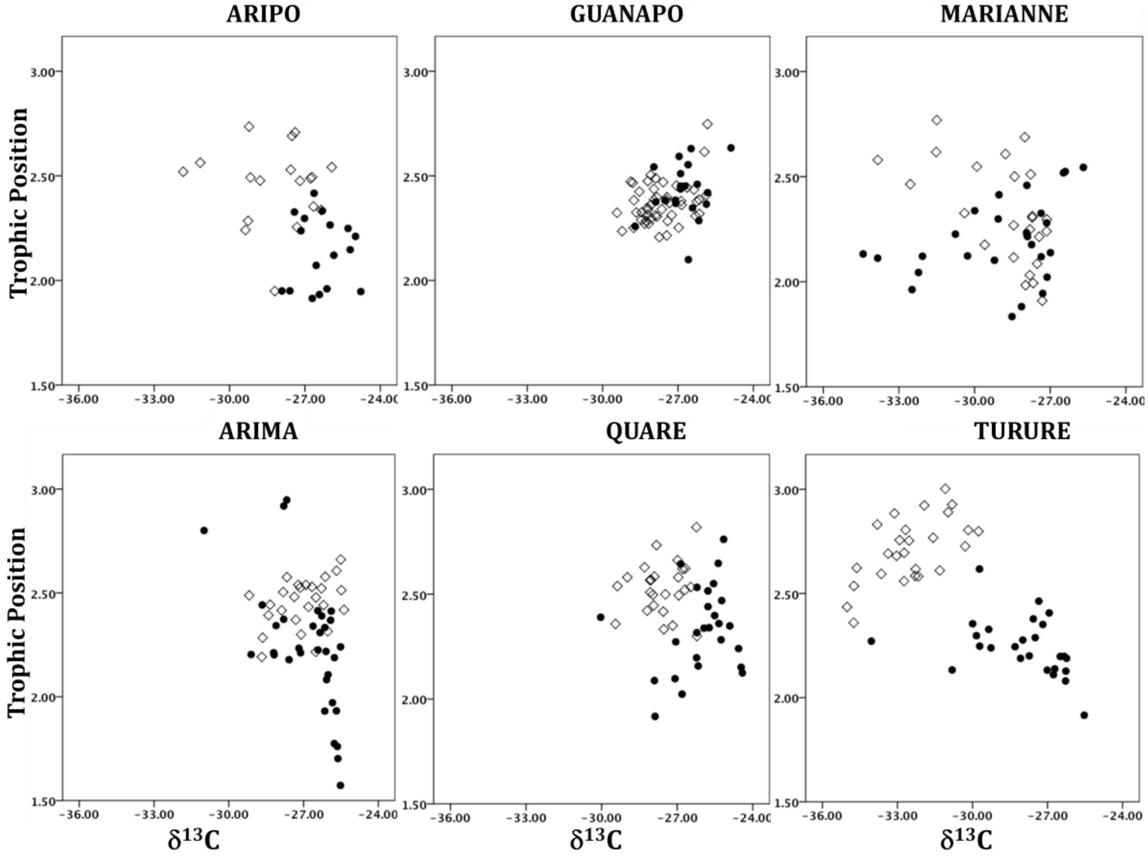


Figure 4-10. Bi-plots of guppy trophic position (y-axis) and $\delta^{13}C$ signatures (x-axis) for the 6 rivers sampled in Trinidad. In the top row are the rivers sampled in 2007, while in the bottom row are the rivers sampled in 2008. The HP guppies are indicated in filled circles, while the LP guppies are in open diamonds. Each data point represents an individual guppy. The Arima HP site was contaminated with manure, thus guppies' trophic position values for this site should not be considered comparable to the other sites.

CHAPTER 5: Conclusions and future directions

In my thesis I reported the existence of intraspecific variation in the trophic ecology of guppies. Predation regime, season, and the river of origin all affected guppies' diets but I did not find an indication of ontogenetic shift in diets. In the dry season, the differences in diet between guppies from high and low predation sites were the greatest, and they correlated with guppy life history trait patterns (Chapter 2). Guppies from sites with high predation pressures had higher overall reproductive allotment, matured earlier, and produced more and smaller offspring. HP guppies were also more insectivorous with high degrees of prey selectivity, selecting higher quality. Guppies from low predation sites showed the opposite life histories patterns, with lower allocation to reproduction, later maturation, and production of fewer and larger offspring. Concurrently, LP guppies were more herbivorous and did not show to be selective. The diet patterns found were confirmed by gut length measurements. In general, guppies from LP sites that consumed lower quality diets had longer guts compared to HP guppies that consumed higher quality diets (Chapter 3). More information regarding the role of the quantity and quality of resources in the environment in determining guppies' diets is needed. In particular, we know that guppies in high predation sites had high quality diet and also show higher reproductive output, earlier maturation, and faster growth rates (Arendt and Reznick

2005), but we do not know the causative mechanisms between these traits. Experiments showing the effects of high quality diet on growth rates and life history traits are needed, as well as studies looking at the genetic basis of the observed differences in diet between guppies from high and low predation.

The plasticity of gut physiology in response to changes in diet is also unknown. My data confirmed the inverse relationship between diet quality and gut length, and raised questions related to the performance of nutrient and energy assimilation. For instance, I found that guppies from low predation sites in the dry season have much higher percentages of detritus and algae in their guts compared to guppies from high predation, and they also have longer guts. Does this mean that guppies from low predation sites are better herbivores? Or else, are they better at assimilating nutrients from low quality food than high predation guppies? More detailed studies on the differences between high and low predation guppies' guts plasticity, assimilation efficiencies, and metabolism are needed to address these unanswered questions.

In Chapters 3 and 4 I showed that guppies's diets shifted during the wet season and that the differences found during the dry season between guppies from high and low predation sites disappeared. The data on guppies' diets were all collected at one point. Data collected along a time series would provide a profile of the diet dynamics throughout the year, which would help understanding the patterns of changes between dry and wet seasons. In particular, information on the factors causing the observed changes in diet between seasons is needed. The change in diet during the wet season could be attributed to a decrease in stream resources or to a decrease in guppy density. Both effects could alter the per capita resources available to guppy and the competitive environment in which they live, hence affecting their feeding choices. Also, abiotic factors, such as flood frequency and magnitude or other types of disturbance, could influence guppies’ diets; however our knowledge on this topic is very limited. Studies that are conducted along a time series and concurrently investigate the patterns of multiple variables such as life histories, diet, guppy density, per capita resource availability, and abiotic disturbance (e.g. floods) could be useful to pin point the cause-effect relationship among all these factors. An investigation focusing on the causative mechanisms underlying the dynamics of guppy diets in high and low predation environments would help elucidate the relationships among the aforementioned variables.

The river of origin had an effect on guppy diets and trophic position, as well as on biological and environmental characteristics of the streams (Chapter 4). This is not surprising considering that streams are natural systems and that it is almost impossible to find streams that are the exact replicate of each other. There could be many factors, such as the geology of the landscape and the extent of suitable habitats, that could affect the stream and the organisms living in it. For instance, the amount of resources and the guppy density in two distinct low predation streams could be different, which could affect guppies’ diets. The river of origin should be considered as an important source of variation. It should not be overlooked when exploring common patterns between high and low predation guppies. Studies that aim to find repeatable pattern should thus incorporate guppies from several drainages, and the factors causing differences between drainages should be investigated into greater detail.

Overall, my thesis calls for more studies on the mechanisms by which guppies’ phenotypes can differentially affect the environment in which they evolved. It also promotes further investigations on physiological, behavioral, and morphological adaptations of guppy phenotypes.

LIST OF REFERENCES

Abrams, P. A. and L. Rowe. 1996. The effects of predation on the age and size of maturity of prey. Evolution **50**:1052-1061.

Agard, J., M. Alkins-Koo, A. Cropper, K. Garcia, F. Homer, S. Maharaj, and other contributors. 2005. Northern Range Assessment 2005. Report of an Assessment of the Northern Range, Trinidad and Tobago: People and the Northern Range. State of the Environment Report 2004.

Anderson, C. and G. Cabana. 2005. $\delta^{15}N$ in riverine food webs: effects of N inputs from agricultural watersheds. Canadian Journal of Fisheries and Aquatic Sciences **62**:333-340.

Aravena, R., M. L. Evans, and J. A. Cherry. 1993. Stable isotopes of Oxygen and Nitrogen in source identification of nitrate from septic systems. Ground Water **31**:180-186.

Arendt, J. D. and D. N. Reznick. 2005. Evolution of juvenile growth rates in female guppies (*Poecilia reticulata*): predator regime or resource level? Proceedings of the Royal Society B-Biological Sciences **272**:333-337.

Bashey, F. 2008. Competition as a selective mechanism for larger offspring size in guppies. Oikos **117**:104-113.

Bassar, R. D., A. Lopez-Sepulcre, M. R. Walsh, M. M. Turcotte, M. Torres-Mejia, and D. N. Reznick. 2010a. Bridging the gap between ecology and evolution: integrating density regulation and life-history evolution. The Year in Evolutionary Biology **1206**:17-34.

Bassar, R. D., M. C. Marshall, A. Lopez-Sepulcre, E. Zandonà, S. K. Auer, J. Travis, C. M. Pringle, A. S. Flecker, S. A. Thomas, D. F. Fraser, and D. N. Reznick. 2010b. Local adaptation in Trinidadian guppies alters ecosystem processes. Proceedings of the National Academy of Sciences of the United States of America **107**:3616-3621.

Ben-David, M. and D. M. Schell. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia **127**:180-184.

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.

Berra, T. M. 2001. Freshwater Fish Distribution. Academic Press.

Bolnick, D. I. 2001. Intraspecific competition favours niche width expansion in Drosophila melanogaster. Nature **410**:463-466.

Brooks, J. L. and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science **150**:28-35.

Buddington, R. K., J. W. Chen, and J. M. Diamond. 1991. Dietary-regulation of intestinal brush-border sugar and amino-acid-transport in carnivores. American Journal of Physiology **261**:R793-R801.

Cappuccino, N. and P. W. Price. 1995. Population dynamics: new approaches and synthesis. Academic Press, San Diego.

Charlesworth, B. 1980. Evolution in age-structured populations. Cambridge University Press, Cambridge, U.K.

Croft, D. P., B. J. Arrowsmith, J. Bielby, K. Skinner, E. White, I. D. Couzin, A. E. Magurran, I. Ramnarine, and J. Krause. 2003. Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. Oikos **100**:429-438.

De Niro, M. J. and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta **45**:341-351.

Drewe, K. E., M. H. Horn, K. A. Dickson, and A. Gawlicka. 2004. Insectivore to frugivore: ontogenetic changes in gut morphology and digestive enzyme activity in the characid fish *Brycon guatemalensis* from Costa Rican rain forest streams. Journal of Fish Biology **64**:890-902.

Dussault, G. V. and D. L. Kramer. 1981. Food and feeding-behavior of the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). Canadian Journal of Zoology-Revue Canadienne De Zoologie **59**:684-701.

Eklov, P. and R. Svanback. 2006. Predation risk influences adaptive morphological variation in fish populations. American Naturalist **167**:440-452.

Fajen, A. and F. Breden. 1992. Mitochondrial-dna sequence variation among natural-populations of the Trinidad guppy, *Poecilia reticulata*. Evolution **46**:1457-1465.

FAO. 1997. Database on introduced aquatic species, Rome.

Ffrench, R. 1992. Birds of Trinidad and Tobago. Christopher Helm, London.

Fraser, D. F. and J. F. Gilliam. 1992. Nonlethal Impacts of Predator Invasion - Facultative Suppression of Growth and Reproduction. Ecology **73**:959-970.

Fraser, D. F., J. F. Gilliam, B. W. Albanese, and S. B. Snider. 2006. Effects of temporal patterning of predation threat on movement of a stream fish: evaluating an intermediate threat hypothesis. Environmental Biology of Fishes **76**:25-35.

Fry, B. 2006. Stable Isotope Ecology. Springer.

Gadgil, M. and W. H. Bossert. 1970. Life Historical Consequences of Natural Selection. American Naturalist **104**:1-24.

Gannes, L. Z., D. M. Obrien, and C. M. delRio. 1997. Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. Ecology **78**:1271-1276.

Gende, S. M., T. P. Quinn, and M. F. Willson. 2001. Consumption choice by bears feeding on salmon. Oecologia **127**:372-382.

German, D. P. and M. H. Horn. 2006. Gut length and mass in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. Marine Biology **148**:1123-1134.

German, D. P., D. T. Neuberger, M. N. Callahan, N. R. Lizardo, and D. H. Evans. 2010. Feast to famine: The effects of food quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **155**:281-293.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology **21**:394-407.

Gilliam, J. F., D. F. Fraser, and M. Alkinskoo. 1993. Structure of a Tropical Stream Fish Community - a Role for Biotic Interactions. Ecology **74**:1856-1870.

Gore, J. A. 2007. Discharge measurements and streamfolw analysis. Pages 51-78 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology. Academic Press.

Grether, G. F., D. F. Millie, M. J. Bryant, D. N. Reznick, and W. Mayea. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. Ecology **82**:1546-1559.

Haskins, C. P., E. F. Haskins, J. J. A. McLaughlin, and R. E. Hewitt. 1961. Polymorphism and population structure of *Lebistes reticulatus*, an ecological study. Pages 320-395 *in* W. F. Blair, editor. Vertebrate Speciation. University of Texas, Austin.

Hess, A. D. 1941. New limnological sampling equipment. Limnological Society of America Special Publication **6**:1-5.

Hill, W. R., P. J. Mulholland, and E. R. Marzolf. 2001. Stream ecosystem responses to forest leaf emergence in spring. Ecology **82**.

Hobson, K. A. and R. G. Clark. 1992a. Assessing avian diets using stable isotopes .1. Turnover of C-13 in tissues. Condor **94**:181-188.

Hobson, K. A. and R. G. Clark. 1992b. Assessing avian diets using stable isotopes .2. Factors influencing diet-tissue fractionation. Condor **94**:189-197.

Jensen, T. and A. Verschoor. 2004. Effects of food quality on life history of the rotifer *Brachionus calyciflorus* Pallas. Freshwater Biology **49**:1138-1151.

Karasov, W. H. and C. Martinez del Rio. 2007. Physiological Ecology: How Animal Process Energy, Nutrients, and Toxins. Princeton University Press.

Kenny, J. S. 1995. Views from the bridge: a memoir on the freshwater fishes of Trinidad. Maracas, St. Joseph, Trinidad and Tobago. .

Kilham, S. S., M. Hunte-Brown, P. Verburg, C. M. Pringle, M. R. Whiles, K. R. Lips, and E. Zandona. 2008. Challenges for interpreting stable isotope fractionation of carbon and nitrogen in tropical aquatic ecosystems. Verh. Internat. Verein. Limnol. **30**:166-170.

Kozlowski, J. and R. G. Wiegert. 1987. Optimal age and size at maturity in annuals and perennials with determinate growth. Evolutionary Ecology **1**:231-244.

Kramer, D. L. and M. J. Bryant. 1995a. Intestine length in the fishes of a tropical stream. 1. Ontogenic allometry. Environmental Biology of Fishes **42**:115-127.

Kramer, D. L. and M. J. Bryant. 1995b. Intestine length in the fishes of a tropical stream. 2. Relationships to diet - the long and short of a convoluted issue. Environmental Biology of Fishes **42**:129-141.

Langerhans, R. B. and T. J. DeWitt. 2004. Shared and unique features of evolutionary diversification. American Naturalist **164**:335-349.

Law, R. 1979. Optimal life histories under age-specific predation. American Naturalist **114**:399-417.

Liley, N. R. and B. H. Seghers. 1975. Factors affecting the morphology and behaviour of guppies in Trinidad. Pages 92-118 *in* G. Baerends, C. Beer, and A. Manning, editors. Function and evolution in behavior, Clarendon, Oxford.

Lloyd, D. G. 1987. Selection of offspring size at independence and other size-versus-number strategies. American Naturalist **129**:800-817.

MacArthur, R. H. and E. R. Pianka. 1966. On optimal use of a patchy environment. American Naturalist **100**:603-609.

MacAvoy, S. E., S. A. Macko, and G. C. Garman. 2001. Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. Canadian Journal of Fisheries and Aquatic Sciences **58**.

Magurran, A. E. 2005. Evolutionary Ecology. The Trinidadian Guppy. Oxford University Press.

Magurran, A. E. and B. H. Seghers. 1990. Population differences in the schooling behavior of newborn guppies, *Poecilia reticulata*. Ethology **84**:334-342.

Magurran, A. E. and B. H. Seghers. 1994. Sexual Conflict as a Consequence of Ecology - Evidence from Guppy, *Poecilia reticulata*, Populations in Trinidad. Proceedings of the Royal Society of London Series B-Biological Sciences **255**:31-36.

Maharaj, L. D. 1994. The ecology of selected aquatic insects in the Maracas River. M.Sc Thesis. University of the West Indies, St. Augustine, Trinidad.

Martin, R. A. and D. W. Pfennig. 2010. Field and experimental evidence that competition and ecological opportunity promote resource polymorphism. Biological Journal of the Linnean Society **100**:73-88.

Martinez del Rio, C., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews **84**:91-111.

Mattingly, H. T. and M. J. Butler. 1994. Laboratory predation on the Trinidadian guppy - implications for the size-selective predation hypothesis and guppy life-history evolution. Oikos **69**:54-64.

McCutchan, J. H., W. M. Lewis, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**:378-390.

McIntyre, P. B. and A. S. Flecker. 2006. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. Oecologia **148**:12-21.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2007. An Introduction to the Aquatic Insects of North America. 4th Edition. Kendall/Hunt Publishing Company.

Minagawa, M. and E. Wada. 1984. Stepwise Enrichment of N-15 Along Food-Chains - Further Evidence and the Relation between Delta-N-15 and Animal Age. Geochimica Et Cosmochimica Acta **48**:1135-1140.

Moore, J. W. and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters **11**:470-480.

Morrell, L. J., K. L. Hunt, D. P. Croft, and J. Krause. 2007. Diet, familiarity and shoaling decisions in guppies. Animal Behaviour **74**:311-319.

Murdoch, W. W., S. Avery, and M. E. B. Smyth. 1975. Switching n predatory fish. Ecology:1094-1105.

Naya, D. E., W. H. Karasov, and F. Bozinovic. 2007. Phenotypic plasticity in laboratory mice and rats: a meta-analysis of current ideas on gut size flexibility. Evolutionary Ecology Research **9**:1363-1374.

Olson, M. H. 1996. Ontogenetic niche shifts in largemouth bass: Variability and consequences for first-year growth. Ecology **77**:179-190.

Olsson, J., M. Quevedo, C. Colson, and R. Svanback. 2007. Gut length plasticity in perch: into the bowels of resource polymorphisms. Biological Journal of the Linnean Society **90**:517-523.

Owens, D. C. 2010. Seasonal variation in terrestrial insect subsidies to tropical streams and implications for the diet of *Rivulus hartii*. M.Sc. Thesis. University of Nebraska.

Palkovacs, E. P. and D. M. Post. 2008. Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feed back to shape predator foraging traits? Evolutionary Ecology Research **10**:699-720.

Palkovacs, E. P. and D. M. Post. 2009. Experimental evidence that phenotypic divergence in predators drives community divergence in prey. Ecology **90**:300-305.

Parenti, L. R. 1981. A Phylogenetic and Biogeographic Analysis of Cyprinodontiform Fishes (Teleostei, Atherinomorpha). Bull. Am. Museum Nat. History **168**.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source Partitioning Using Stable Isotopes: Coping with Too Much Variation. Plos One **5**.

Perez, G. R. 1996. Guia para el estudio de los macroinvertebrados acuaticos del Departimento de Antioquia. Universidad de Antioquia, Colombia.

Peterson, B. J. and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics **18**:293-320.

Phillip, D. 1998. Biodiversity of Freshwater Fishes of Trinidad and Tobago, West Indies. PhD thesis. University of St. Andrews.

Phillips, D. L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia **127**:166-170.

Phillips, D. L. and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia **127**:171-179.

Phillips, D. L. and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia **136**:261-269.

Phillips, D. L. and P. L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia **130**:114-125.

Piersma, T. and A. Lindstrom. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. Trends in Ecology & Evolution **12**:134-138.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. John Hopkins University Press, Baltimore.

Pilastro, A. and A. Bisazza. 1999. Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). Proceedings of the Royal Society of London Series B-Biological Sciences **266**:1887-1891.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**:703-718.

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.

Pringle, C. M. and T. Hamazaki. 1997. Effects of fishes on algal response to storms in a tropical stream. Ecology **78**:2432-2442.

Ramirez, A., C. M. Pringle, and M. Douglas. 2006. Temporal and spatial patterns in stream physicochemistry and insect assemblages in tropical lowland streams. Journal of the North American Benthological Society **25**:108-125.

Reznick, D. 1982a. Genetic determination of offspring size in the guppy (*Poecilia reticulata*). American Naturalist **120**:181-188.

Reznick, D. 1982b. The impact of predation on life-history evolution in Trinidadian guppies - genetic-basis of observed life-history patterns. Evolution **36**:1236-1250.

Reznick, D., M. J. Bryant, and F. Bashey. 2002. r- and K-selection revisited: The role of population regulation in life-history evolution. Ecology **83**:1509-1520.

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 life-history evolution in trinidadian guppies (*Poecilia reticulata*). Evolution **36**:160-177.

Reznick, D. and A. P. Yang. 1993. The influence of fluctuating resources on life-history - patterns of allocation and plasticity in female guppies. Ecology **74**:2011-2019.

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. 1989. Life-history evolution in guppies .2. Repeatability of field observations and the effects of season on life histories. Evolution **43**:1285-1297.

Reznick, D. N., M. J. Bryant, D. Roff, C. K. Ghalambor, and D. E. Ghalambor. 2004. Effect of extrinsic mortality on the evolution of senescence in guppies. Nature **431**:1095-1099.

Reznick, D. N. and H. Bryga. 1987. Life-history evolution in guppies (*Poecilia reticulata*) .1. Phenotypic and genetic changes in an introduction experiment. Evolution **41**:1370-1385.

Reznick, D. N. and H. A. Bryga. 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae) .5. Genetic basis of parallelism in life histories. American Naturalist **147**:339-359.

Reznick, D. N., M. J. Butler, F. H. Rodd, and P. Ross. 1996a. Life-history evolution in guppies (*Poecilia reticulata*) .6. Differential mortality as a mechanism for natural selection. Evolution **50**:1651-1660.

Reznick, D. N., F. H. Rodd, and M. Cardenas. 1996b. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae) .4. Parallelism in life-history phenotypes. American Naturalist **147**:319-338.

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.

Robinson, B. W. and D. S. Wilson. 1994. Character release and displacement in fishes - a neglected literature. American Naturalist **144**:596-627.

Robinson, B. W., D. S. Wilson, A. S. Margosian, and P. T. Lotito. 1993. Ecological and morphological-differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. Evolutionary Ecology **7**:451-464.

Rodd, F. H. and D. N. Reznick. 1997. Variation in the demography of guppy populations: The importance of predation and life histories. Ecology **78**:405-418.

Roff, D. A. 1992. The evolution of life histories: Theory and analysis. Chapman and Hall, New York.

Roff, D. A. 2002. Life history evolution. Sinauer Associates, Sunderland, MA.

Rostant, W. G. 2005. Freshwater Decapod Community of Trinidad and Tobago. Master thesis. The University of the West Indies.

Schluter, D. 2001. Ecology and the origin of species. Trends in Ecology & Evolution **16**:372-380.

Seghers, B. H. 1974. Geographic variation in the responses of guppies (*Poecilia reticulata*) to aerial predators. Oecologia **14**:93-98.

Seghers, B. H. 1978. Feeding behavior and terrestrial locomotion in the cyprinodontid fish *Rivulus hartii* (Boulenger). Verh. Internat. Verein. Limnol. **20**.

Semmens, B. X., P. J. Auster, and M. J. Paddack. 2010. Using Ecological Null Models to Assess the Potential for Marine Protected Area Networks to Protect Biodiversity. Plos One **5**.

Semmens, B. X., E. J. Ward, J. W. Moore, and C. T. Darimont. 2009. Quantifying Inter- and Intra-Population Niche Variability Using Hierarchical Bayesian Stable Isotope Mixing Models. Plos One **4**.

Sibly, R. M. 1981. Strategies of digestion and defecation. Pages 109-139 *in* C. R. Townsend and P. Calow, editors. Physiological ecology: an evolutionary approach to resource use. Blackwell Publishing, Oxford.

Sinervo, B. and S. C. Adolph. 1994. Growth plasticity and thermal opportunity in sceloporus lizards. Ecology **75**:776-790.

Skulason, S. and T. B. Smith. 1995. Resource polymorphisms in vertebrates. Trends in Ecology & Evolution **10**:366-370.

Smith, C. C. and S. D. Fretwell. 1974. The optimal balance between size and number of offspring. American Naturalist **108**:499-506.

Smith, T. B. 1987. Bill size polymorphism and intraspecific niche utilization in an African finch. Nature **329**:717-719.

Smith, T. B. 1990. Patterns of morphological and geographic-variation in trophic bill morphs of the African finch *Pyrenestes*. Biological Journal of the Linnean Society **41**:381-414.

Smith, T. B. and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annual Review of Ecology and Systematics **27**:111-133.

Starck, J. M. 1999. Structural flexibility of the gastro-intestinal tract of vertebrates - Implications for evolutionary morphology. Zoologischer Anzeiger **238**:87-101.

Starck, J. M. 2003. Shaping up: how vertebrates adjust their digestive system to changing environmental conditions. Animal Biology **53**:245-257.

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, Oxford, U.K.

Steinman, A. D., G.A. Lamberti, and P.R.Leavitt. 2007. Biomass and Pigments of Benthic Algae. Pages 357-379 *in* F. R. Hauer and G.A.Lamberti, editors. Methods in Stream Ecology. Academic Press.

Sterner, R. W. 1993. Daphnia growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. Ecology **74**:2351-2360.

Strauss, R. E. 1979. Reliability estimates for Ivlev's electivity index, the forage ratio, and a proposed linear index of food selection. Transactions of the American Fisheries Society **108**:344-352.

Svanback, R. and D. I. Bolnick. 2005. Intraspecific competition affects the strength of individual specialization: an optimal diet theory method. Evolutionary Ecology Research **7**:993-1012.

Svanback, R. and D. I. Bolnick. 2007. Intraspecific competition drives increased resource use diversity within a natural population. Proceedings of the Royal Society B-Biological Sciences **274**:839-844.

Svanback, R. and L. Persson. 2004. Individual diet specialization, niche width and population dynamics: implications for trophic polymorphisms. Journal of Animal Ecology **73**:973-982.

Templeton, C. N. and W. M. Shriner. 2004. Multiple selection pressures influence Trinidadian guppy (*Poecilia reticulata*) antipredator behavior. Behavioral Ecology **15**:673-678.

Travis, J. 2009. Phenotypic plasticity.*in* S. A. Levin, editor. The Princeton Guide to Ecology. Princeton University Press.

Twombly, S., N. Clancy, and C. Burns. 1998. Life history consequences of food quality in the freshwater copepod *Boeckella triarticulata*. Ecology **79**:1711-1724.

Urabe, J. and R. W. Sterner. 2001. Contrasting effects of different types of resource depletion on life-history traits in *Daphnia*. Functional Ecology **15**:165-174.

Vander Zanden, M. J., G. Cabana, and J. B. Rasmussen. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ^{15}N) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences **54**:1142-1158.

Vander Zanden, M. J. and J. B. Rasmussen. 1999. Primary consumer δ^{13}C and δ^{15}N and the trophic position of aquatic consumers. Ecology **80**:1395-1404.

Vander Zanden, M. J. and J. B. Rasmussen. 2001. Variation in δ^{15}N and δ^{13}C trophic fractionation: Implications for aquatic food web studies. Limnology and Oceanography **46**:2061-2066.

Vanderklift, M. A. and S. Ponsard. 2003. Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis. Oecologia **136**:169-182.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences **37**:130-137.

Wagner, C. E., P. B. McIntyre, K. S. Buels, D. M. Gilbert, and E. Michel. 2009. Diet predicts intestine length in Lake Tanganyika's cichlid fishes. Functional Ecology **23**:1122-1131.

Wainwright, P. C., C. W. Osenberg, and G. G. Mittelbach. 1991. Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus) - effects of environment on ontogeny. Functional Ecology **5**:40-55.

Wallace, J. B., J. J. Hutchens, and J. W. Grubaugh. 2007. Transport and storage of FPOM. Pages 249-271 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in stream ecology. Academic Press.

Walsh, M. R. and D. Reznick. 2010. Influence of the indirect effects of guppies on life-history evolution in *Rivulus hartii*. Evolution **64**: 1583-1593

Walsh, M. R. and D. N. Reznick. 2008. Interactions between the direct and indirect effects of predators determine life history evolution in a killifish. Proceedings of the National Academy of Sciences of the United States of America **105**:594-599.

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.

Werner, E. E. and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size structured populations. Annual Review of Ecology and Systematics **15**:393-425.

Werner, E. E., J. F. Gilliam, D. J. Hall, and G. G. Mittelbach. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology **64**:1540-1548.

Werner, E. E. and D. J. Hall. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish *Lepomis macrohirus*. Ecology **55**:1042-1052.

Werner, E. E. and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology **84**:1083-1100.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

Winemiller, K. O. 1990. Spatial and temporal variation in tropical fish trophic networks. Ecological Monographs **60**:331-367.

Winemiller, K. O. and D. B. Jepsen. 1998. Effects of seasonality and fish movement on tropical river food webs. Journal of Fish Biology **53**:267-296.

Winer, B. J. 1971. Statistical principles in experimental design. McGraw-Hill, New York.

Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics **25**:443-466.

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EDUCATION

PhD Environmental Science/Ecology 2010	Drexel University
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AWARDS, FELLOWSHIPS, AND GRANTS

2010, 2008	Best Poster Presentation, 1st prize. Drexel Research Day ($500 each)
2009, 2006	Honorable Mention. Drexel Research Day
2009	ASLO Outstanding Student Poster Award ($50)
2006-2010	6 Travel grants. Drexel University (Dept. of Biology) ($500 each)
2010	Travel grant. Drexel University (Office of Graduate Studies) ($310)
2010	ASLO Student Travel Award – ($250)
2005-2010	J.Mozino scholarship (6 years). Drexel University (total amount $69,586)
2009	Office of International Travel award. Drexel University ($500)
2005, 2003	Fondaz. “Ing. Aldo Gini” grant. Università di Padova (total €8,200)
2001	Erasmus scholarship-Kopenhagen University. (€1,500)

PUBLICATIONS

1. **Zandonà E.**, S. Auer, S. Kilham, A. López-Sepulcre, M. O’Connor, R. Bassar, A. Osorio, C. Pringle, D. Reznick. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. Submitted to *Functional Ecology*.
2. Bassar R.D., M.C.Marshall, A.López-Sepulcre, **E. Zandonà**, S.K.Auer, J.Travis, C.M.Pringle, A.S.Flecker, S.A.Thomas, D.Fraser, D.Reznick. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *PNAS.* 107(8): 3616-21.
3. Dadda, M., **E. Zandonà**, C. Agrillo, A. Bisazza. The cost of hemispheric specialization in a fish. 2009. *Proceedings of the Royal Society B: Biological Sciences.* 276(1677): 4399-4407.
4. Sieg A.E., **E. Zandonà**, V.M. Izzo, F.V. Paladino, J.R. Spotila. 2010. Population level “flipperedness” in the eastern Pacific leatherback turtle. *Behavioral Brain Research*. 206(1): 135-138.
5. Kilham S.S., M.E. Hunte-Brown, P. Verburg, C.M. Pringle, M.R. Whiles, K.R. Lips **E. Zandonà**. 2009. Challenges for interpreting stable isotope fractionation of carbon and nitrogen in tropical aquatic ecosystems. *Verhand. Internat. Verein. Limnol.* 30(5): 749-753.
6. Dadda M., **E. Zandonà**, A. Bisazza. Emotional responsiveness in fish from lines artificially selected for a high or low degree of laterality. 2007. *Physiology and Behaviour*. 92(4): 764-772.

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