Characterizing the effects of urbanization on stream biota using a trait-based approach

by

Piatã Marques
B.Sc., Universidade do Estado do Rio de Janeiro, 2011
B.Ed., Universidade do Estado do Rio de Janeiro, 2011
M.Sc., Universidade do Estado do Rio de Janeiro, 2013

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Supervisory Committee

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Supervisory Committee

Dr. Rana El-Sabaawi, Department of Biology
Supervisor

Dr. Francis Juanes, Department of Biology
Departmental Member

Dr. John Taylor, Department of Biology
Departmental Member

Dr. Chris Darimont, Department of Geography
Outside Member

Abstract

We live in an increasingly urban world and ecologists are being called upon to provide thorough information on the effect of urbanization on ecosystems. However, urban ecology has historically focused almost exclusively on describing changes in species richness. Although this has been important as a first characterization of the effect of urbanization, the focus on describing species richness has restricted our understanding of the mechanisms determining ecological patterns and processes in cities. In this thesis, I apply a trait-based approach to a widespread urban invasive species, the guppy, *Poecilia reticulata*, in order to explore the mechanisms through which urbanization can affect reproductive and feeding ecology traits of the stream biota. I first review studies that use trait-based approaches in stream ecosystems and develop an intraspecific trait framework that can be used to link urbanization to changes in traits of the stream biota. Then, I combine this framework with existing information on trait evolution of guppies in their non-urban, native range in Trinidad, to explore the effect of urbanization on guppy life history related traits and population density in Brazil. Next, building on a study of drivers of diet and trophic morphology in Trinidadian guppies, I use a trait-based framework to explore the effect of urbanization on guppy diet and feeding morphology in Brazil.

My review shows that intraspecific trait approaches in urban streams are rare, but have the potential to provide a mechanistic understanding of the effects of urbanization on stream biota. By using an intraspecific trait approach, I show that urbanization increases guppy body length, increases fecundity and improves condition. Concurrent investment in reproduction and somatic tissues suggests that urbanization relaxes life history traits trade-offs in guppies. Urban guppies also attain far higher densities than non-urban guppies.

These changes in traits and populations are related to the large amount of high-quality food (i.e. chironomids) available for guppies in urban streams. Urban-induced changes in traits enhance guppy invasive potential. By studying guppies in Trinidad, I have found that each population is composed of two resource-use phenotypes with distinct diets and gut morphology (carnivorous guppies with short guts and detritivorous/algivorous guppies with long guts). The frequency of each resource-use phenotype appears to be determined by guppy density: low density appears to increase the frequency of the carnivorous phenotype. Guppy populations in Brazil are also composed of two resource-use phenotypes, and the existence of these phenotypes is related to the variation in individual feeding morphology (i.e. cranium shape) that affect feeding efficiency. Neither density nor urbanization appears to shift the distribution of the two resource use phenotypes. However, urban guppies have larger and wider crania, thus a more efficient insect feeding morphology, than non-urban guppies. Overall my study suggests that consumption of chironomids is important for the success of guppies in urban streams, and it is possible that similar mechanism also facilitates the success of other urban dwellers. My study also highlights the power of intraspecific trait approaches for understanding the ecology urban dwellers. Such knowledge can help us refine and advance ecological theories to better predict future ecological change in an increasingly urbanized world.

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Acknowledgments

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Dedication

To those who can see the wonders beyond the concrete of our cities.

Chapter 1- Introduction

From the beginning of hunter-gather and agricultural societies to the rise of modern urban societies, humans have caused changes to hydrologic, biochemical and biological processes (Zalasiewicz, Waters & Williams, 2014; Ellis, 2015; Pecl *et al.*, 2017; Pokhrel *et al.*, 2017). The effect of human activities is so profound and widespread that now the Earth is going through a new epoch, the Anthropocene, where humans have become the main force changing the environment (Corlett, 2015). An important anthropogenic driver of contemporary ecological change is the modification of the landscape through urbanization (Seto, Guneralp & Hutyra, 2012; Song *et al.*, 2015; Delphin *et al.*, 2016) which is expected to increase as the global population grows and becomes more urbanized (United Nations, 2014). Thus, one of the current challenges for ecologists is to assess how ecological patterns and processes change and interact with humans in an increasingly urbanized world (McPhearson *et al.*, 2016). In this thesis, I explore the effects of urbanization on stream ecosystems using a trait-based approach applied to the guppy, *Poecilia reticulata,* a widespread invasive species that is commonly found in Tropical urban ecosystems.

1.1 Urban ecology and ecosystems

The discipline of urban ecology is relatively new (Wu, 2014). Narratives on urban ecology can be found on Darwin`s book “On the origin of species” published in 1859. While the first records of empirical research come from Europe, Asia and North America in late 1940s and 1950s (McDonnell, 2011). But for decades following that urban areas were largely avoided by ecologists, seen as not legitimate subjects for ecological research

(McDonnell, 2011). Only in the late 1990s did urban ecology establish and flourish as a multidisciplinary field which aims to understand how humans and ecology interact in urban areas (Alberti, 2008; Wu, 2014; Hahs & Evans, 2015).

Urban ecologists focus their attention on urban ecosystems where there is a high density of people, built structures cover most of the land surface and where human-environment interactions determine the ecosystem structure, function and dynamics (Wu, 2014). Studies have suggested that although urban ecosystems are widespread across the globe, geographically distant cities are more similar to each other than their natural surrounding because they have similar climate, hydrology, soils and biodiversity (McKinney, 2006; Groffman *et al.*, 2014). This suggests that, converting natural land cover to impervious urban surfaces has a homogenizing effect on the ecosystem (McKinney, 2006; Olden, 2006). This effect lead to the emergence of generalizations known as "urban syndromes" that describe the effects of urbanization to ecosystems (Pickett *et al.*, 2016). Among these, the "Urban Stream Syndrome" (Walsh *et al.*, 2005) is of special interest for my thesis because it summarizes the effects of urbanization to streams. It suggests that across the globe, and despite differences in ambient biota, urban streams have similar conditions including high productivity, low biodiversity, disappearance of sensitive species, proliferation of invasive hardy species, increased magnitude and frequency of flash floods, and increased contamination (Walsh *et al.*, 2005).

1.2 Urban biodiversity and urban dwellers

Urbanization poses a major threat to biodiversity worldwide, causing massive declines in sensitive taxa and apex predators (Seto *et al.*, 2012). Yet, urban areas still harbour many different species, often at high abundances, and a major effort is ongoing for characterizing and conserving biodiversity in cities (Aronson *et al.*, 2014, 2017; Ives *et al.*, 2016). Species that thrive in urban areas have been referred to as synanthropes (McKinney, 2006), synurbic species (Francis & Chadwick, 2012), urban exploiters or urban adapters (Shochat *et al.*, 2006). Each of these terms uses a different set of criteria (e.g. occurrence, population density) to determine what is an urban species. In this thesis I use the term "urban dweller" as defined by Fischer *et al.* (2015), which is based on differences in population dynamics in urban *versus* non-urban areas. The urban areas are characterized by being heavily modified for residential, recreational, commercial or industrial human use (this excludes agricultural areas). Species that persist in urban areas and maintain viable populations (i.e. maintain positive growth rate) independent of immigration from natural areas, are referred to as urban dwellers (Fischer *et al.*, 2015). It is vital to understand the ecology of urban dwellers because they dominate the biomass in urban ecosystems, which can have consequences for ecosystem structure and function. They are also thought to outcompete more sensitive species (Shochat *et al.*, 2010).

1.3 Trait-based approaches in urban ecosystems

Studies characterizing ecosystem processes often begin by quantifying biodiversity. It is widely recognized that biodiversity affects many aspects of ecosystem structure and function, but the mechanisms underlying the effects have been difficult to characterize (Duncan, Thompson & Pettorelli, 2015). Trait-based approaches have long been used in ecological studies to provide a more mechanistic understanding of the links between species composition, population dynamics, community structure and ecosystem processes (Mcgill *et al.,* 2006; Bolnick *et al.,* 2011; Trussell & Schmitz, 2012). Trait approaches focus on how the characteristics of the species (i.e. traits), rather than their taxonomic identities, relate to and respond to the environment. A trait can be defined as any morphological, physiological or phenological characteristic measured at the individual level which has consequences to fitness (i.e. functional trait), or the ecosystem (i.e. an ecosystem effect trait) (Violle *et al.*, 2007; Matthews *et al.*, 2011). Most trait-based studies focus on non-urban systems, and trait-based studies in urban systems focus on interspecific rather than intraspecific trait variation (e.g. Lizée *et al.*, 2011; Palma *et al.*, 2017). Urbanization imposes rapid changes in the traits of aquatic and terrestrial organisms (Alberti, 2015; Alberti, Marzluff & Hunt, 2017b; Alberti *et al.*, 2017a). Trait-based approaches therefore have the potential to reveal the relationship between urban selective pressure, biodiversity, and changes in ecosystem structure and function (Hahs & Evans, 2015). I use an intraspecific-trait perspective in my thesis because it can provide a much-needed understanding of the mechanisms through which urbanization affect the biota (McDonnell & Hahs, 2013; LaPoint *et al.*, 2015; Hamblin, Youngsteadt & Frank, 2018).

1.4 Guppies as a model species

The Trinidadian guppy, *Poecilia reticulata* is native to the northeast of South America and to the Caribbean (Magurran, 2005). However, guppies are widespread, having invaded at least 69 countries out of their native range (Deacon, Ramnarine & Magurran, 2011) and established populations in both urban and non-urban systems (Lindholm *et al.*, 2005; Alexandre, Esteves & de Moura e Mello, 2010). This range expansion was facilitated by the introduction of guppies for mosquito control in countries with epidemic episodes of mosquito borne diseases and as a result of the aquarium trade (Seng *et al.*, 2008; Strecker, Campbell & Olden, 2011). This impressive range expansion is also aided by the guppy`s reproductive traits. Female guppies copulate with multiple males, store sperm for up to six months, and can give birth to multiple broods fathered by several males (Evans & Magurran, 2001; Hain & Neff, 2007). A single female guppy has an 86% chance of establishing a viable population following a new introduction (Deacon *et al.*, 2011).

In their native range on the island of Trinidad, guppies have been studied for decades and provided one of the best-known examples of life history trait change and evolution, specifically in response to predation (Magurran, 2005; Travis *et al.*, 2014). In Trinidad, guppies naturally occur in stream reaches with large fish predators (high predation, HP), and in stream reaches without large fish predators (low predation, LP). These different communities often occur in upstream and downstream reaches of the same river and are isolated by barrier waterfalls that limit the upward dispersal of fish. Guppies from HP and LP reaches have different life history traits, which are thought to evolve as the result of differences in predation pressure. In HP reaches, guppies have greater reproductive allotment, smaller but greater number of offspring, and smaller size at maturity when compared to LP guppies (Reznick & Endler, 1982; Reznick, Butler IV & Rodd, 2001). These predator-induced differences are heritable, thus they are considered to be a result of evolutionary process, although considerably plasticity also exists (Reznick, 1982; Torres-Dowdall *et al.*, 2012). In order to test how fast the reported trait evolution could arise in natural settings, several controlled transplant experiments were performed in Trinidadian streams (Reznick, Ghalambor & Crooks, 2008). In these experiments guppies were collected from downstream HP reaches, and then transplanted to upstream, previously guppy-free LP environments, and their trait changes observed in time. Following transplant, major changes in life history traits were rapid, occurring within a few years of guppy transplant (Reznick & Endler 1982).

Differences between HP and LP guppies are also seen in diet and feeding related traits. Guppies are omnivorous/generalist feeders, but their diets change with context. In LP reaches guppy diets are dominated by low quality items such as detritus and algae, while in HP reaches guppies feed mostly on insects, which are more nutritious food because they have high nitrogen content (Zandonà *et al.*, 2011). Guppies also vary in cranium shape, which is thought to affect their efficiency for feeding on invertebrates (Palkovacs, Wasserman & Kinnison, 2011). Unlike life history traits, which are extensively studied, little is known about how guppy diets and feeding related traits vary in relation to predators, or with other factors such as food availability. In addition, there are contradictory observations of guppy diets in the literature. For example, while the majority of studies show that LP guppies feed on higher proportions of detritus and algae than HP guppies, some studies show little dietary differences between the two populations (Zandonà *et al.*, 2011, 2015, 2017; Sullam *et al.*, 2015). In addition, LP guppies have shorter and wider crania which suggests more efficient feeding on insects (Palkovacs *et al.*, 2011). Therefore, the study of trophic ecology in Trinidad is on-going (Travis *et al.*, 2014).

Guppies are one of the few vertebrate species for which the drivers of trait change and evolution are relatively well known (Travis *et al.*, 2014), but so far studies of guppy and trait change have been largely limited to preserved ecosystems with minimal human impacts. Guppies are found in many urbanized streams in the tropical region (Widianarko *et al.*, 2000; Alexandre *et al.*, 2010). This provides a unique opportunity to use a well-studied model organism to explore the effect of urbanization to the traits of stream biota. Throughout this thesis I use what we know about the evolution of guppy traits in their native range to make and test hypotheses about the drivers of trait change in urban guppies.

1.5 Thesis goals and structure

My goal in this thesis is to characterize the effect of urbanization on guppy life history traits, diet and feeding related traits. I also explore the mechanisms through which urbanization changes such characteristics. I begin by reviewing trait-based studies in aquatic urban biota and develop a theoretical framework that relates the effects of urbanization to changes in traits (Chapter 2). Next, I combine this framework with existing life history trait information from studies in non-urban streams of Trinidad and use this information to make and test hypotheses regarding the effect of urbanization on life history traits of urban guppies that have invaded streams in Brazil (Chapter 3). In the second half of this thesis, I further explore diet and feeding related traits variation in guppies. Because there exists little information on guppy nutrition in their native range, I start by investigating the factors that affect diet and feeding related traits of guppies in their non-urban, native range in Trinidad (Chapter 4). I then use this information to derive hypothesis and predictions about the effect of urbanization on diet and feeding related traits of urban guppies in Brazil (Chapter 5).

1.6 Collaborations

International collaborations are key for building current scientific knowledge, especially knowledge needed to tackle global problems such as urbanization (Ribeiro *et al.*, 2018). My research would not have been possible without my collaboration with scientists from Brazil, Canada and Trinidad & Tobago. Throughout the thesis I use the first-person pronoun “I” because I lead the planning of the experimental design, data collection, data analysis and the writing. I detail specific contributions and affiliations of each of my co-authors at the end of each chapter.

Chapter 2 - Intraspecific trait variation in urban stream ecosystems: towards understanding the mechanisms shaping urban stream communities

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2.1 Abstract

The rapid expansion of urban centers is a critical threat to stream ecosystems, yet we currently lack mechanistic understanding of the effects of urbanization on stream communities. In this chapter I explore how an intraspecific trait perspective can unveil mechanisms of change in urban stream communities. Intraspecific trait approaches are rarely used in urban aquatic ecosystems although their potential has been widely demonstrated in terrestrial systems. I begin by identifying several biotic and abiotic agents that can drive intraspecific trait changes in life history, behavior, morphology, and feeding in a range of urban stream organisms. Then I propose that intraspecific trait-based approaches in urban streams can help explain the mechanisms underlying species persistence, biodiversity responses, functionality, and evolution and how they can potentially improve biomonitoring in urban streams. This trait-based information is essential to better understand, predict, and manage the impacts of urbanization on stream biota.

2.2 Introduction

Over the last few decades, ecosystems have been under an increasing number of threats associated with human activities, many of which are related to land use change and urbanization (Strayer & Dudgeon, 2010). Currently, 54% of the world’s population lives in cities, and this number is expected to reach 66% by 2050 (United Nations, 2014). This trend suggests potential increases in the magnitude of urban impact to ecosystems worldwide in the near future. Urbanization affects natural ecosystems through habitat degradation, species loss, disruption of ecosystem processes, and biological interactions (Alberti, 2008). In stream ecosystems, urban development leads to a collection of symptoms known as “the Urban Stream Syndrome” which include severe environmental degradation, species loss and dominance of a few, tolerant taxa (Walsh *et al.*, 2005).

Research on the effects of urbanization on ecosystems has produced a large volume of literature, but the mechanisms driving the effects of urbanization on biodiversity remain unclear (McDonnell & Hahs, 2013). The majority of studies focus on the community-level numeric responses to urbanization, emphasizing the lethal effects on species (e.g. species loss) and changes to biodiversity (McDonnell & Hahs, 2013; McDonnell & MacGregor-Fors, 2016). However, species known as urban dwellers, are able to persist despite urban disturbance (Fischer *et al.*, 2015). How and why species thrive in urbanized ecosystems remains a fundamental, yet unanswered question (Mouillot *et al.*, 2013).

Changes in environmental conditions can cause plastic or genetic responses in life history, morphology, and behavior within and between populations (Mouillot *et al.*, 2013). Humans can cause dramatic intraspecific trait changes through harvesting, pollution, climate change, species introduction, and landscape alteration (Darimont *et al.*, 2009, 2015; Palkovacs *et al.*, 2012). Possibly the most classical example is the industrial melanism of the peppered moths, *Biston betularia* (Kettlewell, 1959). Intraspecific traits can be highly sensitive to environmental change because selective pressure operates on individuals, creating plastic and genetic responses in phenotype that can affect a range of ecological processes (Reznick *et al.*, 2001; Matthews *et al.*, 2010; Verberk, van Noordwijk & Hildrew, 2013). Therefore, characterizing intraspecific trait responses is an important component of understanding the impacts of anthropogenic change.

Recent work in terrestrial systems has shown that urbanization can be a major force driving intraspecific trait change (Alberti *et al.*, 2017a). Temperature can drive the phenology of urban brittlebush, *Encelia farinosa,* populations (Neil *et al.*, 2014); city noise causes blackbirds, *Turdus merula,* to sing at higher frequencies (Nemeth *et al.*, 2013); and increased predation risk in cities affects the wing morphology of the European starling, *Sturnus vulgaris* (Bitton & Graham, 2015). Such studies have been instrumental in revealing mechanisms by which terrestrial organisms respond to urban pressure because they can link specific urban impacts to phenotypic change (Alberti *et al.*, 2017a). For instance, feeding plasticity has been hypothesized to be a key mechanism for the success of terrestrial urban species because it allows continued access to high quality foods under altered food availability conditions (Shochat *et al.*, 2010).

In contrast, much less is known about how urbanization affects intraspecific traits in aquatic ecosystems in general, and in stream systems specifically. In this review, I propose that understanding how intraspecific traits vary in response to urbanization can help us tackle important mechanistic questions in urban stream ecosystems. I begin by identifying potential selective forces that can operate in urban streams. I use examples from urban and non-urban studies to highlight which traits can come under selection in the urban environment. Then, I identify important mechanistic questions about urban streams that can be addressed by characterizing intraspecific trait variability.

2.3 What can cause intraspecific trait to change in urban streams?

The Urban Stream Syndrome (USS) describes a collection of symptoms commonly observed in urban streams: reduced biotic richness, altered hydrography, elevated concentrations of inorganic dissolved nitrogen and phosphorus, and high contaminants concentration (Walsh *et al.*, 2005). Local environmental conditions and socio-economic aspects of human communities can alter local symptoms (Parr *et al.*, 2016; Booth *et al.*, 2016), but the USS is nonetheless useful for describing the general state of urban streams. Here we use the USS as a starting point to facilitate the identification of agents of selection operating in urban streams. I use research from non-urban streams to explore how organismal traits might respond to these agents. However, in all of these non-urban stream examples I only focus on species that are known to occur in urban environments (Appendix A, Table A1). Finally, I use examples from urban systems, when they are available, to confirm whether trait responses that occur in non-urban systems also occur in urban systems. My goal is to identify various pathways for intraspecific trait change to occur in streams altered by urban development. I highlight some of these pathways in Figure 2.1.

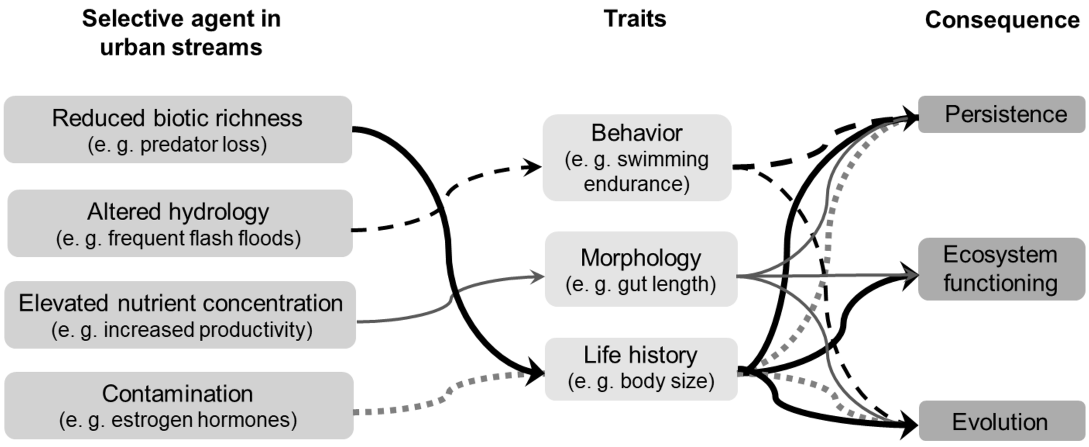

Figure 2. 1. Examples of how an intraspecific trait perspective can help reveal and explain mechanistic change in urban streams. Examples are based on studies cited or mechanisms proposed in the text. Different arrows connect specific selective agents in urban streams to changes in intraspecific traits, and the consequences of trait changes to ecological processes. The selective agents are derived from the Urban Stream Syndrome (Walsh *et al.*, 2005).

2.3.1 Reduced biotic richness

Urbanization can affect species richness by removing sensitive species and promoting the establishment of tolerant or invasive species (McKinney, 2002; Alberti, 2008; Shochat *et al.*, 2010). The combination of local extirpation and introduction/invasion determines community composition in urban ecosystems (Aronson *et al.*, 2014). These changes in community composition can lead to intraspecific trait variability by disrupting ecological interactions (Bolker *et al.*, 2003; Schmitz, Krivan & Ovadia, 2004).

Extirpation is likely to disproportionately affect predator-prey interactions in urban streams because predators are especially vulnerable to environmental changes (Woodward, 2009; Woodward *et al.*, 2012). Studies in natural freshwater systems suggest that both predation release (i.e. absence of predator *via* extirpation) and predation risk (i.e. presence of predator via introduction) can drive changes in prey behavior, morphology, and life history with implications for prey survival (McCollum & Leimberger, 1997; McCoy & Bolker, 2008; Ahlgren, Åbjörnsson & Brönmark, 2011; Hoverman, Cothran & Relyea, 2014). For example, predation risk decreases size at maturity while increasing reproductive allotment in the guppy, *Poecilia reticulata* (Reznick & Endler, 1982; Reznick *et al.*, 2001). Predators can also affect the life history traits of invertebrates and frogs (Laurila, Kujasalo & Ranta, 1997; Latta *et al.*, 2007).

Invasion likely affects competitive interactions in urban systems. Invasive species can take advantage of altered urban environments to proliferate at large densities (Havel *et al.*, 2015; Alberti *et al.*, 2017b). High density of invasive species can increase intra- and interspecific competition (Shochat *et al.*, 2010), which can alter intraspecific traits in both the introduced and resident species. For example, in non-urban streams, invasive larval bullfrogs, *Rana catesbeiana,* reduce the growth rate of native larval frogs (Kupferberg, 1997).

Predation and competition can interact to drive trait changes in multiple species (Schmitz *et al.*, 2004; Ohgushi, Schmitz & Holt, 2012). This interaction suggests that reported extirpation and species introductions in urban streams can also result in a cascading series of direct and indirect trait changes in multiple consumers. Although the effects of species removal or addition on intraspecific traits in urban streams have not been studied, urban stream food webs are typically highly altered (Warren *et al.*, 2006; Yule *et al.*, 2015a), increasing the potential of intraspecific trait responses (black bold arrows Fig. 2.1).

2.3.2 Altered hydrography

Urbanization alters stream hydrology mainly through its effects on stormwater runoff (Burns *et al.*, 2015). The large amount of impervious surfaces in cities prevents stormwater from percolating into the soil. This increases the volume of stormwater that runs off to streams. Runoff water can alter base flow and increase the frequency and intensity of high flow events (Walsh *et al.*, 2005; Luthy *et al.*, 2015). This effect is exacerbated by the stormwater drainage system, which concentrates and directly discharges stormwater runoff into streams (Walsh, Fletcher & Burns, 2012). Urban stream hydrology can be further altered by channelization in which stream channels are often straightened, deepened and lined with concrete (Paul & Meyer, 2001).

Variation in hydrology has been shown to drive intraspecific trait change in many taxa in non-urban systems. High flow increases egg size in *Cyprinella venusta* (Machado, Heins & Bart, 2002). Changes in streamflow can also affect morphological traits in both snails and fish (Franssen *et al.*, 2013; Gustafson *et al.*, 2014). In urban streams, measuring how intraspecific traits respond to differences in the flow regime can help us understand how organisms cope with the variability in hydrology (Blanck & Lamouroux, 2007; Mims & Olden, 2013). For example, Nelson, Atzori and Gastrich (2015) have shown that flashier hydrology boosts the swimming performance of the blacknose dace, *Rhinichthys atratulus* in urban settings. Shifts in swimming performance can be a mechanism that allows the biota to survive the extreme flows and flash floods in urban streams (black dashed arrows Fig. 2.1).

2.3.3 Elevated nutrient concentrations

The urban environment is a major source of nutrients to streams. Roads, lawns and landfills are sources of both dissolved inorganic nitrogen (N) and inorganic phosphorus (P) that are washed into urban streams by stormwater runoff (Carey *et al.*, 2013). This effect is exacerbated by stormwater drainage systems that quickly deliver large volumes of stormwater runoff to streams (Bernhardt *et al.*, 2008; Walsh *et al.*, 2012). The N and P found in stormwater can have various origins ranging from pet waste to household fertilizers (Carey *et al.*, 2013). Wastewater can also be a major source of N and P to urban streams. The specific sources vary with economic development and the condition of the sewer system. In developing countries, the lack of sewer infrastructure causes untreated wastewater to be directly delivered to urban streams (Capps, Bentsen & Ramírez, 2016). The use of either combined sewers, leaky septic tanks and pipes, or both, allows untreated wastewater to reach the stormwater system that drains into streams in some old cities (Bernhardt *et al.*, 2008). Outflows from wastewater treatment plants often drain to streams and can also carry high loads of N and P (Carey & Migliaccio, 2009).

The addition of N and P to urban streams increases nutrient concentrations causing eutrophication (Conley *et al.*, 2009). Inorganic N and P are important limiting nutrients governing both primary productivity and the availability and nutritional quality of basal food resources in freshwater systems (Stelzer & Lamberti, 2001; Murdock, Roelke & Gelwick, 2004; Fields & Kociolek, 2015). Food quantity (i.e. food abundance) (Robinson & Parsons, 2002) and food quality (i.e. food nutrient content) (Jonsson, Jonsson & Finstad, 2013) affect the traits of many aquatic taxa in non-urban systems. For example, changes in nutrient concentrations can affect the lipid content of diatoms (Fields & Kociolek, 2015). Food availability affects fecundity and timing of sexual maturity in freshwater snails

(Tamburi & Martín, 2011). It also affects foraging behavior, habitat use, brood size, offspring size, interbrood interval, and morphology in guppies (Reznick & Yang, 1993; Robinson & Wilson, 1995; Kolluru & Grether, 2005). Increased food availability has recently been suggested to relax life history trade-offs and affect sexual traits in many aquatic taxa, including *Daphnia* sp. and fish (Snell-Rood *et al.*, 2015).

Food quality can affect growth and time of maturity in amphipods (Delong, Summers & Thorp, 1993) and fish (Jonsson *et al.*, 2013), and morphology in *Spea* sp. tadpoles (Pfennig, 1990) and cichlids (Muschick *et al.*, 2011). For species that heavily depend on body coloration for mating (e.g. guppies), the quality of food may also restrict the expression of color pigments, therefore, affecting individual reproductive success (Grether & Kolluru, 2011).

Studies from urban streams confirm that altered food availability and quality can produce intraspecific changes in feeding strategy, morphology, and life history. For example, Tófoli et al. (2013) have suggested that altered prey diversity induces a generalist feeding strategy on the urban catfish *Imparfinis mirini.* Mutchler, Ensign and Yates (2014) have proposed that altered food availability in urban streams changes gut morphology of the stoneroller, *Campostoma oligolepis*. Filgueira et al. (2016) suggest that increased food availability changes body size of the central mud minnow, *Umbra limi*. Therefore, intraspecific changes in trophic traits are likely ubiquitous in urban streams, and are likely important for explaining patterns of persistence and extirpation (grey solid arrows, Fig. 2.1).

2.3.4 Contaminants

Cities are major sources of water contaminants, defined here as chemicals that can cause sublethal effects on aquatic organisms. Contaminants such as heavy metals, pesticides, and road salt are washed from lawns and roads by stormwater runoff and delivered to urban streams mainly through the stormwater drainage system (Kim *et al.*, 2005; Weston, Holmes & Lydy, 2009; Gardner & Royer, 2010; Zgheib, Moilleron & Chebbo, 2012). Contaminants such as pharmaceuticals are found in wastewater, which is directly discharged into urban streams when sewage systems are unavailable (Thomas *et al.*, 2014). Such contaminants can also reach streams in cities where faulty or combined sewers allow wastewater to enter the stormwater drainage system (Panasiuk *et al.*, 2015). Outflows from wastewater treatment plants often drain into urban streams and can also carry high concentrations of pharmaceuticals (Batt, Bruce & Aga, 2006).

Contaminants such as heavy metals, pesticides, and road salt are known to lead to intraspecific trait changes in non-urban stream biota. For example, cadmium and copper impair growth and reproduction in *Daphnia magna* (Knops, Altenburger & Segner, 2001). The Poeciliid fish, *Gambusia affinis,* has lower reproductive investment and smaller male size in sites affected by lead-zinc mining effluent (Franssen, 2009). Laboratory experiments suggest that pesticides reduce the growth of the midge larvae, *Chironomus javanus* (Somparn, Iwai & Noller, 2017). High salt concentrations can potentially indirectly affect intraspecific traits by altering biotic richness (e.g. excluding salt intolerant species, such as salamanders (*Ambystoma maculatum*) or frogs (*Rana sylvatica*) (Collins & Russell, 2009).

Pharmaceuticals such as sterols, caffeine, antidepressants, antibiotics, environmental estrogens, and, in some cases, cocaine compounds have all been reported in urban streams

(Kolpin *et al.*, 2002, 2004; Thomas *et al.*, 2014). This problem is exacerbated by increases in flow variability (Kolpin *et al.*, 2004). Evidence from non-urban streams suggest these chemicals can cause trait changes. Norfluoxetine, a residue from antidepressant (Prozac®), induces spawning in *Dreissena polymorpha* bivalves (Fong & Molnar, 2008), while plant sterols can lead to masculinization of female Poeciliid fish (Bortone & Davis, 1994). In urban streams, estrogens from wastewater effluents cause intersexualization of white suckers, *Catostomus commersoni*, and demasculinization in fathead minnows, *Pimephales promelas* (Woodling *et al.*, 2006; Vajda *et al.*, 2011). Changes in traits related to reproduction are likely to disrupt population dynamics and affect species persistence in urban streams (Hutchings *et al.*, 2012) (grey doted arrows, Fig. 2.1).

2.3.5 Interacting agents of trait change in urban streams

I have thus far outlined how individual stressors can influence the intraspecific traits of stream biota. It is important to note that stressors can interact and be confounded (Craig *et al.*, 2017). For example, urban stormwater runoff and associated stormwater drainage network is an important source of stress to urban streams (Walsh *et al.*, 2012). Stormwater input not only changes hydrology but can also contribute to thermal stress, change turbidity, and increased nutrient and contaminant concentrations. Each of these additional stressors are known to produce changes in traits such as life history patterns (Robinson, Reed & Minshall, 1992; Seehausen, Alphen & Witte, 1997; Mladenka & Minshall, 2001; Engström-Öst & Candolin, 2007; Somparn *et al.*, 2017). Also, to cope with added water volume, the morphology of urban stream channels is often altered. Channel modification affects hydrology and reduces species richness through simplification and homogenization of habitats (Paul & Meyer, 2001; Walsh *et al.*, 2005). Both changes in hydrology and species loss can lead to changes in species morphological traits (Pfennig & Murphy, 2002; Franssen *et al.*, 2013; Gustafson *et al.*, 2014). In addition, riparian deforestation occurs in association with urbanization (Paul & Meyer, 2001; Walsh *et al.*, 2005). Loss of riparian vegetation increases instream temperature and light incidence, which can affect algal traits (Butterwick, Heaney & Talling, 2005). Interacting stressors can have synergistic, antagonistic, and additive effects on traits (Coors & De Meester, 2008). However, such effects have not yet been assessed in urban stream organisms.

2.4 Application of trait-based approaches to urban streams

By viewing the components of the USS as drivers of trait change, I have demonstrated that there is a large potential for intraspecific trait changes to occur in urban streams (Fig. 2.1). It is therefore likely that an ‘urban phenotype’ emerges as a response to urbanization across a wide range of aquatic taxa (Alberti, 2015). These trait changes can be either plastic or heritable and have either ecological or evolutionary consequences. Yet, to my knowledge there are few studies on how intraspecific traits of stream organisms change in response to urbanization, which I have highlighted in the previous section (Woodling *et al.*, 2006; Chaves *et al.*, 2011; Tófoli *et al.*, 2013; Mutchler *et al.*, 2014; Nelson *et al.*, 2015; Filgueira *et al.*, 2016; Murphy *et al.*, 2016). While these studies describe intraspecific trait changes that appear to be caused by some of the urban stream selective agents described here, many questions remain regarding the mechanisms responsible for these shifts. Answering these questions can benefit from an intraspecific trait perspective in urban streams.

2.4.1 Why do some species persist in urban ecosystems?

Urbanization is an important driver of species decline globally (Aronson *et al.*, 2014). Despite significant loss, some species are able to persist in urban settings (McKinney & Lockwood, 1999; Shochat *et al.*, 2010). Characterizing how urbanization affects intraspecific traits can help us understand mechanisms promoting species persistence in urban streams. For instance, increased sprint and endurance swimming can ensure survival and persistence of fish in flashy urban streams (Nelson *et al.*, 2015). Plasticity in feeding strategy traits can facilitate survival under the altered food availability conditions of urban streams (Tófoli *et al.*, 2013). Changes in life history traits can increase fitness of urban stream species (Filgueira *et al.*, 2016; Murphy *et al.*, 2016). For example, Murphy *et al.* (2016) have suggested that high temperature and high food availability increases the body size of salamanders, which potentially increases their survival and subsequently their fitness in urban streams.

Investigating intraspecific trait change can further help understand the success of invasive species in urban streams. Urbanization increases the occurrence of invasive species in aquatic ecosystems (Havel *et al.*, 2015). Intraspecific trait plasticity of invasive species allows them to take advantage of the urban environments (Davidson, Jennions & Nicotra, 2011). For example, a global meta-analysis suggests the persistence and proliferation of invasive species in novel aquatic systems is related to traits that enhance food consumption and growth rate (McKnight *et al.*, 2017).

2.4.2 Can we better understand patterns of biodiversity in urban streams?

Explaining observed biodiversity patterns is a central goal in urban ecology because this information can facilitate the management and conservation of species in cities (McDonnell & Hahs, 2013). Interspecific trait-based approaches have been commonly used to understand how biodiversity is influenced by urbanization (Evans *et al.*, 2011; Twardochleb & Olden, 2016) (Appendix A, Table A2). Such studies typically use average trait data published in the literature to examine trait similarities among species in different assemblages. However, the importance of including intraspecific trait information to clarify mechanisms determining community structure and biodiversity is increasingly recognized (Bolnick *et al.*, 2011; Violle *et al.*, 2012). Including intraspecific information in urban stream studies can clarify links between urbanization, species traits, and ecological interactions that shape community structure, allowing us to better understand the response of biodiversity to urbanization (Bolnick *et al.*, 2011; Verberk *et al.*, 2013; Brans *et al.*, 2017).

2.4.3 Can urbanization cause evolution?

Characterizing intraspecific trait responses allow us to determine if and how urbanization can cause evolution, and whether evolution plays an important role in explaining biodiversity patterns of urban ecosystems. Evidence suggests that urbanization has a great potential to drive contemporary evolution, and that rapid evolution can be fundamental to prevent species extirpation in rapidly changing environments (Gonzalez *et al.*, 2012; Alberti, 2015; Donihue & Lambert, 2015; Johnson & Munshi-South, 2017). However, to empirically demonstrate that urbanization causes evolution requires establishing a direct causal link between the urban impact on a population, changes in trait distribution, and genetic divergence (Bull & Maron, 2016). Recent studies from terrestrial ecosystems are already on this path. For example, Winchell et al. (2016) suggest that large and smooth human-made surfaces, such as concrete, led Anolis lizards to evolve longer limbs and more subdigital scales, which improve clinging ability in urban environments.

In addition to clarifying evolutionary mechanisms, studying urban systems can help us advance existing evolutionary theory because constraints and trade-offs shaping evolution in urban systems might differ from those in natural ecosystems and current theory might not always be applicable. For instance, classic theory on life history evolution assumes that nutritional resources are limited, and therefore life history evolution is mainly shaped by nutritional trade-offs between somatic growth and reproductive investment (Roff, 1992; Stearns, 1992). However, urban streams are eutrophic and resource rich (Paul & Meyer, 2001; Meyer, Paul & Taulbee, 2005). Increased resource availability might facilitate the consumption of highly nutritious food, which in turn can decouple life history trade-offs between somatic growth and reproductive investment (Snell-Rood *et al.*, 2015). Research on evolution in urban landscapes can benefit from existing approaches such as breeding experiments and genomic sequencing tools to link urban trait change to trait heritability (Donihue & Lambert, 2015; Messer, Ellner & Hairston, 2016).

2.4.4 Does the functional role of an organism change in an urban environment?

Intraspecific trait changes can alter the role of organisms in the ecosystem. Plastic changes in behavior or physiology can induce non-consumptive trophic cascades (Ohgushi *et al.*, 2012; Trussell & Schmitz, 2012). Evolutionary divergence in life history traits can change many ecosystem parameters such as nutrient recycling, primary production, and leaf litter decomposition (Bassar *et al.*, 2010, 2012; El-Sabaawi *et al.*, 2015). These ecosystem changes can further alter organism's traits (i.e. eco-evolutionary feedback) (Post & Palkovacs, 2009), and it has recently been suggested that urban-mediated intraspecific trait changes can cause such eco-evolutionary feedbacks (Alberti, 2015; Alberti *et al.*, 2017b). However, to empirically demonstrate the existence of these feedbacks, future studies need to couple field observations with both empirical tools such as common garden experiments and conceptual frameworks that link trait changes to their ecosystem consequences (Travis *et al.*, 2014; Jeyasingh, Cothran & Tobler, 2014).

2.4.5 Can we improve biomonitoring approaches in urban streams?

Trait-based approaches are commonly used in biomonitoring assessments. Such approaches often rely on mean trait values calculated across species to infer ecosystem integrity (Zuellig & Schmidt, 2012; Nichols, Hubbart & Poulton, 2016). However, within species variation in traits can be significant, and overlooking this aspect may increase error in biomonitoring assessments. For example, measuring individual body size increases accuracy of size structure estimates which are an important tool for assessing stream integrity in some situations (Orlofske & Baird, 2014). Such improvements can be especially important in monitoring restored urban ecosystems, because biotic assessments are commonly used to infer effectiveness of urban stream restoration practices (Stranko, Hilderbrand & Palmer, 2012; Bain *et al.*, 2014).

2.5 Future challenges

When characterizing intraspecific trait variability in urban systems, researchers need to choose which traits to focus on. In general, the choice of traits is an important and often controversial issue in all trait-based approaches (Violle *et al.*, 2007). In urban streams, focusing on traits that affect fitness (e.g. body size, growth, fecundity) can be useful for studying questions related to persistence and evolution, while characterizing traits that affect ecosystem function (as defined by Matthews et al. (2011), examples include trophic and nutrient processing traits) can be more useful for studying questions relating to trait-mediated ecosystem effects (Fig. 2.1).

A major challenge when identifying mechanisms that shape the ecology of stream organisms is to isolate agents of trait change. Urban impacts interact and are confounded in streams (Wenger *et al.*, 2009). Multiple agents of stress can lead to additive, synergistic, or antagonistic effects in traits which can impair our ability to link specific impacts to changes in traits (Schinegger *et al.*, 2016). This challenge could be overcome by combining field research with data modeling and common garden experiments to identify potentially important agents of trait change, test for the effect of specific agents on changes in traits, and assess the strengths of their interactions. For example, in a series of experiments, Coors and De Meester (2008) were able to untangle the additive, synergistic, and antagonistic effects of predators, parasitism, and pesticides on life history traits of *Daphnia magna.*

It is also important to determine the agents of selection in urban streams as they will differ from terrestrial systems. Detailed research on how urbanization affects intraspecific traits comes mostly from terrestrial systems. However, terrestrial and aquatic ecosystems are likely to respond to urbanization differently, and it is unclear whether we can extrapolate from one to the other. In terrestrial systems, urbanization is predicted to decrease natural variation in temperature, water, and food availability, which will produce a more stable, uniform environment (Shochat *et al.*, 2006; Alberti, 2015). Conversely, urbanization along streams increases the frequency and magnitude of flood events, as well as episodes of nutrient and contaminant addition, which result in a more unstable and unpredictable environment (Walsh *et al.*, 2005; Somers *et al.*, 2013). Therefore, the types of selective pressure in urban streams likely differ from terrestrial urban ecosystems, and an intraspecific approach can highlight differences and generalities between and across the systems, leading to a broader understanding of how urbanization affects communities and ecosystems.

2.6 Conclusion

With the expansion of cities, there is a growing need to understand the mechanisms through which urbanization affects the biota (McDonnell & Hahs, 2013; McPhearson *et al.*, 2016). Here I have identified potential agents of trait change in urban streams and have proposed that intraspecific trait-based approaches can reveal mechanisms of change in these altered ecosystems. This approach should provide important insights into the processes through which organisms respond and adapt to impacts caused by urban development, allowing us to better assess and predict the effects of urbanization to stream ecosystems.

2.7 Coauthor contributions

Rana El-Sabaawi[1] helped conceiving the ideas and writing the manuscript;

Luisa R. Manna[2] and Rosana Mazzoni[2] contributed with the writing of the manuscript.

[1] University of Victoria, Victoria BC, Canada;

[2] Universidade do Estado do Rio de Janeiro, Rio de Janeiro RJ, Brazil

Chapter 3 - How urbanization increases the invasive potential of an introduced species

3.1 Abstract

Urbanization is a global process that converts the land into a built landscape, causing changes in habitat and ecological processes with consequences for biodiversity. Alien species often persist and become invasive in urban ecosystems. How and why invaders succeed is an important yet poorly understood question, especially in urban stream ecosystems. In this chapter I investigate how urbanization adjacent to streams affects the invasive potential of the guppy, *Poecilia reticulata*, a widespread alien species. I begin by characterizing the effect of urbanization on streams in Rio de Janeiro. Then I assess how traits that are related to the potential of guppies to invade (i.e. life history traits and condition) respond to urbanization. Next, I explore how urbanization affects the availability of food for guppies and their diets. I also test if biodiversity (i.e. the presence of other fish species) dampens guppy invasive potential. I find that urban streams have high concentrations of ammonium and fecal coliforms, which indicates contamination with sewage. Guppy populations in urban streams have 26x higher density (and larger body sizes than non-urban populations). Urban guppies have on average 5 more offspring and are in better condition than non-urban guppies. Urbanization increases the availability and consumption of chironomids by guppies. The presence of other fish species has a negative but small effect on the guppy traits and density. I find a positive relationship between the consumption of chironomids and both fecundity and condition. Overall my data suggest a relaxation of the trade-offs that shape life history traits caused by an increase of food

resources in urban streams. My findings also suggest that urbanization enhances the invasiveness of guppies by enhancing reproduction and somatic investment.

3.2 Introduction

The human population is urbanizing rapidly, and by 2050, about 66% of the world population is expected to be urban (United Nations, 2014). Urban centers are therefore expanding towards adjacent non-urban systems and transforming them in dramatic ways. Urbanization replaces green spaces with impervious surfaces, increasing pollution, and changing biodiversity patterns: sensitive taxa decline, while tolerant taxa persist (Walsh *et al.*, 2012; Aronson *et al.*, 2014). Understanding the mechanisms that drive these changes in biodiversity is a major goal of urban ecology (McDonnell & Hahs, 2013).

The movement of people and goods between cities provides opportunities for organisms to be transported away from their native range, and introduced into new ecosystems (Wilson *et al.*, 2009; Essl *et al.*, 2011). For example, plants have been commonly introduced in urban areas for horticultural purposes, while invertebrates and vertebrates have been accidentally introduced as stowaways on ships or from the pet trade (Padayachee *et al.*, 2017). As a result, urban ecosystems contain a large number of alien species, defined as non-native species whose presence in a region results from human actions (*sensu* Richardson, Pysek, & Carlton, 2011) (McKinney, 2006; Lososová *et al.*, 2016).

Alien species become invasive when they maintain large self-replacing populations over many life cycles far from the site of introduction (Richardson *et al.*, 2011). Invasive species typically reach very high densities in urban environments, with a range of negative consequences for humans and native organisms. For example, feral pigeons (*Columba* *livia*) and black rats (*Rattus rattus*) are found in high densities in many urban areas around the globe, which raises concerns because they carry multiple human pathogens, contaminate food and damage urban architecture (Haag-Wackernagell & Moch, 2004; Feng & Himsworth, 2014; MacGregor-Fors & Escobar-Ibáñez, 2017). Invasive species can also compete with native fauna for food and resources (Shocat et al., 2010). The diversity of native competitors and predators can provide protection against invasion but evidence for this protective effect in urban systems is rare (Yamanishi *et al.*, 2012; Bjørn *et al.*, 2019).

Although the occurrence of invasive species in urban areas has been reported for many decades, the ecology of such invasions is not clear. Specifically, we do not understand the mechanisms facilitating invasion in urban areas (Cadotte *et al.*, 2017; Cavin & Kull, 2017; Gaertner *et al.*, 2017). This is especially true for systems that have traditionally received less attention from urban ecology researchers, such as aquatic ecosystems in general (El-Sabaawi, 2018), and stream ecosystems in particular (Marques *et al.*, 2019). Urbanization causes profound modifications to stream ecosystems, mainly because of the input of runoff and wastewater that alters stream flow and increases the concentration of nutrients and contaminants (Walsh *et al.*, 2005, 2012). Invasive species proliferate under such conditions (Havel *et al.*, 2015), but it is not clear why invasive species succeed in urban stream systems.

Changes in traits related to life history affect population dynamics which is important for the persistence of invasive species (Capellini *et al.*, 2015; Allen, Street & Capellini, 2017). For example, goldfish (*Carassius auratus*) spawn earlier when occurring in non-native environments which can extend their growing season and facilitate their population persistence (Liu *et al.*, 2015). Changes to life history traits can be either plastic (non-heritable) or evolutionary (heritable), and both can facilitate proliferation in urban systems (Alberti *et al.*, 2017b).

Urbanization can affect life history traits in a wide range of species (Vaugoyeau *et al.*, 2016; Brans & Meester, 2018), but its effects on invasive species traits in general, or on aquatic organisms in particular, are unclear (Cadotte *et al.*, 2017; Gaertner *et al.*, 2017), but highly likely to occur (Chapter 2; Marques *et al.*, 2019). Current life history theory proposes that life history traits are shaped in part by nutritional trade-offs because organisms have limited nutritional resources to allocate into multiple tasks such as somatic growth, reproduction, and navigating biotic interactions (i.e. competition and predator avoidance) (Roff, 1992; Stearns, 1992). However, urbanization has the potential to increase the availability of food resources in many ecosystems (El-Sabaawi, 2018). For example, lawn fertilizers and sewage can provide dissolved nutrients (N and P) that increase productivity and thus the availability of food resources at the base of the food web (Singer & Battin, 2007; Carey *et al.*, 2013). This high food availability has the potential to relax the nutritional tradeoffs that shape life history traits in human impacted systems, allowing organisms to increase their investment in multiple life history processes simultaneously (Snell-Rood *et al.*, 2015), but empirical tests of this hypotheses are lacking in urban ecosystems.

In this study I characterize population density, size structure, life history traits and condition of the invasive guppy, *Poecilia reticulata*, in urban ecosystem in order to better understand the success of invasive species in urban streams. The native range of guppies is the Caribbean and the northern region of South America (Magurran, 2005). However, guppies were introduced worldwide by the aquarium trade or for the biological control of mosquito borne diseases. Guppies are now recognized as a globally invasive species, with established populations in at least 69 countries worldwide (Deacon *et al.*, 2011). Guppies have viable populations and attain high density in many urban ecosystems, suggesting that they are successful urban dwellers (Cunico, Allan & Agostinho, 2011; Fischer *et al.*, 2015). Invasive guppies can introduce parasites, threaten native fish populations and alter ecosystem processes (Valero, Garcia & Magurran, 2008; Bassar *et al.*, 2010; Tadiri, Dargent & Scott, 2013).

Guppies are also a model species for studying the evolution of life history traits (Reznick & Endler, 1982; Magurran, 2005; Travis *et al.*, 2014). A well-documented tradeoff between growth and reproduction is observed in native guppy population of Trinidad in response to predator-induced mortality and food availability (Gordon, Hendry, & Reznick, 2017; Reznick, 1983). Most studies on guppies are restricted to their native range, particularly in Trinidad, and it is not clear how such traits facilitate guppy invasion in non-native ecosystems. Specifically, we do not know how guppy traits respond to anthropogenic landscape changes, or if the responses of their traits facilitate success in disturbed ecosystems.

My goal in this chapter is to use an intraspecific trait approach (Chapter 2) to assess how urbanization affects the potential of guppies to invade ecosystems (i.e. invasiveness *sensu* Richardson et al., 2011) and to describe the mechanisms allowing the invasion success of guppies in urban systems. I have selected urban and non-urban streams in Rio de Janeiro, Brazil. The first record of guppies in Rio de Janeiro dates to 1942, when specimens were catalogued by the National Museum (MNRJ 3646). Currently, guppies have invaded many brackish and freshwater systems in Rio de Janeiro (Rocha, Bergallo & Mazzoni, 2011). Because biodiversity (i.e. the presence of competitors and predators) can also affect life history, within each treatment (urban, non-urban), I have selected streams where guppies are found on their own or with other fish species, including competitors and predators. I begin by comparing environmental metrics in order to confirm that these streams suffer from the "Urban Stream Syndrome", including degraded water quality and increased contamination (Walsh *et al.*, 2005).

My first objective is to assess guppy success and invasiveness in urban streams. I compare density and size structure to test the hypothesis that urbanization increases the success of guppy populations. I predict that urban guppy populations are more successful by attaining higher density and increased body sizes than non-urban populations (Schröder *et al.*, 2009; Shochat *et al.*, 2010; Møller *et al.*, 2012). I also compare life history traits of urban and non-urban guppies. I focus on such traits because they affect invasiveness by facilitating dispersal, production of offspring and the maintenance of viable populations out of the site of first introduction (Richardson *et al.*, 2011). I hypothesize that urbanization increases guppy invasiveness by enhancing traits related to reproduction and somatic growth.

Then, I explore potential mechanisms of change to guppy life history traits. I test the hypotheses that urbanization increases food availability and the consumption of nutritious food by guppies. Urbanization is expected to increase the biomass of invertebrates in urban streams, particularly that of tolerant taxa such as Chironomidae (Paul & Meyer, 2001; Yule *et al.*, 2015b). Chironomids are highly nutritious (have high nitrogen concentration per body mass) and have been shown to be preferred by guppies in their native range (Zandonà *et al.*, 2011). The consumption of nutritious food has been suggested to affect guppy life history traits in their native range (Reznick, 1983; Zandonà *et al.*, 2011). I expect that increased consumption of chironomids enhances reproduction and somatic growth in urban environments. I predict that improved nutrition in urban streams allows guppies to invest simultaneously towards somatic growth and reproduction, providing evidence for a relaxation of life history tradeoffs.

Finally, I investigate whether fish biodiversity (the presence of potential fish predators and competitors in the stream) dampens guppy success and invasiveness by decreasing guppy density, body size, investment in reproduction and condition. Biodiversity can affect invasive species by increasing competition and predation (Kennedy *et al.*, 2002; Yamanishi *et al.*, 2012). I therefore predict that the presence of competitors and predators will reduce the success and invasiveness of guppies.

3.3 Materials and methods

My study took place in Rio de Janeiro, Brazil, where urban streams receive direct sewage discharges from the nearby households, while non-urban streams are found within parks and forest reserves and receive no direct input of sewage. I have selected 6 urban stream reaches and 6 non-urban stream reaches, with the selection of urban streams based on the presence of development and evident direct sewage input. Most reaches are located in small first to second order streams in different watersheds. Two urban reaches are located downstream of the non-urban reaches in the same stream (Eldo Sujo and Water Planed Sujo reaches). In such cases, reaches are separated by natural and artificial barriers forming waterfalls which prevent the upstream movement of fish. Each reach is 30 meters in length. Within each treatment, half the sites have guppies as the only fish, while in the other half guppies co-occurred with potential competitors and predators (Appendix B, Table B1). In reaches where guppies occurred with other fish, the total number of fish species ranges from 3-10, and each site has at least one potential predator, the catfish *Rhamdia quelen* (Bonato, Delariva & Silva, 2012) and one potential omnivorous competitor such as the poecilid *Phalloceros* sp. or the pearl cichlid *Geophagus brasiliensis*. Reaches where guppies co-occur with other fish species (GF) are considered to have increased competition and predation pressure. While reaches where guppies are the only fish species (GO) are considered to have reduced competition and predation pressure. Whenever possible I have replicated the sampling in two years (2016-2017), and in total there are 20 sampling events.

3.3.1 Environmental variables

At each reach, I measured pH, conductivity (Spec µS/cm), temperature (^{o}C), dissolved oxygen (mg/L) and % canopy. Ammonium (NH_4) is an important limiting nutrient in aquatic ecosystems and is often elevated in urban streams (Walsh *et al.*, 2005, 2012), where it can originate from various sources including sewage (Bernhardt *et al.*, 2008). I collected and filtered five water samples along each reach and analyzed them for NH_4 concentration (µg/L) using a fluorometric method (Holmes *et al.*, 1999). I also took a composite sample, composed of three subsamples, of water to estimate fecal coliforms (*Escherichia coli* counts, FC). E. *coli* is commonly used to indicate the contamination of water with human feces (Edberg *et al.*, 2000). The samples were refrigerated and sent (within 24 hours of collection) to a private analytical laboratory where the most probable number of E. *coli* cells (MPN/100mL) was estimated following a dilution method (Rice *et al.*, 2012).

3.3.2 Population density, size structure and fish biodiversity

I estimated guppy density following a depletion method (Carle & Strub, 1978). Each stream reach (~30m in length) was blocked with fine mesh nets and electrofished 3 consecutive times using a backpack electrofisher (LR-24 Smith-Root® Vancouver WA, USA). There was a decreasing number of guppies captured at each fishing event. The difference in the number of guppies captured (both male and females) in each of the 3 fishing events allowed me to estimate guppy density (GD, ind/m^2), using the *FSA* package for *R* (Ogle, 2015; R Core Team, 2018). A total of 3224 female guppies (411±84 urban and 126±39 non-urban individuals per reach) were either measured for standard length (SL, mm) and returned to the stream or euthanized, fixed in formalin (10%) and brought to the lab for standard length measurements and further analyses. Guppies were collected and euthanized following protocols approved by the University of Victoria (2016-008) and the State University of Rio de Janeiro (UERJ CEUA/005/2016) animal care committees, as well as the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA 16152-1). I used body length to build cumulative length distributions in order to determine the size class distribution of the guppies in each site (Neumann & Allen, 2007). All co-occurring fish species were identified and counted to estimate fish biodiversity.

3.3.3 Guppy reproductive traits and condition

From the euthanized female guppies, a total of 494 urban (82±12 per reach) and 241 non-urban (40±12 per reach) were used for assessing life history traits. Female guppies are viviparous and produce a new brood every 3-4 weeks (Reznick & Endler, 1982; Magurran, 2005). Therefore, I used only mature females (having mature eggs or embryos) for the life history study. Mature eggs can be distinguished from immature eggs by size and color (Haynes, 1995). For each female I estimated three reproduction-related traits: fecundity measured as the number of offspring (number of embryos and mature eggs, NO), the gonad weight (dry weight, mg), and reproductive allotment (gonad dry weight / total body dry weight minus gonads). I assessed the condition (CO) of each female using the hepatosomatic index (HSI) which was calculated as the ratio of liver dry weight (mg) and eviscerated body dry weight (mg). This provided an estimate of the amount of stored nutrients and health of the guppies (Lloret, Shulman & Love, 2014).

3.3.4 Invertebrate availability

In each reach three randomly placed samples of benthic invertebrates were collected using a surber sampler (area=$0.09m^2$). For each sample, invertebrate families were identified, measured (mm) and mass-length regressions from the literature were used to estimate invertebrate biomass (dry mass mg/m^2) (Benke *et al.*, 1999). Since not all invertebrates can be consumed by guppies, I only considered taxa found in guppy gut contents and invertebrates smaller than 6mm (mean size of invertebrates consumed by guppies) to estimate the invertebrate biomass available as food for guppies (IB, mg/m^2). This corresponded to 32% and 26.7% of the total invertebrate biomass in GF and GO urban reaches respectively, and 0.65% and 32% of the total invertebrate biomass in GF and GO non-urban reaches respectively (Appendix B, Fig. B1). Because chironomids are an important food source for guppies (Zandonà *et al.*, 2011), I also reported the biomass of chironomids (CB, mg/m^2).

3.3.5 Diet analysis

From the euthanized female guppies, I retained a total of 116 urban (29±3 per reach) and 120 non-urban (20±3 per reach) for gut content analysis. For each guppy, the foregut was sectioned to the point where the gut turns 180^o and the gut contents were analyzed. I used only the foregut because gut contents in this area are still relatively intact (Zandonà *et al.*, 2015). Gut content analysis was performed using the gridded microscope slide technique (Zandonà *et al.*, 2011). The slide area occupied by invertebrates, algae and detritus (i.e. silt and amorphous material) was estimated (mm^2). I identified the invertebrates to the lowest taxonomic level possible, generally family, using published taxonomic keys (Merritt & Cummins, 1996; Mugnai, Nessimian & Baptista, 2010). Then, I used the total area of the slide occupied to estimate the proportion of invertebrates, algae and detritus consumed, following: $P_i = A_i / A_t$. Where, P_i = proportion of the food item, A_i = area of the food item, A_t = total area occupied by all diet items. The proportion of invertebrates consumed was further divided into proportion of chironomids (PC) and proportion of other invertebrates. Individuals with empty guts were removed from all analysis.

3.3.6 Statistical analyses

3.3.6.1 Comparisons between urban and non-urban systems

I performed a PCA analysis based on the correlation matrix (standardized) of the environmental variables: pH, conductivity (Spec µS/cm), temperature, dissolved oxygen, % canopy, ammonium concentration, fecal coliforms and sampling year (Appendix B, Table B2). This analysis assessed whether urban reaches were more similar to each other than to non-urban reaches, as indicated by how the reaches clustered in the PCA plot. The dimensions that best represent the variation among reaches (PC1 and PC2, %) were estimated and plotted using the function prcomp and the package factoextra for R (Kassambara & Mundt, 2016). I removed one reach with missing data and estimated DO and NH_4 for two reaches based on the mean and treatment (urban or non-urban) (Appendix

B, Table B2). Discharge in each reach was measured only for the 2017 survey and was therefore not included in the PCA, but there were no differences between urban and non-urban reaches (~0.02 avg m/s in both).

I further compared NH_4 concentration, fecal coliforms, invertebrate biomass (all invertebrates (IB) and chironomid (CB)) between urban and non-urban reaches using generalized linear mixed models (GLMMs). I included urbanization (Urban vs. Non-urban), year, and fish biodiversity (GO vs. GF) as fixed factors and reach identity as a random factor. In analyses of NH_4, IB, and CB, I added guppy density as a fixed factor since it can potentially influence these variables through consumption or excretion (Zandonà *et al.*, 2011; El-Sabaawi *et al.*, 2015).

I tested for changes to the average proportion of chironomids in gut contents between urban and non-urban guppies using GLMMs. I also tested for differences in the number of offspring, condition, gonad weight and reproductive allotment. In all models I included urbanization, year, fish biodiversity (GF vs. GO), body length, and guppy density as fixed factors, and reach identity as a random factor.

I used cumulative length distributions to estimate variation in body size between urban and non-urban guppies in GO or GF reaches. I tested for differences in length distribution using a bootstrapped Kolmogorov-Smirnov test (K-S two-sample test) from the *Matching* package for R, which is appropriate for discrete length data (Sekhon, 2011; Ogle, 2017). I also tested for differences in guppy density between urban and non-urban reaches using GLMMs. In this model I included year and fish biodiversity as fixed factors and reach identity as random factor.

I fitted all models with the *lme4* package for R (Bates *et al.*, 2015). I choose the link function based on the best fit for the data. The model fit was evaluated visually using Q-Q plots. I tested the models for collinearity with the VIF function of the *car* package for R (Fox & Weisberg, 2011).

3.3.6.2 Testing the link between chironomid consumption and guppy traits

I used linear mixed models (LMMs) to explore the relationship between the consumption of chironomids and guppy traits across urban and non-urban reaches. I built three separate models using the mean for each trait per reach; number of offspring (NO), condition (CO) and body length (SL), as response variable. I used the proportion of chironomids consumed (PC), sampling year (YR), fish biodiversity (GO vs GF), and guppy density (GD) as fixed factors, and reach identity (RI) as a random factor in all the models. Body length (SL) was included as a fixed factor when modeling condition and number of offspring. The global models for number of offspring, condition and body length were subjected to model selection (Grueber *et al.*, 2011). The selection of the best model was based on corrected Akaike`s information criteria AIC_c. Low AIC_c scores indicate a good model fit, while delta AIC_c values (ΔAIC_c) show the magnitude of the difference between the model with the best fit (ΔAIC_c=0) and alternative models. Only the best models (ΔAIC_c <2) were considered because they have substantial empirical support (Burnham & Anderson, 2002). The best models were retrieved and averaged to estimate the coefficients of the predictor variables (Grueber *et al.*, 2011).

All models were fitted with the *lme4* package for R (Bates *et al.*, 2015). Models were tested for multicollinearity with the VIF function of the *car* package for R (Fox &

Weisberg, 2011). VIF values ~3 suggest low/moderate collinearity (Zuur, Ieno & Elphick, 2010). Whenever applicable, model selection and averaging were performed with the *MuMIn* package for R (Barton, 2018). The variance explained by both fixed plus random factor (conditional R square, R^2_c), and the variance explained only by the fixed factors (marginal R square, R^2_m) were estimated using the "r.squaredGLMM" function of the R package *MuMIn*. I assessed the significance of each fixed factor included in the best model using the likelihood ratio test from the "lrtest" function of the *lmtest* package for R (Hothorn *et al.*, 2018). All plots were built with package *ggplot2* for R and deviations reported are standard errors (Wickham, 2009)

3.4 Results

3.4.1 Environmental variables

The PCA showed that urban reaches were environmentally more similar to each other than non-urban reaches in both years (Fig. 3.1; Appendix B, Table B3). The PCA explained 56% of the total variation among reaches. The PC1, explained 33.5% of the variation and was driven by differences in ammonium, dissolved oxygen and pH between urban and non-urban reaches (Fig. 3.1; Appendix B, Table B3). PC2 explained 23% of the variation and was driven mainly by differences between sampling years (Fig. 3.1; Appendix B, Table B3). However, there was no obvious clustering pattern by year, suggesting that urban effects were consistent between years. The variation in abiotic factors between sites differing in fish biodiversity were also minor (Fig. 3.1).

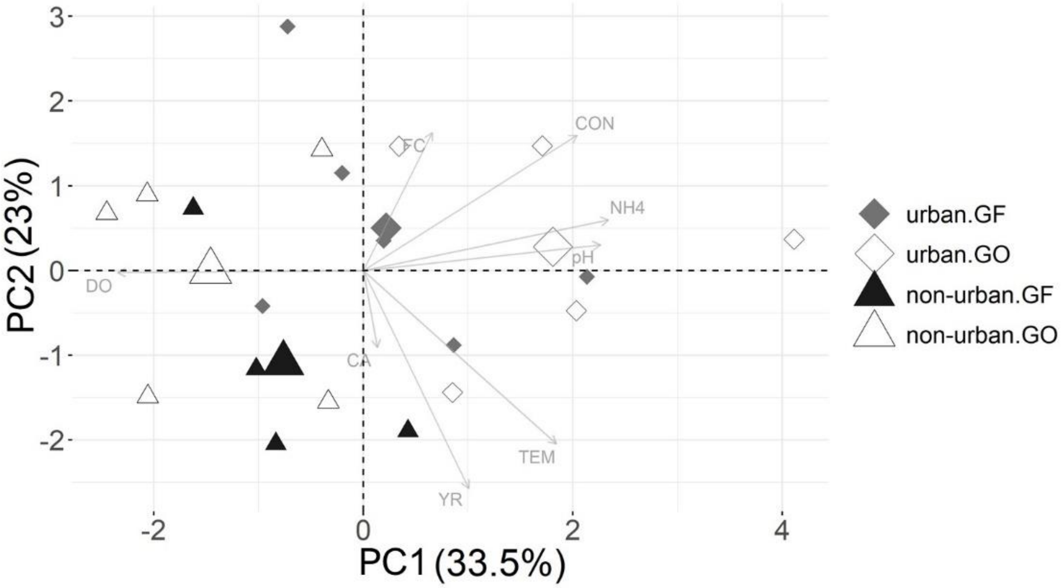

Figure 3. 1. Principal Component Analysis (PCA) showing urban reaches where guppies co-occur with other fish species (increased competition and predation, solid black diamonds, urban.GF), urban reaches where guppies are the only fish species (reduced competition and predation, hollow black diamonds, urban.GO), non-urban reaches where guppies co-occur with other fish species (increased competition and predation, solid grey triangles, non-urban.GF) and non-urban reaches where guppies are the only fish species (reduced competition and predation, hollow grey triangles, non-urban.GO). Large symbols represent the mean for all the reaches in each condition. The analysis is based on the environmental variables: CON=Specific conductivity (Spec µS/cm), TEM=Temperature (ºC), DO= Dissolved oxygen (mg/L), CA=canopy cover (%), FC= fecal coliforms (E. *coli*, MPN/100mL), NH_4= Ammonium concentration (µg/L) and sampling year (YR).

Generalized linear models confirmed urban vs. non-urban differences observed in the PCA. Urban reaches had higher ammonium concentration than non-urban reaches (2756±1150 and 15±3 µg/mL, respectively) (GLMM, t=12.5, p<0.001), with a positive effect of guppy density on ammonium concentration (GLMM, t=3.5, p<0.001) (Appendix B, Table B4). There were higher concentrations of fecal coliform in urban reaches compared to non-urban reaches (197.673±100.650 and 1.801±867 MPN/100mL, respectively) (GLMM, t=8.1, p<0.001), with higher E. *coli* counts in 2016 than 2017 (136.5±129.6 and 76.3±40.6, respectively) (GLMM, t=-2.4, p<0.01) (Appendix B, Table

B4). Otherwise, there were no significant differences between years or in abiotic conditions between sites that differed in fish diversity (Appendix B, Table B4).

3.4.2 Guppy success, invasiveness and the effect of biodiversity

Guppy density was higher in urban than non-urban in reaches (78±34 and 3±1 ind/m^2, respectively) (GLMM, t=6.7, p<0.001), with a lower density in 2016 compared to 2017 (33±16 and 52±35 ind/m^2, respectively) (GLMM, t=2.4, p=0.02) and no effect of biodiversity (Fig. 3.2a; Appendix B, Table B4). The cumulative length frequency distribution showed that urban guppies had larger body lengths than non-urban guppies (K-S test p<0.001). The presence of other fish species slightly reduced guppy body size but did not entirely remove the effect of urbanization (K-S test p<0.01) (Fig. 3.2b).

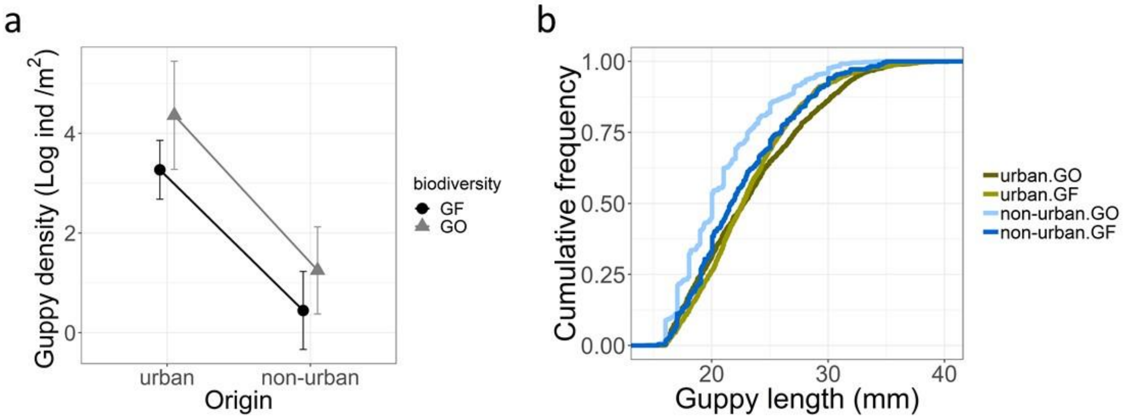

Figure 3. 2. Guppy population metrics. Panel (a) shows the guppy density estimated as mean number of individuals, both males and females, per meter square. Bars represent the standard error of the mean. Black circles indicate reaches where guppies are the only fish species (GO) and grey triangles indicate reaches where guppies co-occur with other fish species (GF). Panel (b) shows the Empirical Cumulative Distribution Function (ECDF) curves for guppy length. Lines represent the proportion of guppies at each length category in urban reaches where guppies occur with other fish (dark grey, urban.GF), urban reaches where guppies are the only fish species (dark grey, urban.GO), non-urban reaches where guppies occur with other fishes (light grey, non-urban.GF) and non-urban reaches where guppies are the only fish species (light grey, non-urban.GO). Data shown combine both sampling years (2016 and 2017).

Urban guppies had more offspring than non-urban guppies (13±0.5 and 8±0.5, respectively) (GLMM, z=2.1, p=0.04) (Fig. 3.3a; Appendix B, Table B5). Although figure 3.3a suggests an effect of biodiversity on fecundity, the GLMM model showed no interaction between the two (Appendix B, Table B5). This is likely an effect of body size which was strongly and positively correlated with fecundity (GLMM, z=49.8, p<0.001), while guppy density caused a small reduction in fecundity (GLMM, z=-6, p<0.001) (Appendix B, Table B5). Although there was a tendency for increased gonad weight and reproductive allotment in urban guppies, there were no statistically significant differences between urban and non-urban guppies, or between reaches that differed in fish diversity (Appendix B, Fig. B3a,b).

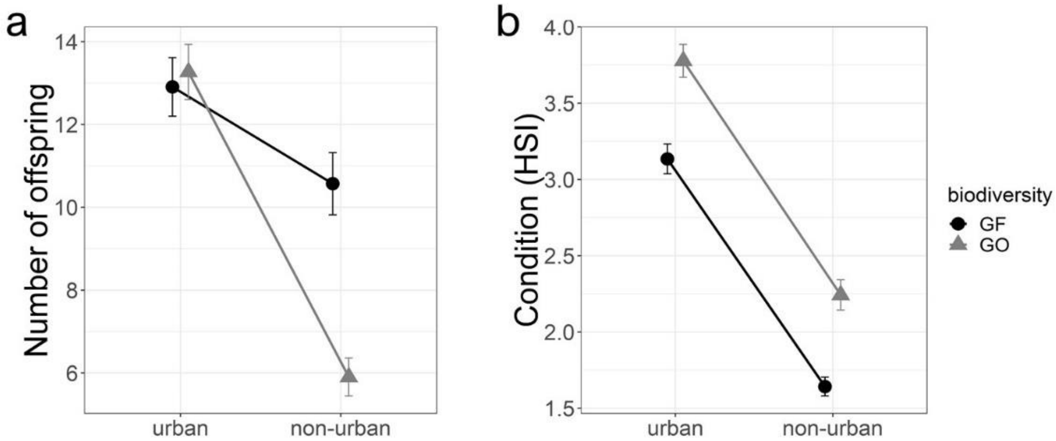

Figure 3. 3. Comparing traits of urban and non-urban guppies. In both conditions guppies occur in stream reaches with other fish species (GF, black circles) and in reaches where guppies are the only fish species (GO, grey triangle). The panels show: (a) the number of offspring, estimated as the mean total counts of embryos and mature eggs for all the females, (b) guppy condition estimated for the female guppies using the mean hepatosomatic index (I). Symbols represent the means and bars are the standard error of the mean. Data shown include two sampling years (2016 and 2017).

Urban guppies had better condition compared to non-urban guppies regardless of biodiversity (3.4±0.1 and 2±0.1 I index, respectively) (Fig.3.3b; Appendix B, Table B5). Overall guppy condition was slightly better in 2016 compared to 2017 (3.3±0.1 and 2.6± 0.1 HSI, respectively) (GLMM, t=-9.4, p<0.001). Reaches with denser guppy populations also had guppies with higher condition, but the effect was small (GLMM, t=4.4, p<0.01) (Appendix B, Table B5).

3.4.3 Mechanism of change to guppy life history traits and the effect of biodiversity

Urbanization increased the availability of food for guppies. The biomass of all invertebrate taxa together was higher in urban reaches compared to non-urban reaches, regardless of fish biodiversity (122±40 and 38±9 mg/m2, respectively) (GLMM, t=2.9, p<0.01) (Fig. 3.4a; Appendix B, Table B4). The biomass of chironomids was also higher in urban reaches than in non-urban reaches, regardless of fish biodiversity (114±40 and 19±5 mg/m2, respectively) (GLMM, t=3.7, p<0.001) (Fig. 3.4a; Appendix B, Table B4). There were no significant differences in the biomass of other invertebrates between urban and non-urban reaches, independent of fish diversity (Fig. 3.4a).

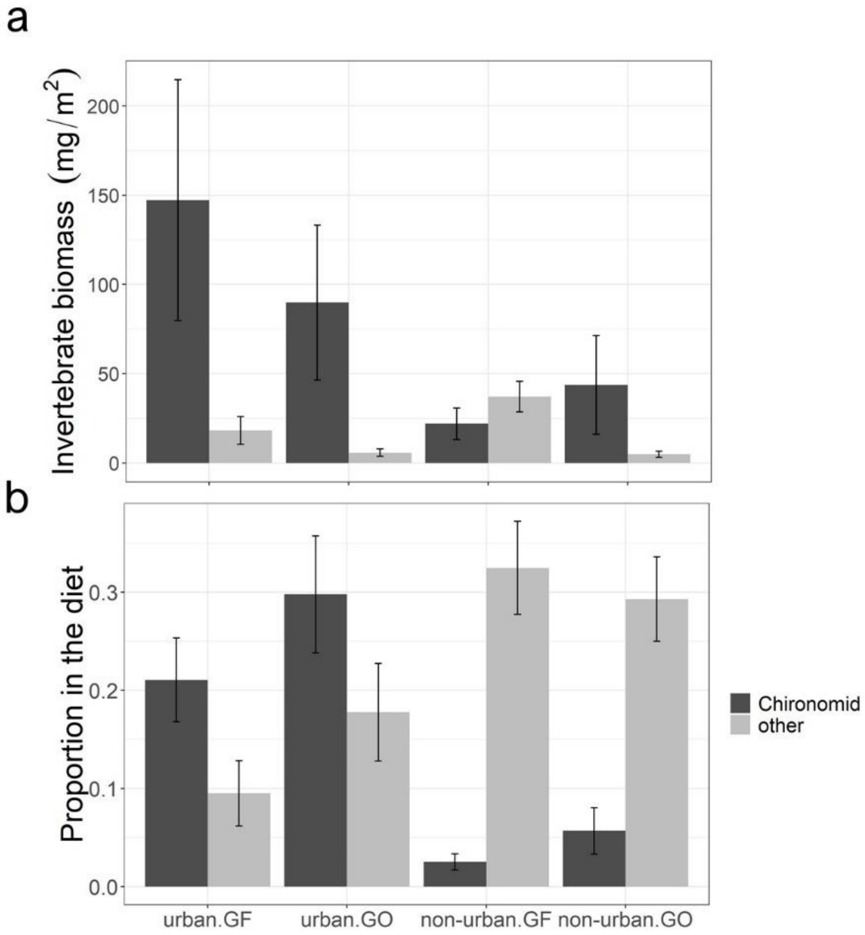

Figure 3. 4. Chironomids (dark grey) and other invertebrates (light grey) measured in the streams as biomass available for consumption (panel a) and found in the guts of female guppies, expressed as proportion of total diet (panel b). The data include samples urban reaches where guppies occur with other fish species (urban.GF), urban reaches where guppies are the only fish species (urban.GO), non-urban reaches where guppies occur with other fish species (non-urban.GF) and non-urban reaches where guppies are the only fish species (non-urban.GO). Bars represent the mean and lines are the standard error of the mean. Data shown includes both sampling years 2016 and 2017.

Guppy diets were dominated by detritus regardless of urbanization or fish diversity (proportion = 0.5 to 0.7 of the gut contents; Appendix B, Fig. B2). Invertebrates were the second most important diet items, with proportion of 0.3-0.5 of the gut contents, with no clear differences between urban or non-urban sites, or with differences in fish diversity (Appendix B, Fig. B2). However, chironomids were consumed in higher proportion in urban reaches than in non-urban reaches, regardless of fish biodiversity (0.25±0.04 and 0.04±0.01, respectively) (GLMM, $z=3.8$, $p<0.001$) (Fig. 3.4b; Appendix B, Table B5). There was a small effect of density which slightly reduced the consumption of chironomids at higher guppy density (GLMM, $z=-2.8$, $p=0.01$) (Appendix B, Table B5). The consumption of other invertebrates was low in 2017 (GLMM, $z=-2.59$, $p<0.01$) but there were no differences between urban and non-urban reaches, independent of fish biodiversity (Fig. 3.4b; Appendix B, Table B5).

A relationship was observed between diet, fecundity and guppy condition. Following model selection, the best LMM models (R^2_m = 71-74%) suggested that the consumption of chironomids had a positive relationship with the number of offspring ($coef_{avg}=19.49$). This model also showed that fecundity increased with body size ($coef_{avg}=1.58$) and declined when other fish species were present ($coef_{avg}=-0.66$) (Fig. 3.5a). Fecundity was elevated in 2017, compared to 2016 ($coef_{avg}=1.18$) (Table 3.1). The best models (R^2_m =24-29%) also suggested that the consumption of chironomids ($coef_{avg}=3.35$) had an overall positive effect on guppy condition, despite a reduction in condition in 2017 (Fig. 3.5b) (Table 3.1). The likelihood ratio test showed that the proportion of chironomid consumed was a significant predictor of fecundity ($\chi^2_{(1)}=17.8$, $p<0.001$) and condition ($\chi^2_{(1)}=3.8$, $p=0.05$). I could not confidently assess the relationship between diet and body length because following model selection, the null model was included as a best model. A table with the full model selection for each LMM model can be found in the supplement (Appendix B, Table B6).

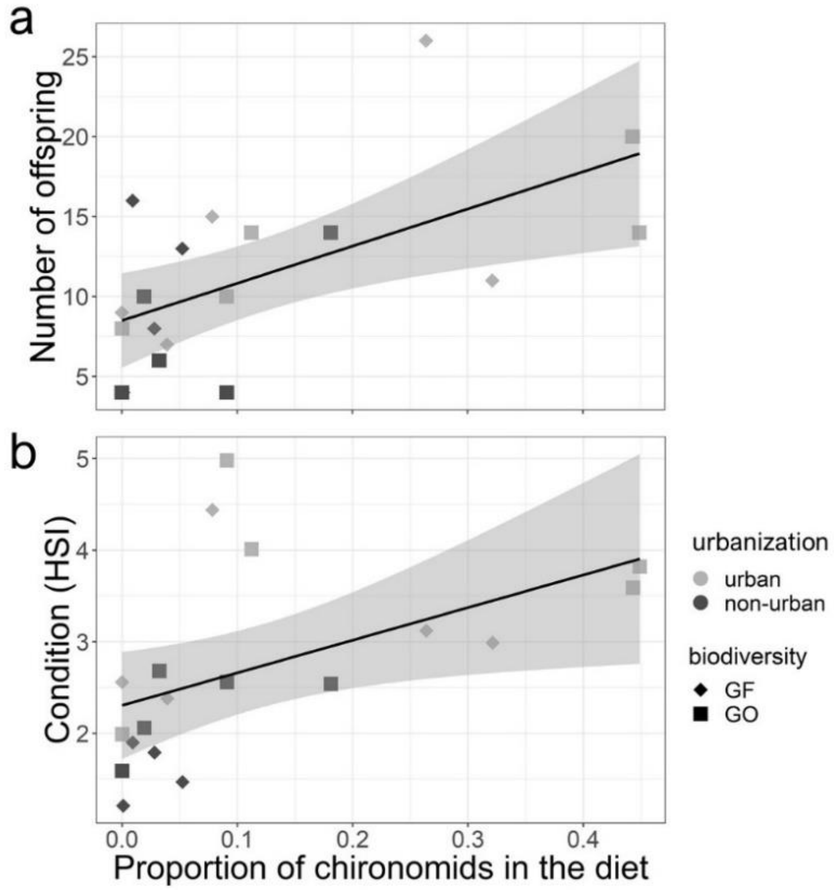

Figure 3. 5. Relationship between diet (proportion of chironomids consumed) and (a) number of offspring, (b) condition. Each symbol represents the average value in urban (grey) and non-urban (black) reaches where guppies co-occur with competitors and predators (high biotic interactions, diamonds) and reaches where guppies are the only fish species (low biotic interactions, squares). Lines represent the linear model fit and shades are confidence intervals. Model results are in Table 4.

Table 3. 1. LMM models testing the relationship between guppy traits and diet. I built separate models using body length (SL, mm), number of offspring (NO) and guppy condition (CO, I) as response variables and sampling year (YR), fish biodiversity (guppy only and guppy and other fish, GO vs GF), body length (SL, mm), guppy density (GD, ind/m^2) and the proportion of chironomids consumed (PC, %) as fixed factors. I used reach identity as a random factor. Following model selection, the coefficients of the best models (ΔAICc <2) were estimated and the averaged coefficients are shown. The R^2_c and R^2_m show the range of conditional and marginal R^2 values for the best models. A table with the full model selection showing all the candidate models can be found in the appendix (Appendix B, Table B6)

			Averaged coefficients					
Response variable	R^2_c	R^2_m	(Int)	YR	GOvsGF	SL	GD	PC
NO	0.83-0.88	0.71-0.74	-28.76	1.18	-0.66	1.58	–	19.49
CO	0.24-0.36	0.24-0.29	2.43	-0.20	–	–	–	3.35

(-) indicates a factor not included in the model

3.5 Discussion

Invasive species are pervasive in urban systems, but the mechanisms that facilitate invasion and success are not clear (Cadotte *et al.*, 2017; Gaertner *et al.*, 2017). In this chapter I show that guppies, a widespread invasive species, proliferates in urban ecosystems, reaching high densities, and that urbanization enhances traits that are likely to make guppies good invaders. My data suggest a relaxation of life history tradeoffs because urban guppies simultaneously invest more in both somatic and reproductive traits, reaching far higher densities in urban vs. non-urban streams. Differences between urban and non-urban guppies vary slightly between years and are only slightly dampened by the presence of other fish species.

Urbanization causes profound environmental changes in stream ecosystems. Within the literature on urban streams, the term “urban stream syndrome” is used as a metaphor to summarize environmental changes resulting from urbanization along streams, including water quality degradation, increased flashiness, increased proliferation of tolerant taxa, and declines in sensitive taxa (Walsh *et al.*, 2005). However, because symptoms of urbanization can be expressed differently depending on local socioeconomic conditions, the universality of the urban stream syndrome has been recently debated, especially for tropical streams (Capps *et al.*, 2016). The streams of Rio de Janeiro show some of the symptoms of the syndrome, including high concentration of ammonium. In addition, these streams are especially contaminated with fecal coliforms (E. *coli*), exceeding on average 196 times the maximum concentration allowed by local regulations (Conselho Nacional do Meio Ambiente, 2000). This high concentration likely results from the discharge of raw sewage because of the poor sewer infrastructure (Borges *et al.*, 2015).

Despite the degraded environmental conditions, urban guppies attain higher population density and body sizes than non-urban guppies (Fig 3.2). Successful urban dwellers maintain higher population density in urban than in nearby non-urban systems (Faeth, Bang & Saari, 2011; Møller *et al.*, 2012; Fischer *et al.*, 2015). Whether urban dwellers also maintain increased body sizes in urban than in non-urban populations is debated. For instance, house sparrows, *Passer domesticus,* have reduced body sizes in urban systems (Meillère *et al.*, 2015). While the body size of frogs, *Pelophylax perezi*, and newts, *Triturus marmoratus* and *Salamandra canalicula*, is larger in urban systems (Iglesias-Carrasco, Martín & Cabido, 2017). The larger body size of urban guppies, as seen in this study, can potentially help them resist high waterflow and flash floods which can in turn improve population success (Walsh *et al.*, 2005; Hockley *et al.*, 2014).

Urbanization enhances guppy invasiveness as evidenced by large body sizes, increased number of offspring and enhanced condition of guppies in urban compared to non-urban streams (Figs. 3.2b and 3.3). In general, the capacity of species to invade a new ecosystem (i.e. invasiveness) is related to traits that affect dispersal, production of offspring and the maintenance of viable populations (Richardson *et al.*, 2011; Liu, Comte & Olden, 2017). The larger body size, better condition and high number of offspring are known to enhance guppy invasiveness. Larger female poecilids travel longer distances facilitating dispersal (Brown, 1985; Croft et al., 2003). Increased body size and condition allow guppies to have more offspring which can facilitate the establishment of new populations in the site of introduction (Brown, 1985; Liu et al., 2017; Lloret et al., 2014; Sibly & Hone, 2002).

The effects of urbanization on food resources in stream ecosystems is debated. While increased nutrient inputs and declines in canopy cover can increase primary production, the increased discharge can scour these resources away (Walsh *et al.*, 2005; Wenger *et al.*, 2009). I show that urbanization increases food availability for guppies, as seen by the increased biomass and consumption of chironomids (Fig. 3.4). Chironomids are considered to be an indicator of urbanization because they are highly tolerant to degraded conditions (Paul & Meyer, 2001; Yule *et al.*, 2015b). They also happen to be a preferred food source for guppies in their native range (Zandonà *et al.*, 2011). While the density of chironomids observed in the non-urban reaches of my study is similar to the density of chironomids observed in the guppy`s native range in Trinidad (19±5 and 10±1 mg/m^2, respectively), the urban reaches considered here have more than ten times the biomass of chironomids observed in the guppy’s native range (114±40 and 10±1 mg/m^2, respectively) (Eugenia Zandonà personal communication). Therefore, one of the ways in which urbanization facilitates guppy success is by increasing the biomass and consumption of chironomids which are highly nutritious food (have high nitrogen concentration per body mass)

(Zandonà *et al.*, 2011). To the best of my knowledge, this is the first time that a trophic interaction between two aquatic urban dwellers has been reported. However, such interactions are likely widespread because both guppies and chironomids are globally distributed (Ferrington, 2008; Deacon *et al.*, 2011).

Urbanization potentially relaxes life history trade-offs, because urban guppies show increased investment in both reproduction and somatic growth despite high density and biodiversity. Life history traits are shaped by trade-offs between somatic growth, reproduction and energy required for biotic interactions (Stearns, 1989). For example, studies on guppies in non-urban streams in Trinidad show a tradeoff between reproduction and somatic growth in response to changes in mortality due to predation (Reznick & Endler, 1982; Reznick, 1983; Reznick & Bryga, 1987). In addition to mortality, such tradeoffs can occur because organisms have finite energy budgets that are limited by resource availability that can be regulated by population density (Reznick, Butler, Rodd, & Ross, 1996). However, increased resources in human altered systems have been hypothesized to relax life history trade-offs (Snell-Rood *et al.*, 2015). My data provide preliminary support for this hypothesis by showing that urban guppies have increased body sizes, more offspring and better condition than non-urban guppies even when density is high, independent of fish biodiversity (i.e. presence of competitors and predators) (Figs. 3.2b and 3.3).

The effect of nutrition on life history trade-offs in guppies can be confounded. To clearly demonstrate the relaxation of trade-offs requires estimation of mortality and growth rates because they help explain the patterns of resource allocation (Roff, Heibo, & Vøllestad, 2006). Other factors, such as pollution (e.g. pharmaceutical hormones) can also influence life history traits, but the effects in this system and others are not clear (Overturf *et al.*, 2015). The occurrence of such chemicals in urban streams is highly influenced by water flow and the residence times/concentration necessary to influence guppy life history traits can vary (Brown, Gunnarsson, Kristiansson, & Tyler, 2014; Thomas et al., 2014).

Urbanization dampens the effect of population density on guppies. In non-urban systems, high density is predicted to reduce body size, fecundity and condition because of the competition for food resources (Roff, 1992; Stearns, 1992). However, I found that enhanced density does not reduce guppy body size, has only a relatively small negative effect on the number of offspring and has a positive effect on condition (Appendix B, Table B5). This is likely related to the consumption of chironomids, as suggested by the LMM models (Table 3.1; Fig. 3.5). Although none of the model factors were good predictors of body length (i.e. the null model was included as best model), laboratory experiments have shown that increased consumption of nutritious food increases guppy body size (Arendt & Reznick, 2005; Reznick & Yang, 1993). The mechanisms by which guppies are potentially released from the effect of density requires further testing. It is not clear whether high food availability alone can dampen the effect of density or if that effect is also related to traits that increase guppy tolerance to high densities (Whitney & Gabler, 2008; Colautti & Lau, 2015).

The value of conserving biodiversity in cities is debated because the ecological and human benefits are not always clear (Dearborn & Kark, 2010). A potential benefit of urban biodiversity is to provide protection against invasion, as seen in non-urban systems (Kennedy *et al.*, 2002; Smith-Ramesh, Moore & Schmitz, 2017). Biodiversity was found to regulate the invasion of the apple snail *Pomacea canaliculata* in urban streams

(Yamanishi *et al.*, 2012). However, this protective role of biodiversity is not very strong in my data. The presence of other fish has a small, non-significant effect on guppy population density, number of offspring and condition, although these effects are always in a negative direction, suggesting protection from invasion. Here I assessed the effects of biodiversity using categories, but other aspects of biodiversity, such as species identity, dominance and rarity can also be important to determine its protective role against invasion in urban streams (Henriksson *et al.*, 2015).

An important question for future studies is whether the observed changes in traits that increase invasiveness are plastic (non-heritable) or evolutionary (heritable). Urbanization is a strong selective agent for trait change which causes contemporary evolution (Alberti, 2015; Alberti *et al.*, 2017b). Rapid evolution is poorly studied in urban aquatic ecosystems, but recent evidence suggests urbanization leads to evolutionary changes towards “faster” life history traits associated with increased fecundity, smaller body size at maturity and early reproduction (Brans & Meester, 2018). Studies on guppies in non-urban streams in their native range suggest that body size and the number of offspring have a heritable component, but there is also likely to be considerable plasticity, especially in body size (Arendt & Reznick, 2005; Gordon et al., 2017; Reznick, 1997; Reznick & Bryga, 1987). In the future, laboratory breeding experiments could test if urbanization drives the evolution of increased body sizes and “fast” life history traits in urban guppies. This information can be fundamental for managers because the evolution of a highly invasive phenotype can promote range expansion and secondary invasions in urban and non-urban systems (Bertelsmeier & Keller, 2018). Because guppies are globally distributed, abundant in urban environments and have been extensively studied for evolution of life history traits, they offer a great opportunity to study urban evolution, a topic that is only recently being explored (Johnson & Munshi-South, 2017).

3.6 Coauthor contributions

Eugenia Zandonà[2], Rana El-Sabaawi[1] and Rosana Mazzoni[2] helped with the experimental design; Luisa R. Manna[2] and Therese C. Frauendorf[1] helped with collecting data; Rana El-Sabaawi[1] and Therese C. Frauendorf[1] helped with data analysis; Rana El-Sabaawi[1] helped with the writing of the manuscript.

[1] University of Victoria, Victoria, BC, Canada;

[2] Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Chapter 4 – Disentangling the effects of predation and density on resource-use phenotypes among and within populations

4.1 Abstract

The effect of predation on prey is often studied at the population level, with little regard to the effects within the prey population. In this chapter I evaluate intrapopulation variation in resource use and gut length of the guppy, *Poecilia reticulata*, in native non-urban populations of Trinidad under different predation regime. I specifically test whether predation regime, food availability or population density drive resource use in the guppy. I also evaluate if differences in resource use and gut length emerge on contemporary timescales after guppies are released from predators. I show that resource use has a bimodal distribution along the resource use axis, suggesting that guppy populations have two ecologically distinct resource-use phenotypes (carnivorous and detritivorous/algivorous). These phenotypes exist in both ancestral and derived populations. The difference in phenotype is also reflected in gut length: carnivorous guppies have shorter guts than detritivorous/algivorous. I find that the frequency of the carnivorous phenotypes is related to guppy density suggesting that shifts in the frequency of the resource-use phenotypes are likely driven by changes in intraspecific competition among guppies. A shift in resource use distribution (towards a detritivorous/algivorous phenotype) is established after a few generations (15-18) post-predator release, suggesting changes to resource use can occur on contemporary timescales. Typically, bimodality in resource use is associated with stark differences in morphology and feeding habitats. My study shows that it can also occur in a much more subtle way, and that it can be altered by population density, raising the question that intrapopulation bimodal variation might be a widespread phenomenon.

4.2 Introduction

The causes and ecological consequences of intraspecific trait variation are of increasing interest because of their importance in conservation, ecosystem processes and eco-evolutionary interactions (Mimura *et al.*, 2017; Des Roches *et al.*, 2018). A trait refers to any characteristic measured at the individual level (Violle *et al.*, 2007). Trait differences among phenotypes of the same species are widespread and can emerge in response to different environmental factors (Boyle, Sandercock & Martin, 2015). Intraspecific variation in traits related to diet and excretion can alter the role of the organism in its community and change ecosystem processes (Bassar *et al.*, 2012; Simon *et al.*, 2017).

Predation is thought to be a driver of short-term and long-term intraspecific variation. Predation risk can directly affect prey traits related to feeding, morphology and life history (Riesch, Martin & Langerhans, 2013; Hoverman *et al.*, 2014; Miller, Barrueto & Schluter, 2017). Predation can also cause indirect trait changes through its effects on prey behavior and density (Preisser, Bolnick & Benard, 2005; Holt & Barfield, 2012). Disentangling the direct and indirect effects of predators on prey traits is an important goal for ecology, as human activity is disrupting predator-prey relationships worldwide (Rodewald, Kearns & Shustack, 2011; Schmitz, 2017).

Traditionally, studies on the effects of predators have compared mean trait values between prey populations under different predation risks (Eklov & Svanback, 2006; Hawlena, Hughes & Schmitz, 2011). However, traits can vary not only between populations but also within populations (i.e. intrapopulation variation) (Horswill *et al.*, 2016; Carvalho *et al.*, 2018). Intrapopulation variation in traits can originate from genetic or phenotypic variability and both can affect predator-prey population dynamics, community structure (e.g. food web dynamics), and ecosystem processes (e.g. trait-mediated ecosystem effects) (Trussell & Schmitz, 2012; MacLeod *et al.*, 2014; Nakazawa, 2017). Despite the potential implications, most ecological studies on the effects of predators to prey do not consider intrapopulation variation (Pettorelli *et al.*, 2015).

Intrapopulation variation can be visualized as a change in distribution along a trait axis from unimodality to bimodality (Hendry *et al.*, 2009) (Fig. 4.1). This often results from noticeable phenotypic/morphological differences, not related to sex or ontogeny, found among two groups of individuals within the population. These morphotypes have been often related to differences in habitat, such as in benthic and limnetic sticklebacks (Matthews *et al.*, 2010) and Arctic char (Malmquist, 1992). In such cases, morphotypes are considered to be ecologically different and are assigned to distinct categories in ecological studies.

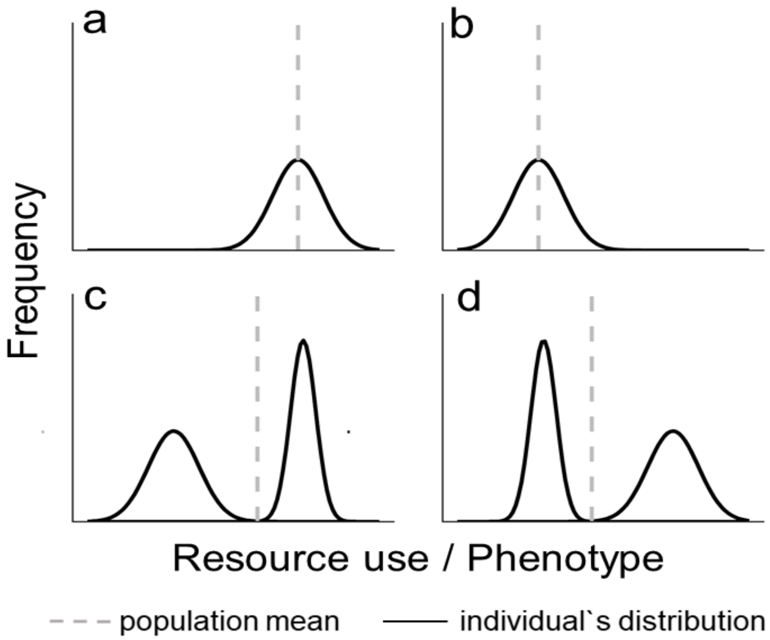

Figure 4. 1. Hypothetical scenarios for resource use distributions within populations. In (a) and (b) individuals follow a unimodal distribution. In (a) most individuals are carnivorous (increased frequency of individuals towards the right), while in (b) most individuals are detritivorous/algivorous (increased frequency of individuals towards the left). In both cases (a,b) the population mean (represented by the dashed line) adequately describes the population (i.e. there is low individual variation). Cases (c, d) show bimodal distributions in which the population has a high frequency of carnivorous individuals (c), or detritivorous/algivorous individuals (d). In both cases (c,d) the population means poorly describe populations (i.e. high intrapopulation variation).

However, traits that are not related to marked morphological differences can also lead to bimodality with important ecological significance. For example, dark-eyed Juncos, *Junco hyemalis*, show bimodal breeding activity in response to an altitudinal gradient (LaBarbera & Lacey, 2018). The pipistrelle bat, *Pipistrellus pipistrellus*, has bimodal phonic types which are related to the location of different types of prey (Gareth & van Parijs, 1993).

The Trinidadian guppy *Poecilia reticulata* has emerged as an important model for studying intraspecific trait variation in the context of predation (Magurran, 2005). In

Trinidad, guppy populations naturally occur in stream reaches under high (HP) and low (LP) predation risk due to the presence/absence of major fish predators (Reznick & Endler, 1982). Guppies from HP and LP populations differ genetically in many life history traits (Reznick & Endler, 1982). Experiments where guppies from HP reaches were transplanted into guppy free, LP reaches have shown that the evolution of life history traits can occur rapidly, within a few generations after transplant (Gordon et al., 2009; Reznick & Endler, 1982).

Predation has also been hypothesized to cause differences in resource use and gut morphology between HP and LP guppies (Zandonà *et al.*, 2011, 2015; Sullam *et al.*, 2015). Guppy populations under high predation risk have been shown to consume more invertebrates, while low predation guppies feed predominantly on algae and detritus. This difference in resource use quality is reflected in gut morphology. HP populations have on average shorter guts than LP populations (Zandonà *et al.*, 2015). Intraspecific variation in diet and gut morphology between HP and LP guppies are related to differences in nutrient processing that can affect the rates and ratios at which guppies excrete nutrients back to the ecosystem (El-Sabaawi *et al.*, 2012; Sullam *et al.*, 2015; Dalton *et al.*, 2017). These differences are invoked to explain why HP and LP guppy phenotypes have differing effects on ecosystem processes (El-Sabaawi *et al.*, 2015; Simon *et al.*, 2017). Such ecological effects are thought to be important for generating eco-evolutionary feedbacks (Bassar *et al.*, 2012; Travis *et al.*, 2014).

However, the causes of feeding differences between HP and LP guppies are unclear. We do not know if predators affect resource use directly, or if their effects are mediated by density (LP guppies have larger population densities than HP guppies), or if the differences result from environmental variation in resource availability that can be confounded with predation regimes (Reznick *et al.*, 2001). Density-mediated effects would have important implications because density can change due to a variety of factors, not just predators. Guppy density can change in response to flooding causing variation in density within predation regimes (Grether *et al.*, 2001). Density-dependence in resource use might, for example, explain why dietary differences between HP and LP guppies vary between studies conducted in the dry and wet seasons (Zandonà *et al.*, 2015, 2017).

Here, I investigate intrapopulation variation in guppy trophic traits and examine the drivers of this variation. I begin by asking whether guppy populations have bimodality in resource-use phenotypes and assess how this is related to the mean population resource use. I propose two different scenarios (Fig. 4.1). First, individuals having similar resource-use phenotype are normally distributed around a mean resource use (unimodal) (Fig. 4.1a,b). In this scenario, there is low intrapopulation variation and changes to the mean resource use arises because most individuals shift from one resource (Fig. 4.1a) to another (Fig. 4.1b). This scenario has been implicitly assumed in all previous guppy studies where means are used to report a shift from feeding on invertebrates (i.e. carnivorous) in HP conditions, to feeding on detritus and algae (i.e. detritivorous/algivorous) in LP conditions (Zandonà *et al.*, 2011; Sullam *et al.*, 2015). An alternate scenario is the bimodal distribution (Fig. 4.1c,d), where there is high intrapopulation variation with individuals forming distinct feeding groups and changes in mean resource use occur because of shifts in the frequency of the phenotypes within each population. Under this scenario, all guppy populations have a group of carnivorous and a group of detritivorous/algivorous individuals, but HP populations have a higher frequency of carnivorous than LP populations (Fig. 4.1c).

My second objective is to evaluate if resource-use phenotype is related to gut length. Across species, organisms with a low-quality diet (e.g. high fiber content) need longer guts to maximize nutrient absorption than organisms with a high-quality diet (e.g. high protein content) (Wagner *et al.*, 2009). Changes in gut length are critical for nutrient processing and assimilation and incur a large metabolic cost (Karasov & Douglas, 2013). However, studies on intraspecific variation of gut length in response to predators are rare. Early studies on guppies have shown that, on average, HP populations, which are carnivorous (insectivorous), also have shorter guts (Zandonà *et al.*, 2011), but the links between resource use and gut length have not been examined within populations.

My third objective is to explore the mechanisms through which predators affect the distribution of resource-use phenotypes. I specifically test whether changes to resource use are driven by changes in predation regime (presence of predators), food availability or population density. The removal of predators can increase prey density, which increases intraspecific competition, by reducing per-capita resource availability for the prey.

Lastly, by using an on-going guppy transplant experiment (Travis *et al.*, 2014), I assess whether differences in resource use and gut length can emerge in transplanted populations on a contemporary timescale. Such experiments have shown that life history traits evolve relatively quickly post-transplant, within 2-10 years depending on the trait (Reznick, Bryga & Endler, 1990; Reznick *et al.*, 1997). However, the rates of change of diets or feeding related traits that affect ecosystem processes are unknown. I quantify diets and gut length in guppies after 15-18 generations of transplant, at the earliest point where sampling of such traits became possible. Since I predict that density is likely to be an important factor in determining resource use, and since guppy density increases rapidly within the first years after a transplant (Travis *et al.*, 2014), I expect LP-like resource use and gut length traits to have emerged in these populations.

4.3 Materials and methods

In Trinidad, HP guppy populations co-occur with predatory fish such as *Crenicichla alta* which is restricted to downstream reaches by waterfalls. Upstream LP populations co-occur with a small omnivorous killifish, *Anablepsoides hartii* (formerly known as *Rivulus hartii*), that occasionally preys on guppy juveniles (Mattingly & Butler, 1994; Phillip *et al.*, 2013). In February 2014, during the early phase of the dry season, I sampled HP and LP reaches in the Aripo (ARI), El-Cedro (CED), and Quare (QUA) rivers, where predation risk and life history trait differences have been confirmed previously (Grether et al., 2001; Reznick et al., 1997).

In this study I also use guppies from four recent, on-going experiments described in Travis *et al.* (2014). In these experiments, guppies from the downstream HP reach of the Guanapo river have been transplanted into upstream, predator free, previously guppy free reaches in the same watershed. These experiments are monitored for a number of response variables, including traits, densities, and ecological responses (Travis *et al.*, 2014). The overall goal is to measure the rate of trait evolution as guppies evolve from HP to LP phenotypes after introduction. Transplants were performed in March 2008 at the Upper La Lajas (UPL) and Lower La Lajas (LOL) reaches and in March 2009 at the Caigual (CAI) and Taylor (TAY) reaches (Handelsman *et al.*, 2013; Travis *et al.*, 2014). I sampled the extralimital reaches of the experiment, as to not disrupt current mark recapture efforts. These reaches (hereafter referred as transplants) were sampled in 2014 to assess if trophic traits had evolved after 5 years (15 generations) in TAY and CAI, and 6 years (18 generations) in LOL and UPL.

At each of the HP, LP and transplanted reaches, I sampled 2-3 pools for guppy resource use (diet), gut length, invertebrate biomass, algal biomass and guppy density, totaling 8 pools per treatment (HP, LP and transplanted). I sampled pools because these are preferred guppy habitat, and because guppies have high pool fidelity (Croft et al., 2003; Reznick, Butler, Rodd, & Ross, 1996). I also measured environmental variables to characterize each pool including canopy cover (%), dissolved oxygen (mg/L), temperature (^{o}C) and conductivity (µS/cm) (Appendix C, Table C1).

4.3.1 Diet analysis, body size and gut length

I analyzed guppy diets using gut content analysis, which is the most commonly used method for studying trophic ecology in Trinidadian guppies (Zandonà *et al.*, 2011, 2015; Sullam *et al.*, 2015). This approach is preferred over stable isotopes because isotopic signatures vary considerably among individuals, and overlap in some prey resources, making it difficult to identify dietary patterns (Zandonà *et al.*, 2017).

An average of 5 females per pool (3-9 individuals) were captured and euthanized following protocols approved by the University of Victoria Animal Care Committee. I used only mature female guppies (i.e. carrying fully developed eggs) to avoid additional sources of intraspecific variation derived from sex or ontogeny.

In total, 122 female fish were analyzed. Standard body length (SL, mm) and total gut length (GL, mm) were measured. I estimated resource use by analyzing only the foregut to the point where the gut turns 180^{o} because gut contents in this area are still relatively intact (Zandonà *et al.*, 2015). Gut content analysis was performed using the gridded microscope slide technique (Zandonà *et al.*, 2011). The slide area occupied by invertebrates, detritus (silt and amorphous material) and algae (mainly single cell and filamentous diatoms) was estimated (mm^2). I identified the invertebrates to the lowest taxonomic level possible, generally family, using published taxonomic keys (Merritt & Cummins, 1996; Mugnai *et al.*, 2010). I then used the total area of the slide occupied by food to estimate the proportion of invertebrates, detritus and algae consumed. Gut content data were expressed as proportion of total area: $P_i = A_i / A_t$. Where, P_i = proportion of the food item, A_i = area of the food item, A_t = total area occupied by all diet items. Individuals with empty guts were removed from all analyses.

4.3.2 Intrapopulation variation in resource use, body size and gut length

I used gut contents from all individuals per treatment (HP, LP and transplanted populations) to assess the distribution of the proportion of invertebrates, detritus and algae consumed. I tested for bimodality in the distributions by using Hartigan's dip statistic (HDS) from package *diptest* for *R* (Maechler, 2015). The HDS test shows how strongly a distribution deviates from unimodality towards multimodality (Hartigan & Hartigan, 1985). HDS is preferred for detecting bimodality because it is more robust to skew which can significantly bias alternative tests (Freeman & Dale, 2012; Pfister *et al.*, 2013). HDS *p*-values below 0.05 indicate significant bimodality. When bimodality was detected I estimated the antimodes of the resource use distribution (the valley that separate the two modes of the distribution) using the function "amps" from the package *modes* for *R* (Deevi, 2016). I used the antimodes to determine the separation of the population into distinct phenotypes and tested for differences in the proportion of phenotypes between populations using ANOVA followed by Tukey post hoc tests.

I grouped individual guppies into 2mm size categories to assess whether the distribution of body size was related to bimodality in resource use by using the Kolmogorov-Smirnov two-sample test (D). This test quantifies the similarity between the body size distribution and the resource use distribution. Values of D close to 1 indicate low similarity.

I assessed the variation in gut length by examining the distribution of gut ratio (gut length/guppy standard length) from all individuals in all the pools within HP, LP and transplanted populations. I also assessed differences in gut ratio within and between populations using ANOVAs, followed by a Tukey post hoc test whenever necessary. All statistical tests were performed in *R* (R Core Team, 2018).

4.3.3 Food availability, guppy density and the mechanisms for phenotypic change

I collected benthic invertebrates at each pool with a Hess sampler in order to estimate how much was available for guppies to feed on. Invertebrate families were identified, each invertebrate was measured (mm) and mass-length regressions from the literature were used to calculate the total invertebrate biomass (Benke *et al.*, 1999). Because not all invertebrates are targeted as food by guppies, I only considered taxa found in gut contents and invertebrates smaller than 5mm (average size of invertebrates found in the guts) to calculate the invertebrate biomass per pool (IB, dry mass mg/m^2) (Appendix C, Table C2). I used ANOVAs to test for differences in invertebrate biomass per pool among reaches.

The epilithon, a mixture of algae, detritus and microbes was scraped from rock surfaces using a Loeb sampler (Loeb, 1981). I took multiple samples (2-3) per pool, filtered and analyzed for Chlorophyll *a* using a fluorometer (Aquafluor®) (Parsons, Maita & Carol, 1984). Chlorophyll concentration was corrected for sample volume and Loeb area, and used to estimate algal biomass (AB, Chl *a* µg/m^2) (Appendix C, Table C3). ANOVAs were used to test for differences in algae biomass per pool among reaches.

I estimated guppy density following a depletion method (Carle & Strub, 1978). At each reach, three pools were blocked with fine mesh nets and fished for 20 minutes, three consecutive times, with 20-minute intervals between fishing events. The difference in the number of guppies captured in each of the 3 fishing events allowed me to estimate guppy density per pool (GD, ind/m^2), using the *FSA* package for *R* (Ogle, 2017; R Core Team, 2018). Density estimates included both mature male and female guppies (Appendix C, Table C3). I used ANOVAs to test for differences in guppy density per pool among reaches.

To assess which factors drive resource use, I built a binomial GLMM model using pools as replicates. I used the proportion of carnivorous guppies (PC) per pool as the response variable and total invertebrate biomass per pool (IB, dry mass mg/m^2), algae biomass per pool (AB, Chla µg/m^2), total guppy density per pool (GD, ind/m^2), predation regime (HP and LP) and mean body length (SL) as fixed factors, and reach identity (RI) as a random factor (Appendix C, Table C3). I weighted the model by the number of guts analyzed per pool (NG) because the response variable is a proportion of all the guts analyzed. From the global PC~IB+AB+GD+HPvsLP+SL+(1|RI), family=binomial, weight= NG, I built a subset of models with different combinations of fixed factors to check which one best described our data. Akaike weights (W) were calculated in order to assess the weight of evidence in favor of each model. The selection of the best model was based on corrected Akaike`s Information Criterion AIC$_c$. Only models with ΔAIC$_c$ <2 were considered to have empirical support (Burnham & Anderson, 2002). The best models were averaged and the variance explained by both fixed and random factors ($R^2_{conditional}$), and the variance explained only by the fixed factors ($R^2_{marginal}$) were estimated using the "r.squaredGLMM" function of the R package *MuMIn* (Barton, 2018). All continuous factors were scaled by calculating the mean and standard deviation of the entire data vector and scaling each entry in that vector by subtracting the mean and dividing it by the standard deviation. Model fit, selection and averaging were performed using the packages *lme* and *MuMIn* for *R* (Grueber *et al.*, 2011; Bates *et al.*, 2015; Barton, 2018). I also assessed all models for collinearity of fixed factors by assessing the variance inflation ratios using the "vif" function of the *car* package for R (Fox & Weisberg, 2011). VIF values ~3 suggest low/moderate collinearity (Zuur, Ieno & Elphick, 2010). I assessed the significance of each fixed factor included in the best model using the likelihood ratio test from the "lrtest" function of the *lmtest* package for R. The best model was plotted using ggplot2 for R (Wickham, 2009).

4.4 Results

There was strong bimodality in the proportion of invertebrates consumed within HP, LP and transplanted populations (HDS $p<0.05$ for all populations) (Fig. 4.2a). Bimodality was also found in the proportion of detritus and algae (Fig. 4.2b), although the pattern was driven mostly by detritus (Appendix C, Fig. C1).

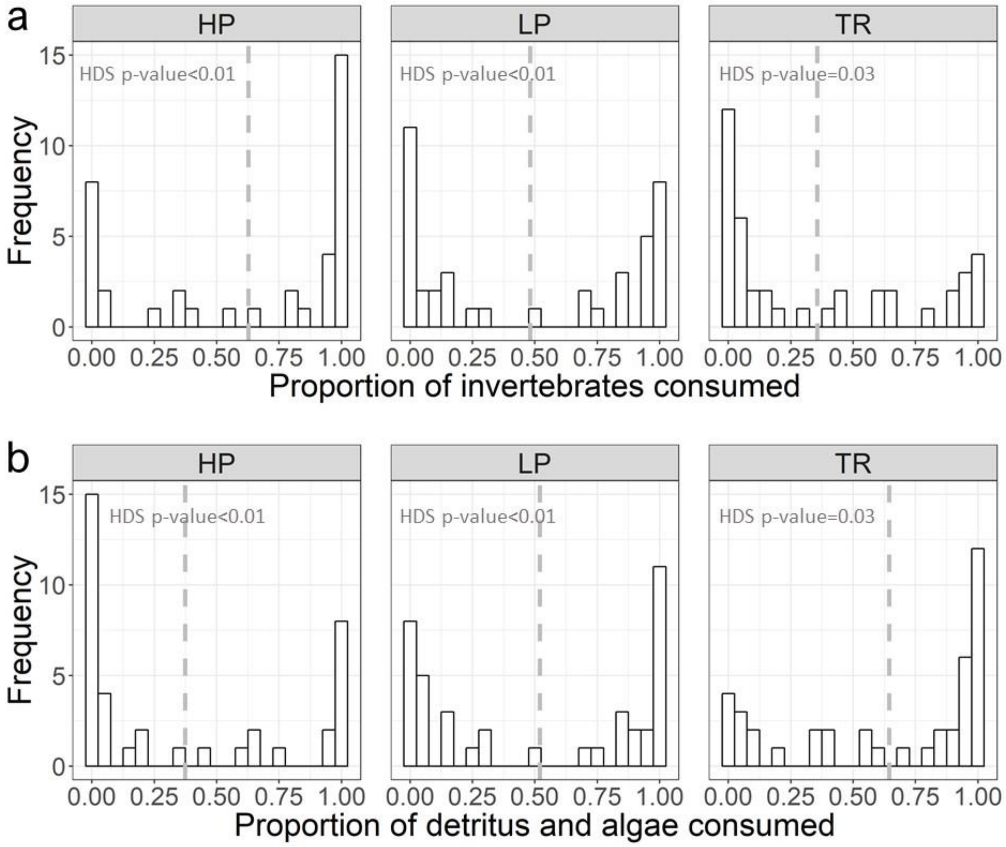

Figure 4. 2. Distribution of resource use phenotypes in Trinidadian guppies. Histograms show the frequency of consumption for all individuals within populations occurring in reaches with predators (HP), without predators (LP) and populations originated from HP reaches that were transplanted into guppy free, predator free reaches (TR). Panel (a) show the proportion of invertebrates consumed. Panel (b) show the proportion of detritus plus algae consumed. Dashed grey lines indicate population mean. HDS show the significance of the Hartigan's dip statistic, where $p<0.05$ indicate a bimodal distribution.

Since the proportion of invertebrates consumed was the mirror image of the proportion of detritus and algae (Appendix C, Fig. C2), I assessed the variation in resource use within HP, LP and transplanted guppy populations by examining only the distribution of the proportion of invertebrates consumed. The separation of the HP, LP and transplanted populations into two distinct feeding groups occurred when the proportion of invertebrates consumed is 0.47, 0.49 and 0.57, respectively (represented by the valley of the bimodal distribution or antimode) (Fig. 4.2a). This suggested that populations can be divided in two resource-use phenotypes with distinct ecological roles. I assigned a carnivorous resource-use phenotype to guppies that feed on high proportion of invertebrates (proportion of invertebrate consumption ≥ antimode). Because there was an inverse relationship between the proportion of invertebrates and detritus/algae consumed (Appendix C, Fig. C2) and because individuals with empty guts were removed from the analysis, I assigned guppies that were not carnivorous to the alternative detritivorous/algivorous resource-use phenotype (proportion of invertebrate consumption < antimode). The carnivorous phenotype in HP, LP and transplanted populations fed on a high proportion of invertebrates (0.93±0.03, 0.92±0.02 and 0.85±0.04, respectively) and low proportions of detritus and algae (0.07±0.02, 0.08±0.02 and 0.15±0.04, respectively). While detritivorous/algivorous phenotypes within HP, LP and transplanted populations fed on high proportions of detritus and algae (0.90±0.02, 0.91±0.03 and 0.90±0.03, respectively) and low proportions of invertebrates (0.10±0.04, 0.08±0.03 and 0.1±0.03, respectively).

The proportional frequency of resource-use phenotypes differed statistically only between HP and transplanted populations where predation release favored a low frequency of the carnivorous phenotype (0.63±0.06 and 0.34±0.06) (Tukey`s post hoc test mean difference in proportion 0.29±0.02, $p<0.01$). Although HP populations have a relatively higher proportion of carnivorous guppies than LP populations (0.63±0.06 and 0.47±0.06), those differences were not statistically significant at $P > 0.05$ (Fig. 4.2a).

Resource use distribution was not related to body length distributions in HP, LP or transplanted populations (K-S test: $D=1$, $P<0.001$ for all populations) (Fig. 4.3a; Appendix C, Fig. C3). There were no differences in body length between carnivorous and detritivorous/algivorous phenotypes within populations (Appendix C, Fig. C4). However, on average, HP populations had smaller body lengths than LP and transplanted populations (18.3±0.3, 21.2±0.4 and 21.8±0.5, respectively) (ANOVA $F_{2,116}$=17.75, p<0.001) (Tukey`s post hoc test, mean difference in length 2.9±1.5 and 3.5±3, p<0.001) (Appendix C, Fig. C4).

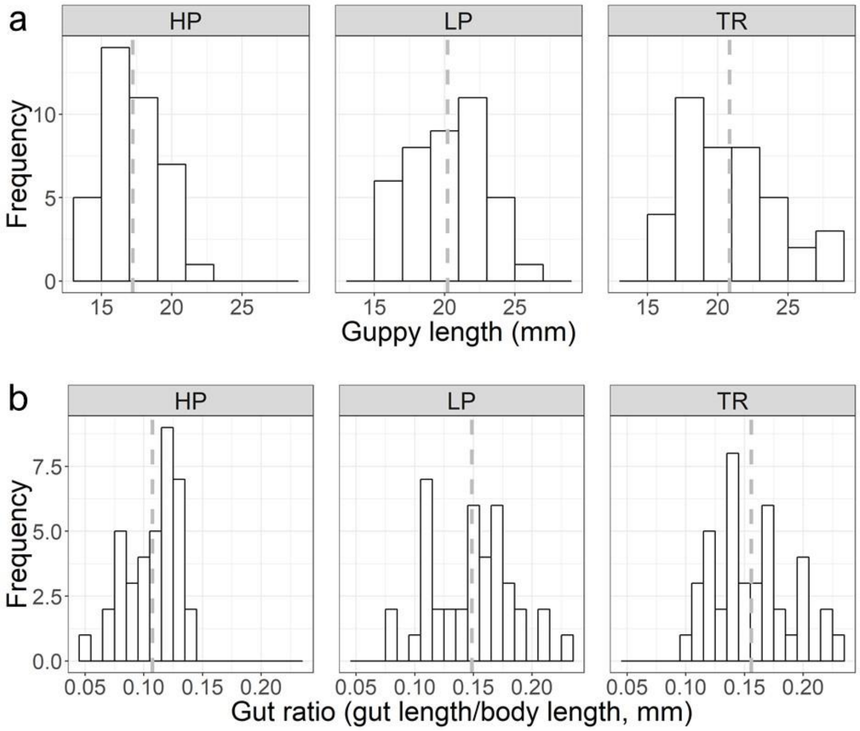

Figure 4. 3. Distribution of guppy length (panel a) and gut ratio (panel b). Histograms show the frequency for all individuals within populations occurring in reaches with predators (HP), without predators (LP) and populations originated from HP reaches that were transplanted into guppy free, predator free reaches (TR). Individual body length was grouped in 2mm length categories. Dashed grey lines indicate population mean.

There were significant differences in mean gut ratio between populations (ANOVA $F_{2,116}$ =27.6, p<0.001). On average HP guppies had shorter guts than LP and the transplanted guppies (Tukey`s post hoc test, mean difference in gut ratio 0.04±0.02 and 0.05±0.03mm, respectively, p<0.001). The overall distribution of gut ratio (defined as gut length/guppy standard length) was not bimodal (Fig. 4.3b). A broad association between resource-use phenotype and gut length was found within LP and transplanted populations, but not within HP populations. Carnivorous guppies had shorter guts than detritivorous/algivorous guppies in LP (gut ratio 0.14±0.09 and 0.16±0.01, respectively) (ANOVA $F_{1,38}$=4.7, p=0.04) and transplanted populations (gut ratio 0.14±0.01 and 0.16±0.01) (ANOVA $F_{1,39}$=8.3, p<0.01) but not in HP reaches (gut ratio 0.11±0.004 and 0.10±0.01) (Fig. 4.4).

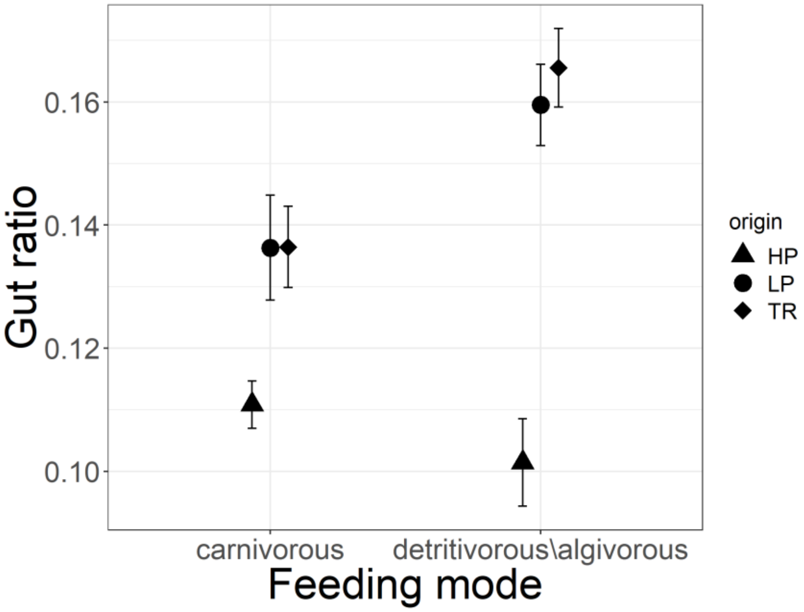

Figure 4. 4. Average gut ratio for each resource-use phenotype in high predation (HP), low predation (LP) and transplanted (TR) populations. Gut ratio (gut length (mm) /guppy length (mm)) is used to account for the effect of body size on gut length. Symbols are means of all individuals and error bars show standard errors.

There were no differences in the invertebrate biomass (119.4±32, 112.5±20 and 103.3±13.7 mg/m^2) and algae biomass ($5x10^{-4}$±$1x10^{-4}$, $3x10^{-4}$ ±$9x10^{-5}$ and $3x10^{-4}$±$3x10^{-5}$, Chl *a* µg/m^2) among HP, LP and transplanted populations respectively. Although there was a trend towards higher guppy density in transplant (15±3 ind/m2) and LP (11±3 ind/m2) compared to HP populations (8±2 ind/m2), these differences were not statistically significant.

I also tested whether predation regime, food availability or population density was the best predictor of resource-use phenotype in HP, LP and transplanted populations. Transplanted populations were modeled separately because they were in the process of adapting (they do not have a defined predation regime, Travis et al. (2014)). The best model for HP and LP populations suggests that guppy density was the main predictor of guppy diet, being negatively related to the proportion of carnivorous guppies (GLMM averaged coefficient=-0.73) (Fig. 4.5; Table 4.1). The models also suggested a negative effect of invertebrate biomass (avg coefficient =-0.41) on the proportion of carnivorous guppies in HP and LP populations (Table 4.1). The likelihood ratio test between a global model with and without population density confirmed that density was a significant predictor of the proportion of carnivorous guppies ($\chi^2_{(1)}$ =5.3, p=0.02). However, the same test performed for invertebrate biomass did not show this factor to be significant. The best model for the transplanted populations was the null model which suggested none of our factors predicted the proportion of carnivorous guppies, although the absence of patterns could have been caused by small sample size. A list of all models derived from the global model for HP+LP and transplanted populations can be found in the supporting information (Appendix C, Table C4).

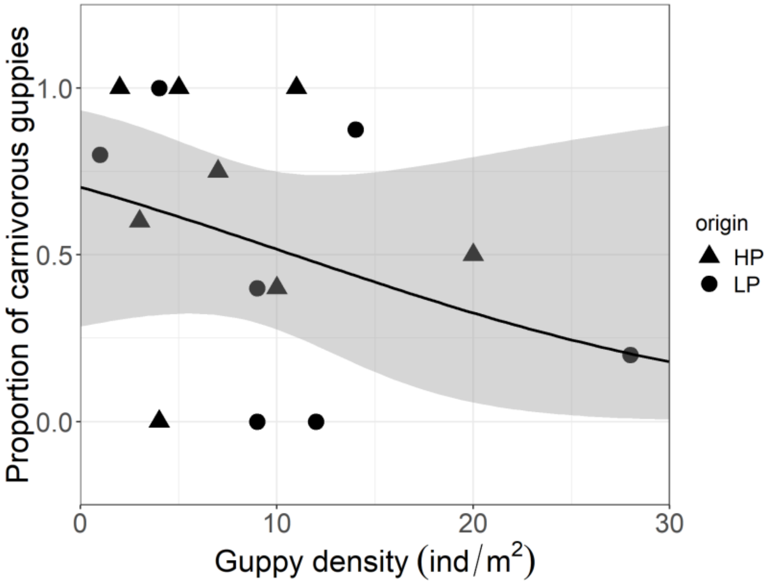

Figure 4. 5. The relationship between guppy density and the proportion of carnivorous guppies in each population. Each point represents a pool (see Appendix C, Table C3). HP are populations that occur with the presence of major fish predators. While LP are populations that occur without the presence of major fish predators. The black line shows the bimodal GLMM model fit and grey shade is the confidence interval.

Table 4. 1. Binomial GLMMs. High predation (HP) and low predation (LP) populations were modeled together. I used the proportion of carnivorous guppies (PC) as response variable and total invertebrate biomass per pool (IB, dry mass mg/m^2), algae biomass per pool (AB, Chla µg/m^2), total guppy density per pool (GD, ind/m^2), predation (with (HP) vs without (LP) the predator), mean guppy length (SL, mm) as fixed factors, and reach identity (RI) as a random factor. The variance explained by both fixed and random factor ($R^2_{contidional}$), and the variance explained only by the fixed factors ($R^2_{marginal}$) was estimated. Only the models with ΔAIC_c <2 are considered and used for data interpretation. Akaike weights (W) show the weight of evidence in favor of each model. I estimated collinearity between fixed factors in the global model based on variance inflation ratios (VIF). A list with the full model selection with all the candidate models can be found in the supplement (Appendix C, Table C4).

Best models							coefficients					
model	R^2_c	R^2_m	df	ΔAICc	W		IB	AB	GD	HPvsLP	SL	Int
1	0.38	0.1	3	0.000	0.324		–	–	-0.737	–	–	0.059
2	0.34	0.12	4	1.788	0.132		-0.415	–	-0.702	–	–	0.075
						VIF	1.17	1.34	1.41	3.04	3.44	

(–) show factors that were not included in the model.

4.5 Discussion

To the best of my knowledge this study is the first to consider the mechanisms that underlie intrapopulation variation in trophic traits in the context of predator-prey interactions. I have shown that guppy populations under high (HP) and low (LP) predation risk contain two feeding groups: carnivorous (high consumption of invertebrates) and detritivorous/algivorous (low consumption of invertebrates). A shift towards detritivory/algivory occurs when guppies are transplanted from HP to previously guppy- and predator-free environments (TR). Variation in resource use is partially mirrored by changes in gut length: in general, carnivorous guppies have shorter guts than detritivorous/algivorous guppies, except in HP populations (Fig. 4.4). Intrapopulation variation in resource use is often associated to sex, ontogeny or size structure of the population (Nifong, Layman & Silliman, 2015; Showalter, Vanni & González, 2016). However, my study uses only mature female guppies and my results show that within population variation in resource use and gut length are not related to body size.

In evolutionary studies, bimodality is typically interpreted as two extremely different phenotypes exploiting resources more efficiently and having better fitness than intermediate phenotypes (Martin & Pfennig, 2009). Different evolutionary processes can cause bimodality (Doebeli & Dieckmann, 2000). Understanding these processes is not the goal of my study, instead I focus on understanding how these resource-use phenotypes vary within predation regimes.

My results suggest that predation regime alone does not explain the frequency of resource-use phenotypes. In other words, guppy diet variation is likely not caused by the direct effects of predators. There were no significant differences between HP and LP populations in the proportion of carnivorous to detritivorous/algivorous phenotypes, and predation was not a significant predictor of resource-use phenotype (Table 4.1). Rather, I found an inverse relationship between the frequency of carnivorous guppies and density in HP and LP populations (Fig. 4.5). Although my model explains a relatively small amount of the variation at ~10%, it suggests that density, rather than predation or food availability, was the strongest driver of resource use in guppies (Table 4.1). Density can regulate the accessibility to food, thus controlling the strength of competition within the population and inducing diversification in resource use (Svanback & Bolnick, 2007). Competitive interaction is known to change the frequency of morphologically distinct phenotypes (Bolnick, 2004). My data suggest that the same mechanism can determine the frequency of resource-use phenotypes within guppy populations.

The density mechanism can help us understand the conflicting evidence for the relationship between predation and resource use in guppies. Mean dietary differences between HP and LP guppies have been observed in some studies (Bassar *et al.*, 2010; Zandonà *et al.*, 2011), but not in others (Zandonà *et al.*, 2017). Such contrasting evidence can be explained if density, rather than predation, was the driver of guppy diets. Although there can be strong differences in density between HP and LP populations, guppy density is also highly sensitive to environmental variation in flow (Grether *et al.*, 2001). This suggests that studies that examine dietary differences in stable flow conditions when HP and LP density differences are large, are likely to see a strong difference in diet between predation regimes (e.g. Zandonà *et al.*, 2011), while studies conducted in periods of high flow such as during the wet season, or during transitional seasons when differences in guppy density are variable are unlikely to see large differences in diet (e.g. Zandonà *et al.*, 2017, and this study).

The association between phenotypic variation and dietary variation is key for understanding how intraspecific changes influences ecological processes including eco-evolutionary interactions in a variety of fishes, not only guppies (El-Sabaawi, 2017). Although phenotypic differences in resource use are widely documented, they are sensitive to environmental variation, and the causes of this “context specificity” is unknown and in need of further research (El-Sabaawi, 2017; Hendry, 2019). Recently it has been hypothesized that differences in density, which are commonly ignored in eco-evolutionary studies, might explain context specificity (El-Sabaawi, 2017; Hendry, 2019). My study provides preliminary evidence to support this hypothesis. Density variation is common in nature and has been suggested to influence ecological differences among phenotypes of the same species in experimental systems (Bassar *et al.*, 2010; Des Roches *et al.*, 2018). Disentangling the effects of density and predation, especially in natural ecosystems, is recognized as a key gap in this area of research (Hendry, 2019).

My study also shows for the first time how rapidly dietary differences can emerge after HP guppies are transplanted into a new, predator-free environment. Such experiments have been used to study the life history evolution of the LP phenotype and have demonstrated that it can begin to emerge on contemporary timescales (between 2-10 years, depending on the trait) (Reznick *et al.*, 1990, 1997). However, the rate of emergence of ecologically relevant traits such as diet related traits or excretion has never been studied. In this chapter I show that transplanted guppies, which have both phenotypes (bimodality with high frequency of the carnivorous phenotype) in ancestral HP populations, shift towards high frequency of the detritivorous/algivorous phenotype within 5-6 years after being released from predation pressure (Fig. 4.2a). Although I was not able to determine the best predictor for the proportion of carnivorous phenotypes in the transplanted population, their diets are consistent with a density-mediated effect. Density in guppy populations newly released from predators is very high, often higher than the density of typical HP or LP guppy populations (Travis *et al.*, 2014 and Frauendorf *et al* in review). Indeed, in my study the density of guppies from the transplanted population was on average 2 times higher than HP and 1.5 times higher than LP populations. Therefore, these extreme densities are likely driving the tendency towards detritivory/algivory in the transplanted populations.

It was not logistically possible to sample the transplanted populations sooner than 5-6 years. Therefore, my findings provide an upper bound for the time of change in resource-use phenotype in this system, showing that it occurs on similar timescales as post-transplant changes in life history traits (Reznick et al., 1990; Reznick & Endler, 1982). The overlap in timescales between traits that influence fitness and traits that influence resource use is important for initiating eco-evolutionary dynamics (Matthews *et al.*, 2011; Hendry, 2016), and is fundamental for a better understanding of predator-prey dynamics in nature (Yamamichi *et al.*, 2011).

Most carnivorous organisms have shorter guts than detritivorous/algivorous ones (Karasov & Douglas, 2013). These differences can determine differences in nutrient processing and excretion, which affect ecosystems nutrient dynamics and productivity (Atkinson *et al.*, 2017). My study shows that distinct resource-use phenotypes can also differ in gut length within populations. Although, gut length distribution does not mirror the bimodal distribution in resource use, I found that carnivorous phenotypes within LP and transplanted populations have shorter guts than detritivorous/algivorous phenotypes.

Factors other than resource use, such as feeding behavior (e.g. feeding rate) also affect gut length, and their influence on intraspecific gut morphology variation requires further study. Evidence suggests that HP guppies (which are mostly carnivorous) have lower feeding rates than LP guppies (which are mostly detritivorous/algivorous), although this remains to be tested in nature (Palkovacs *et al.*, 2011; Zandonà *et al.*, 2017).

Most often, a bimodal resource use distribution has been linked to fairly strong differences in feeding habitats, and in morphology (Hendry *et al.*, 2009). For example, benthic morphotypes of fishes have distinct feeding habitat and body/mouth morphologies suited for capturing benthic food (Malmquist, 1992; Matthews *et al.*, 2010). Although HP and LP guppy populations vary in some obvious traits such as color and body shape (Kemp, Reznick & Grether, 2008; Burns, Nardo & Rodd, 2009), variation among and within traits also exists (Karim *et al.*, 2007). Some studies have suggested that predation drives differences in individual cranium shape (Palkovacs *et al.*, 2011), but these are subtle compared to benthic limnetic morphological differences in temperate fish.

The link between resource-use phenotype and population density can be important to understand how changes to the environment affect guppies. Environmental changes, mainly those related to human activities, cause variation to the density of many taxa, including guppies (Chapter 3; Nater *et al.*, 2018). The link between diet and density that I have observed in my study suggests that density induced changes to diet can be important to understand the effect of human activities to guppy populations. Such information can be important to help explain the success of guppies in human altered environments (Chapter 3). However, this still needs to be tested in the field.

4.6 Coauthor contributions

Rana El-Sabaawi[1] and Eugenia Zandonà[2] helped with experimental design; Therese C. Frauendorf[2], Misha Warbanski[2] and Dawn Phillip[3] assisted with data collection; Rana El-Sabaawi[1] helped with analyzing the data and writing the manuscript.

[1] University of Victoria, Victoria, BC, Canada;

[2] Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil;

[3] University of West Indies, Trinidad, St. Augustine, Trinidad & Tobago

Chapter 5 - Characterizing the effects of urbanization on intrapopulation variation in diet and trophic morphology of a successful urban dweller

5.1 Abstract

Trophic ecology is thought to play a major role in the success of urban dwellers, which are often described as generalist species that can take advantage of food subsidies provided by humans. However, there is increasing evidence that a generalist population can consist of groups of individual specialists, and it is possible for urbanization to change the frequency of specialists within a population. In addition, characterizing intrapopulation variation in diet and trophic traits can reveal important ecological and evolutionary patterns in urban dwellers. In this chapter I explore the effects of urbanization on intrapopulation variation in guppy diet and feeding morphology (cranium shape and gut length) by comparing urban and non-urban guppy populations in Brazil. I assess the drivers of diet and cranium shape variation between urban and non-urban populations and compare them to what is known about trophic ecology of guppies in their native range. My data show that similar to the Trinidadian native populations, both urban and non-urban guppy populations in Brazil are composed of two resource-use phenotypes: carnivorous and detritivorous/algivorous. However, urbanization does not affect the distribution of these resource-use phenotypes. In contrast to studies in the native range, guppy density is not related to the frequency of the resource-use phenotypes in Brazil, in neither urban nor non-urban populations. Resource-use phenotype is related to cranium shape. Carnivorous guppies (i.e. that feed more on midge larvae, chironomids) have shorter/wider crania that increase feeding efficiency. Body size plays an important role in cranium shape. Because

of their large body size, urban guppies had wider and shorter crania than non-urban guppies. This suggests that the high consumption of chironomids by urban guppy populations is not only a result of the increased chironomid availability in urban streams, but it is also related to enhanced feeding efficiency.

5.2 Introduction

The globe is undergoing intense urban land expansion in the 21st century and by 2030, 65% of all land area in the world is expected to become urban (Seto *et al.*, 2012; D’Amour *et al.*, 2017). Urbanization converts the land into a built landscape (Mcdonald, Kareiva & Forman, 2008; D’Amour *et al.*, 2017), causing habitat loss, increasing contaminant concentration (e.g. pesticides, pharmaceuticals), increasing temperature (e.g. urban heat island), altering food and water subsidies (e.g. bird feeders, compost, lawn irrigation) (Alberti, 2008; Birnie-Gauvin *et al.*, 2017; Li *et al.*, 2017). Despite the altered conditions, some species known as urban dwellers persist and take advantage of the urban environment, often reaching high densities (Fischer *et al.*, 2015).

How and why urban dwellers thrive is a critical question for understanding biodiversity in cities. Several hypotheses have been proposed to explain the success of urban dwellers, including tolerance to pollution and “fast” reproduction (Williams, Hahs & Vesk, 2015; Marzluff, 2017). Most of these hypotheses evoke some aspect of the species’ traits. A trait refers to any characteristics measured at the individual level which can affect fitness and/or ecosystem processes (Violle *et al.*, 2007). Traits related to life history and behavior have been used to understand the success of urban dwellers (Chapter 3; Papp *et al.*, 2015; Sol *et al.*, 2018). There is increasing evidence that traits related to trophic ecology are also important as most urban dwellers are generalists species that can exploit a variety of foods, taking advantage of the unique resources in the urban environment (Sorace & Gustin, 2009; Lizée *et al.*, 2011; Bateman & Fleming, 2012; Peressin & Cetra, 2014; Ducatez *et al.*, 2018).

Generalist species have flexible diet and trophic traits, such as those related to feeding morphology, that can shift in response to urbanization. For example, house finches, *Carpodacus mexicanus,* switch from feeding on small seeds of grasses and cacti in non-urban areas to feeding on large sunflower seeds provided in bird feeders in urban areas (Badyaev *et al.*, 2008). Such a shift in diet is facilitated by changes in bill morphology which in turn affect song characteristics with implications for mate choice (Badyaev *et al.*, 2008). Assessing how diet and trophic traits change under urbanization can help us better understand the ecology of urban dwellers (Chapter 2).

Evidence suggesting that urban dwellers are generalist comes from studies that only use the mean trait value of all individuals in the population to characterize trophic strategy. However, considerable variation can exist within populations, even among individuals of the same sex, age and size (Bolnick *et al.*, 2002, 2003). Individuals within populations can differ in such a way that a generalist population can be composed of multiple individual specialists (Vander Zanden *et al.*, 2010; Pagani-Núñez, Valls & Senar, 2015).

Intrapopulation variation can be seen as a collection of specialized dietary groups, where the distribution of individuals along a trait axis deviates from unimodality, towards bimodality or multimodality (Chapter 4; Bolnick *et al.*, 2003; Hendry *et al.*, 2009). For example, a bimodal distribution indicates the presence of two groups of individuals with distinct phenotype/morphology and/or ecological roles, such as seen in benthic and limnetic sticklebacks (*Gasterosteus aculeatus*), Arctic charr (*Salvelinus alpinus),* and carnivorous vs. detritivorous/algivorous guppies (Chapter 4; Malmquist, 1992; Matthews *et al.*, 2010). Intrapopulation variation has been hypothesized to be determined by intraspecific competition because individuals tend to avoid competition by exploring alternative/unique food resources (Roughgarden, 1974; Bolnick *et al.*, 2003; Araújo, Bolnick & Layman, 2011). Thus, intraspecific competition can increase the presence of specialists and increase resource-use modality. The strength of intraspecific competition is related to the accessibility of consumer to food, which is affected by factors such as food availability and population density (Araújo *et al.*, 2011). These factors often interact and are confounded because population density can affect the amount of food per-capita (Svanbäck *et al.*, 2015). Recently, I proposed that density can modulate bimodality in resource use in the Trinidadian guppy (Chapter 4).

Urbanization can potentially disrupt the association between population density and per-capita resource availability in urban dwellers. These species attain high densities in urban areas likely because food resources are highly abundant (Chapter 3; Møller *et al.*, 2012; Šálek, Drahníková & Tkadlec, 2015). In urban areas, the availability of food can increase as a result of a high supply of nutrients for primary production (e.g. ammonium and phosphate) and human-provided food subsidies such as animal feeders, compost and garbage (Alberti, 2008; Becker *et al.*, 2018). Therefore, for urban dwellers, an increase in density is not necessarily correlated with increased resource competition or to declines in per-capita resource availability. Thus, changes in density might not affect dietary intrapopulation variation in urban dwellers.

Here I explore the effect of urbanization on intrapopulation variation using guppies, *Poecilia reticulata* as a model. Guppies are native to northern South America and the

Caribbean, on the islands of Trinidad and Tobago (Magurran, 2005), but they were introduced in many countries as a biological control for mosquito borne diseases and for the aquarium trade (Strecker *et al.*, 2011; Anogwih, Makanjuola & Chukwu, 2015). As a result, guppies have now invaded many urban and non-urban ecosystems across the globe (Lindholm *et al.*, 2005; Araújo *et al.*, 2009; Deacon *et al.*, 2011). Guppies are considered to be successful urban dwellers because they have higher densities, larger body sizes, and better body condition in urban streams (Chapter 3).

In non-urban streams on the island of Trinidad (within the native range), guppies have been extensively studied to clarify the relationship between predation risk, food availability and changes in diet and trophic traits (Chapter 4; Zandonà *et al.*, 2011, 2015; Palkovacs *et al.*, 2011b; Sullam *et al.*, 2015). Although guppies are usually defined as omnivorous/generalist species (Dussault & Kramer, 1981), evidence suggests guppy diet is context dependent. In Trinidad, guppy populations naturally occur in stream reaches with and without the presence of major fish predators (Reznick & Endler, 1982). Guppies that co-occur with predators have been thought to feed mostly on insect larvae (mainly midges, chironomids), while guppies not adapted to predators feed mostly on detritus and algae (Zandonà *et al.*, 2011). Dietary difference is associated with differences in feeding morphology as guppies that are free from predators have wider and shorter crania and have longer guts than guppies adapted to predators (Palkovacs *et al.*, 2011; Zandonà *et al.*, 2015). Until recently, the effect of predation on guppy diet and feeding morphology was unclear because the effect of predation was confounded with differences in guppy density and food availability (Zandonà *et al.*, 2011, 2015). In Chapter 4, I showed that guppies in Trinidad have bimodal resource phenotype distributions, meaning that each population has a group of carnivorous and a group of detritivores/algivorous guppies. This modality appears to be influenced by density, rather than predation regime. Populations under high density have less carnivores and more detritivorous/algivorous guppies, likely because density regulates per-capita access to food. The two resource-use phenotypes differ in feeding morphology with carnivorous guppies having shorter guts than detritivorous/algivorous.

Although much information exists on the trophic ecology of guppies in their native range, studies out of their native range or in disturbed systems are rare. In Chapter 3, I suggested that mean changes in diet (increased consumption of chironomids) facilitates the invasion success of guppies in urban systems. But we do not know how these mean diet tendencies relate to variation within the population, and how they relate to density. In other words, we do not know if guppies outside of their native range display bimodality in resource use, and we do not know if density controls bimodality in highly eutrophic urban systems.

Here I investigate if resource use bimodality exists in guppies in Brazil and how it responds to urbanization. Because density and resources increase concurrently in urban streams (Chapter 3), I expect that density will either have no effect on resource use bimodality in urban guppies, or that it will be positively related to the presence of carnivorous phenotypes in contrast to what I have observed in Trinidad (Chapter 4).

Then, I evaluate how intrapopulation variation in diet relates to trophic morphology. In native non-urban populations of Trinidad, the efficiency of guppies to feed on chironomids is thought to be related to having a shorter/wider cranium which increases gape size (Palkovacs *et al.*, 2011). I have previously shown that urbanization increases guppy density and the mean consumption of chironomids by guppies (Chapter 3). Thus, I predict that urbanization also enhances morphological traits that increase foraging on chironomids (a shorter and wider cranium). I also expect urban guppies to have relatively short guts because of their carnivorous diet (Sullam *et al.*, 2015; Zandonà *et al.*, 2015).

5.3 Material and methods

Here I use the same stream reaches and sampling design as described in Chapter 3. In short, I have selected 6 urban and 6 non-urban stream reaches in Rio de Janeiro, Brazil where guppies are known to occur. Urban stream reaches are characterized by having high concentrations of fecal coliforms and ammonium, which suggests they are contaminated with sewage (Chapter 3). Within each treatment (urban, non-urban) I had 3 reaches where guppies were the only fish present (GO) and 3 reaches where guppies co-occurred with fishes that are potential competitors and predators (GF). In GF reaches, the total number of species is known to range from 3-10, and each reach has at least one potential predator, the catfish *Rhamdia quelen* and one potential omnivorous competitor such as the poecilid *Phalloceros* sp. or the pearl cichlid *Geophagus brasiliensis* (Appendix B, Table B1). Guppy population density is also known to be much higher in urban than in non-urban reaches, irrespective of fish biodiversity (Chapter 3, Fig. 3.2a). Whenever possible I replicated the sampling in two years (2016-2017). In total there were 20 sampling events.

5.3.1 Diet analysis and body size

I reanalyzed the individuals used for mean diet estimates in chapter 3, but now with a focus on intrapopulation variation. As previously described, fishes were captured by electrofishing (detailed in Chapter 3). A total of 116 urban (29±3 per reach) and 120 non-urban (20±3 per reach) fish were euthanized, fixed in formalin (10%) and brought to the lab for gut content analyses. I used only sexually mature female guppies to remove any diet variation related to ontogeny and sex. Each guppy was measured for standard length (mm, SL), dissected and the foregut was sectioned to the point where the gut turns 180^o and the gut contents were analyzed. Gut content analysis was performed using the gridded microscope slide technique (Zandonà *et al.*, 2011). The slide area occupied by invertebrates, detritus (i.e. silt and amorphous material) and algae were estimated (mm^2). I identified the invertebrates to the lowest taxonomic level possible, generally family, using published taxonomic keys (Merritt & Cummins, 1996; Mugnai *et al.*, 2010). The algae were categorized into three broad groups: diatoms, filamentous and others. I then used the total area of the slide occupied to estimate the proportion of each food item: $P_i = A_i / A_t$. Where, P_i = proportion of the food item, A_i = area of the food item, A_t = total area occupied by all diet items. Individuals with empty guts were removed from all analysis.

5.3.2 Intrapopulation diet variation

Since the consumption of invertebrates was inversely related to the total consumption of detritus and algae (Appendix D, Fig. D1), I assessed dietary modality by plotting the distribution of the proportion of invertebrates consumed by all the individuals in all the reaches, separated by population (urban and non-urban). Since preliminary analysis revealed similar resource use distribution between years (2016 and 2017), and between diversity treatments (GO vs. GF) (Appendix D, Figs. D2,3), I combined the data from 2016 and 2017 and GF/GO reaches for this analysis. I tested for bimodality in the distributions of each population by using Hartigan’s dip statistic (HDS) from package *diptest* for *R* (Maechler, 2015). The HDS test is detailed on Chapter 4. Briefly, HDS *p*-values below 0.05 indicated significant bimodality which suggest the existence of different resource-use phenotypes within the population (Hartigan & Hartigan, 1985; Freeman & Dale, 2012; Pfister *et al.*, 2013). I estimated the antimodes of the resource use distribution (valleys of the distribution of the proportion of invertebrates) using the function “amps” from the package *modes* for *R* (Deevi, 2016). I used the antimode values to assign a resource-use phenotype for each individual: carnivorous (proportion of invertebrates consumed ≥ antimode) or detritivorous/algivorous (proportion of invertebrates consumed < antimode) and estimated the proportional frequency of phenotypes per reach (e.g. proportion of carnivorous guppies in the entire population in a reach). I focused on the proportion of carnivorous phenotypes for data analysis in order to stay consistent with Chapter 4. The difference in proportional frequency of resource-use phenotypes between urban and non-urban populations was tested using ANOVA.

I grouped individual guppies into 2mm size categories and assessed whether the distribution of body length was related to bimodality in resource use by using the Kolmogorov-Smirnov two-sample test (D). This test checked the similarity between the body length distribution and the resource use distribution, with values of D close to 1 indicating low similarity. The Kolmogorov-Smirnov test was also used to compare the distribution of body size between urban and non-urban populations.

I plotted the body size distribution and the distribution of the proportion of invertebrates consumed in each population using the package *ggplot 2* for R (Wickham, 2009)

5.3.3 Trophic morphology

Following diet analysis, guppies were assessed for trophic morphology (gut ratio and cranium shape). I used the same individuals analyzed for diet with exception of 36 guppies which had their carcasses damaged. A total of 202 guppies (93 urban and 109 non-urban) were assessed for trophic morphology. I gutted individuals and measured the gut length of each guppy to the nearest millimeter to estimate the gut ratio (gut length / guppy body length) and tested for differences in mean gut ratio among populations (urban and non-urban) using ANOVA. Then I passed the gutted carcasses through a series of chemical solutions that cleared skin and muscles, and stained the bones red, following a protocol modified from Taylor & Van Dyke (1985) and Song & Parenti (1995). After clearing and staining, each guppy was photographed (SPOT Imaging, Diagnostic Instruments, Inc.) under a dissecting microscope (Wild Leica – M420, Leica Biosystems). The microscope magnification (10x) and guppy position on the field of view were kept constant to reduce variation to the images between individuals. Individual images were then analyzed for cranium shape, following a geometric morphometrics approach (Zelditch, Swiderski & Sheets, 2012). Images were uploaded into the software tps.Dig version 2.17 for digitizing the cranium landmarks necessary for the shape analysis (Rohlf, 2013). Landmarks are anatomical loci that are homologous among all individuals and provide adequate description of the shape (Zelditch *et al.*, 2012). I determined 8 landmarks on the dorsal plane of the cranium, which together describe the cranium shape (modified from Palkovacs et al. (2011)) (Fig. 5.1a). The description of the anatomical location of each landmark was based on Hernandez, Ferry-Graham & Gibb (2008) and Jalili & Eagderi, (2017). The position (X, Y coordinate) of each landmark for each individual was uploaded into R software. I used the function “gpagen” of the *geomorph* package for R to perform a Generalized Procrustes Analysis of shape (GPA) (Adams & Otárola-Castillo, 2013). This procedure superimposes each individual (i.e. set of landmarks) onto each other by centering, scaling and rotating them (Zelditch *et al.*, 2012). Centering subtracts the coordinate of the centroid (i.e. distance of all landmarks of one individual to the center of the form, this is the measure of size used in geometric morphometrics) from the corresponding X and Y coordinates of each landmark. While the scaling procedure divides the X and Y coordinate of each landmark by the centroid size of that individual. The rotating process then spins each individual to reduce the distance between homologous landmarks. The resulting aligned coordinates (X and Y) of each landmark (procrustes shape variables) represent the cranium shape of each individual (Fig. 5.1b) (Zelditch *et al.*, 2012). Following GPA, 16 vectors of shape (X and Y coordinates for each one of the 8 landmarks that describe cranium shape) plus one vector that describes the geometric size of each individual (i.e. centroid size) are produced (Zelditch *et al.*, 2012). The separation between shape and size data into distinct vectors allowed me to test for a correlation between the two (i.e. test the presence of allometry) (Zelditch *et al.*, 2012).

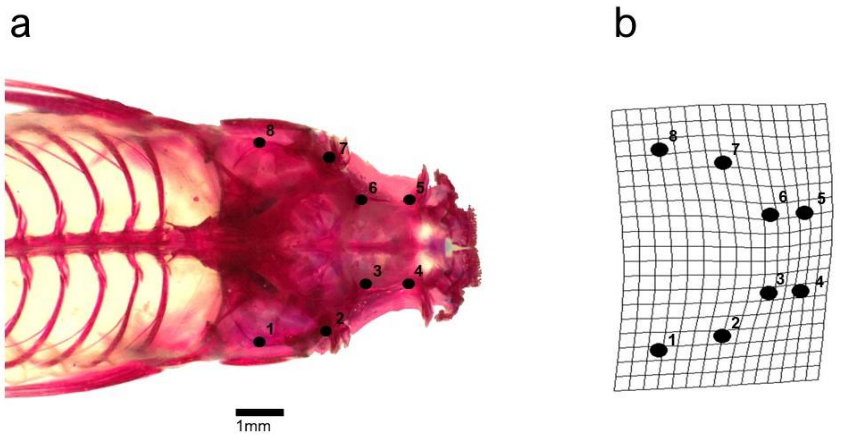

Figure 5. 1. Analysis of the cranium shape of guppies. The panel (a) shows an image from the antero-dorsal view of a female guppy. The bones are stained in red and the numbers show the landmarks used to define the cranium shape. The anatomical loci of each landmark is described as: 1 and 8 = the edge of the pterotic bone, 2 and 7 = posterior region of the sphenotic process, 3 and 6 = the crest of the frontal-parietal bone, 4 and 5 = the intersection between supraorbital part of the frontal bone and the base of the lachrymal bone. The panel (b) shows a thin-

plate spline deformation grid that represents the variation in shape of the individual in panel (a), in relation to the mean of all the individuals in the population. The deformation grid is based on the procrustes shape coordinates obtained after a generalized procrustes analysis using the shape landmarks shown in panel (a).

I performed a Principal Component Analysis (PCA) using the 16 shape vectors from the GPA to assess whether individuals form distinct shape clusters according to the population (urban and non-urban). This analysis was performed on the covariance matrix of the procrustes shape variables from all individuals, using the “prcomp” function of the R software. The PCA was plotted using ggplot2 for R (Wickham, 2009). I used thin-plate spline deformation grids to plot the cranium shape difference between the individuals on the extremes of the main shape axis (PC1) and the mean shape of all individuals using the “plotRefToTarget” function of the *geomorph* package (Adams & Otárola-Castillo, 2013). I tested for differences in cranium shape between urban and non-urban populations using the shape vectors from the GPA through a procrustes ANOVA, with the “procD.lm” function of the *geomorph* package. This function uses a multivariate technique, where the terms in the model are statistically assessed using the procrustes distances among individuals (i.e. the sum of the squared distances between corresponding landmarks after individuals have been centered and rotated) (Adams, Collyer & Kaliontzopoulou, 2019). The procrustes distances are used as a measure of sum of squares which are then evaluated through permutation to obtain a p-value (Goodall, 1991; Anderson, 2001).

I used a Two-block Partial Least Squares analysis to test the allometric relationship between cranium shape and centroid size (i.e. geometric estimate of body size). This analysis described the correlation between two blocks of variables by finding the linear vectors within each block that express the greatest covariance between the blocks (Zelditch *et al.*, 2012). For this, the multidimensional block of shape variables from the GPA was reduced to a linear vector of shape represented by Partial Least Squares scores (PLS scores). This analysis was performed using the “two.b.pls“ function of the *geomorph* package (Adams & Otárola-Castillo, 2013). The distribution of PLS scores for each population and the correlation between the PLS scores describing cranium shape and the centroid size for each individual were plotted using the *ggplot2* package for R.

I further assessed the relationship between intrapopulation variation in diet (resource-use phenotype) and trophic morphology (gut ratio and cranium shape). I tested for differences in gut ratio and cranium shape (16 shape vectors from the GPA analysis) between resource-use phenotypes within each population using ANOVA and procrustes ANOVA, respectively.

5.3.4 Testing the relationship between population density, intrapopulation dietary variation and trophic morphology

I built linear mixed effect models (LMM) for urban and non-urban populations separately because the drivers of diet and trait change can vary between these environments. The response variable was the proportion of carnivores within the reach and the predictor variables were guppy density (GD, ind/m^2), chironomid biomass (mg/m^2), fish biodiversity (guppy only or guppy with other fish, GO vs GF), guppy length (SL, mm) as fixed factors and reach identity (RI) nested within year (YR) as the random factor. I built a second model with mean gut ratio per reach as a response variable and the same fixed and random factors as before, except for guppy length (SL) because gut ratio already accounts for the confounding effect of body length. I included body length in previous models because body size can potentially affect guppy diet (Zandonà *et al.*, 2015). I included fish biodiversity in all models because the presence of predators and competitors can affect guppy diet (Zandonà *et al.*, 2011). I included reach within year as a random factor because geographical and temporal variation in resources availability could affect individual diets.

Then, I built a procrustes LMM which uses the cranium procrustes shape variables (16 vectors of shape from the GPA analysis) as the response variable, following the same statistical technique as the procrustes ANOVA explained previously. I used the same fixed effects as before but replacing body length (SL) with centroid size (CS), which is how body size information is stored after procrustes analysis. I nested reach (RI) as a random effect. I included fish biodiversity in these models because the presence of other fish species such as predators can potentially affect guppy morphology (Torres-Dowdall *et al.*, 2012). Sampling year (YR) was not included in this model because *a priori* analyses showed that cranium shape was not related to year (Appendix D, Fig. D4). The models for gut length ratio were fitted using the "lme" function from the *nlme* package for *R* (R Core Team, 2018). While the models for cranium shape were fitted using the "procD.lm" function from the *geomorph* package for R (Adams & Otárola-Castillo, 2013).

5.4 Results

5.4.1 Diet modality

Guppies had similar mean diets in both urban and non-urban populations. Guppies fed on large proportions of detritus (0.62±0.04 and 0.58±0.03, respectively), followed by invertebrates (0.36±0.04 and 0.35±0.03 respectively) and algae (0.01±0.003 and 0.02±0.01, respectively). Most of the invertebrates found in the diet were aquatic insect larvae (>90% of the total invertebrate consumed in both populations). The bulk diet of all urban populations together was composed of 3 families of aquatic insects, while the bulk diet of all non-urban populations grouped together was more diverse, being composed of 21 aquatic insect families. Chironomids were the most consumed aquatic insect and constitute 23% of the diet of urban population and 3% of the diet of the non-urban population. No mosquitos, family Culicidae, were found in guppy guts (Appendix D, Table D1).

I found significant bimodality in the distribution of the proportion of invertebrates consumed in both urban and non-urban populations (HDS p-value <0.001 for both populations) (Fig. 5.2a). Bimodality was not related to guppy length (Kolmogorov-Smirnov D=1, p<0.01) (Fig. 5.2b), meaning that carnivores and herbivores did not vary in size. The separation of the populations in two groups (marked by the valley of the bimodal distribution or antimode) occurred when the proportion of invertebrates consumed was 0.51 and 0.45 of total gut content area for urban and non-urban populations, respectively (Fig. 5.2a). Based on that, I assigned a carnivorous resource-use phenotype for guppies that feed mostly on invertebrates (proportion of invertebrates consumed ≥ antimode). Because individuals with empty guts were excluded and because there was an inverse relationship between the consumption of invertebrates and the consumption of detritus/algae (Appendix D, Fig. D1), I assigned a detritivorous/algivorous resource-use phenotype for guppies which had proportion of invertebrates consumed smaller than the antimode. Although non-urban populations had relatively larger number of detritivorous/algivorous guppies than urban guppies, I did not find significant difference in the proportion of carnivorous fish between urban and non-urban populations. The LMM models showed no effect of food availability, density, or fish biodiversity on the frequency of the carnivorous phenotypes (Appendix D, Table D2).

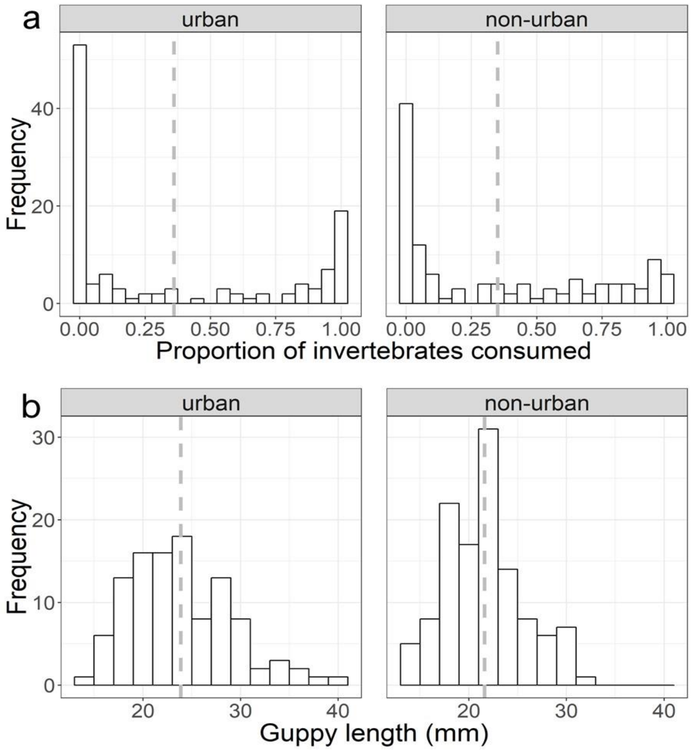

Figure 5. 2. Distribution plots of the (a) proportion of invertebrates consumed (out of the total gut contents area (mm^2) and (b) body length (distributed into 2mm categories) in urban and non-urban populations. Dashed line shows the population average. Data were combined across years (2016-2017) and across biodiversity treatments (streams where guppies are the only fish species or streams where guppies co-occur with other fish).

5.4.2 Trophic morphology

Urbanization had a significant effect on some but not all trophic morphology traits. There was no difference in gut ratio between resource-use phenotypes within urban or non-urban populations. The LMM models showed no effect of guppy density, chironomid biomass or fish biodiversity on gut ratio in both populations (Appendix D, Table D2). A PCA analysis of cranium shape showed a slight tendency for urban guppies to cluster on the right of PC1, towards a shorter-wider cranium shape, while non-urban guppies showed a tendency to cluster towards the left side of PC1 (Fig. 5.3). The PCA loadings can be found in the appendix (Appendix D, Table D3). The procrustes ANOVA test confirmed moderate shape difference between urban and non-urban guppies (ANOVA, $F_{1,200}$ =5.4, p=0.001). An allometric effect also exists because larger individuals had shorter/wider crania (Partial Least Squares correlation coefficient=0.55, p=0.001) (Fig. 5.4). Because urban guppies are larger than non-urban guppies (Fig. 5.2b) (Kolmogorov-Smirnov test, p<0.01), differences in cranium shape between urban and non-urban guppies were enhanced by differences in body size between the populations.

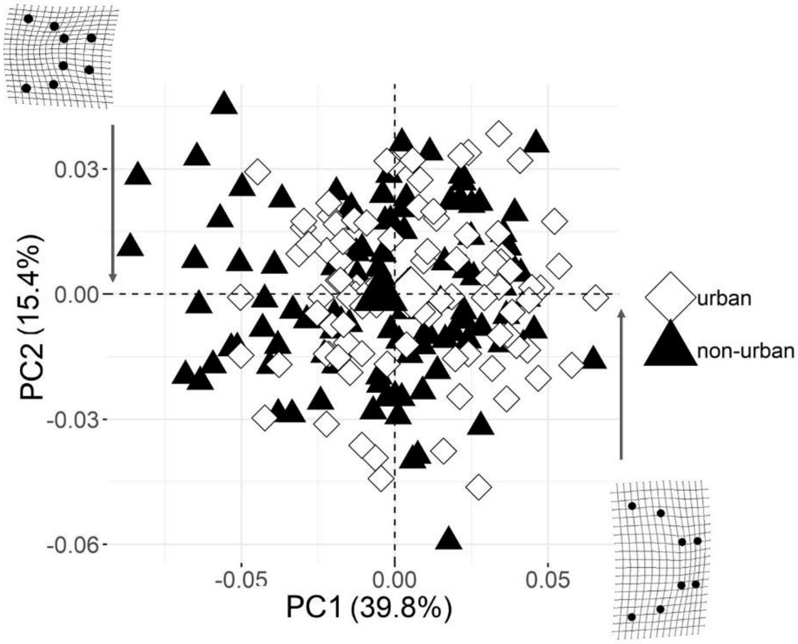

Figure 5. 3. Principal Component Analysis (PCA) of cranium shape. Cranium shape for each individual was obtained from a set of 8 anatomical landmarks. The landmarks were converted into 16 shape variables following a Generalized Procrustes Analysis. The 16 vectors of shape were used the a PCA analysis. Each point represents data from an individual in urban (white diamonds) and non-urban (black triangles) populations. Large symbols represent the mean cranium shape of each population. The shape variation is shown as deformation grids of the difference between the specimens on the extremes of the main shape axis (PC1). Individuals towards the left side of PC1 have more narrow/long cranium shape, while individuals on the right have more wide/short cranium shape. The effect of body size is removed from this analysis. Deformation grids were plotted with 1.5x magnification to facilitate visualization of shape differences Data were combined across years (2016-2017) and across biodiversity treatments (streams where guppies are the only fish species or streams where guppies co-occur with other fish).

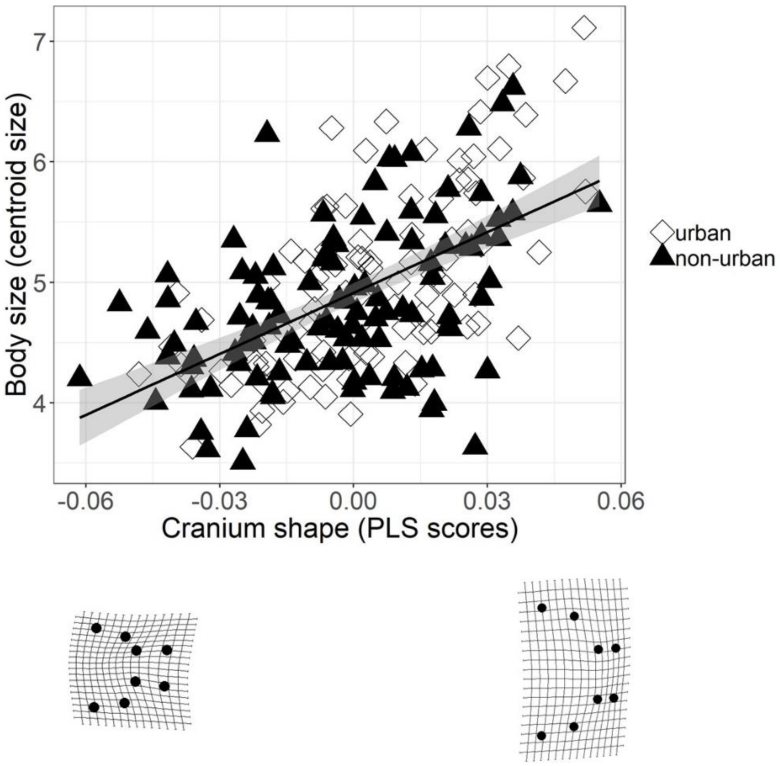

Figure 5. 4. The relationship between cranium shape and geometric body size (i.e. centroid size). Cranium shape for each individual was obtained from a set of 8 anatomical landmarks. The landmarks were converted into 16 shape variables following a Generalized Procrustes Analysis. The 16 vectors of shape were projected into a singular vector represented by the Partial Least Square scores (PLS scores) that describes the cranium shape of each individual were correlated with the centroid size which is a measure of body size used in shape analysis. Each symbol represents one individual in urban (black circles) and non-urban (grey triangles) populations. Lower panels show the shape deformations grids based on the difference between the mean shape of all individuals and the specimens with minimum (left) / maximum (right) Partial Least Square scores. Deformation grids were magnified 1.5x to facilitate visualization of shape differences. Data were combined across years (2016-2017) and across biodiversity treatments (streams where guppies are the only fish species or streams where guppies co-occur with other fish).

Individuals with shorter and wider crania consumed more chironomids (PLS scores correlation coefficient= 0.33, p=0.002) (Fig. 5.5). Urban populations had higher frequency of individuals with high PLS scores than non-urban populations which suggests that urban populations have more guppies with shorter/wider cranium (Fig. 5.6).

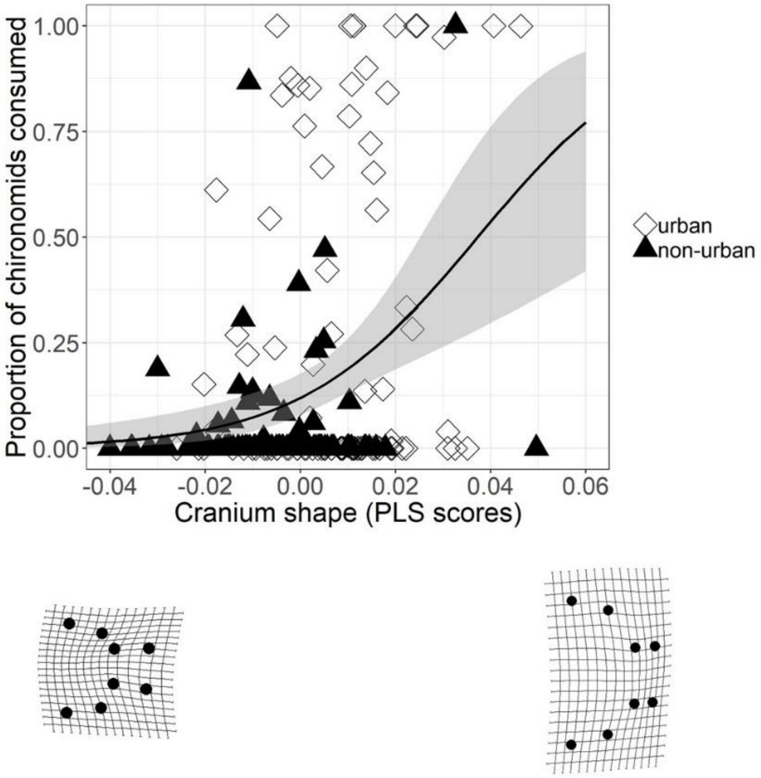

Figure 5. 5. The relationship between cranium shape and the consumption of chironomids (midge larvae). Cranium shape for each individual was obtained from a set of 8 anatomical landmarks. The landmarks were converted into 16 shape variables following a Generalized Procrustes Analysis. The 16 vectors of shape were projected into a singular vector represented by the Partial Least Square scores (PLS scores) that describes the cranium shape of each individual were correlated with the proportion of chironomids consumed. The proportion of chironomids was estimated considering the amount (area of a gridded slide, mm) of all the food items consumed. Each symbol represents one individual in urban (black circles) and non-urban (white triangles) populations. Lower panels show the shape deformations grids based on the difference between the mean shape of all individuals and the specimens with minimum (left) / maximum (right) Partial Least Square scores. Deformation grids were magnified 1.5x to facilitate visualization of shape differences. Data were combined across years (2016-2017) and across biodiversity treatments (streams where guppies are the only fish species or streams where guppies co-occur with other fish).

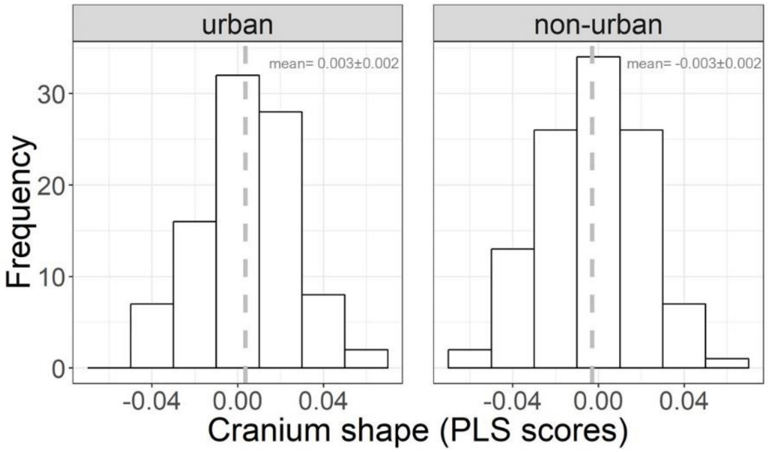

Figure 5. 6. Distribution of the Partial Least Squares scores obtained from 16 vectors of shape that were projected into a singular vector (PLS scores) that describe cranium shape in urban and non-urban populations. Dashed line shows the population average Urban populations have increased frequency of guppies towards positive scores (shorter/wider cranium). Data were combined across years (2016-2017) and across biodiversity treatments (streams where guppies are the only fish species or streams where guppies co-occur with other fish).

In addition to body size, the procrustes LMM showed that chironomid biomass had a small effect favoring shorter/wider crania in both urban and non-urban guppies ($F_{6,82}$=1.8, p<0.01 and $F_{6,96}$=4.8, p<0.01, respectively) (Table 5.1). Guppy density had a small effect, favoring shorter/wider crania ($F_{1,82}$=3.1, p<0.01), while fish diversity slightly reduced cranium length and width ($F_{3,96}$=2.1, p<0.01) (Table 5.1). There were no differences in cranium shape between resource-use phenotypes.

Table 5. 1. Procrustes linear model for cranium shape. Urban and non-urban populations were modeled separately. Each model assessed the amount of shape variation attributed to centroid size (variation on shape attributed to body size, CS), chironomid biomass (CB, mg/m^2), fish biodiversity (guppy only (GO) and guppy co-occurring with other fish species (GF)) and guppy density (ind/m^2). In both models, reach identity was included as a nested random effect (not shown). Where, SS= sum of squares and MS= mean square.

Populations	Fixed factors	Model coefficients				
		df	SS	MS	F	p-value
Urban	CS	1	0.021	0.021	12.1	0.001
	CB	6	0.019	0.003	1.8	0.001
	GOvsGF	2	0.004	0.002	1.2	0.105
	GD	1	0.005	0.005	3.1	0.003
	Residuals	82	0.143	0.002		
Non-urban						
	CS	1	0.015	0.015	8.4	0.001
	CB	6	0.051	0.008	4.8	0.001
	GOvsGF	3	0.011	0.004	2.1	0.002
	GD	2	0.003	0.002	0.95	0.187
	Residuals	96	0.170	0.002		

5.5 Discussion

In this chapter I have investigated how urbanization affects intrapopulation dietary variability and trophic traits in urban stream biota. While other studies have examined mean diet responses of guppies in non-urban streams (Zandonà *et al.*, 2011; Sullam *et al.*, 2015), mine is the first to explore intrapopulation variation, and to link diet and trophic morphology in urban streams. I have shown that guppies, a successful urban dweller, have intrapopulation variation in resource use. Within populations, urban and non-urban guppy populations are bimodal, suggesting they are composed of two groups of individuals with distinct resource-use phenotypes: carnivorous (high consumption of invertebrates, particularly chironomids) and detritivorous\algivorous (low consumption of invertebrates).

I have also shown that urbanization can potentially relax the effect of density in regulating intrapopulation variation. Evidence from non-urban systems often suggests that density regulates intraspecific competition and determines intrapopulation variation through its effect on food availability (Chapter 4, Svanbäck & Persson (2009), Araújo *et al.* (2011)). However, here I show that density has no effect on intrapopulation variation (Appendix D, Table D2). Although urban guppies occur under densities that are up to 26x higher than non-urban guppies (Chapter 3), both urban and non-urban populations have similar frequency of resource-use phenotypes (Fig. 5.2a). In the introduction I hypothesized that this pattern might be caused by differences in food availability because invertebrate biomass is much higher in urban streams than non-urban streams (Chapter 3), and high food availability would dampen the effect of density on intrapopulation variation (Newsome *et al.*, 2015b). However, the LMM model did not show a significant correlation between chironomid biomass and the frequency of the carnivorous phenotypes. It is possible that a per-capita estimate of food availability, which is the ratio between the amount of all food items available and the number of individuals, can clarify these relationships further. Per-capita food resources are likely higher in urban than non-urban streams because of the high productivity and human food subsidies (Alberti, 2008; Becker *et al.*, 2018), which can explain the reduced effect of density to intrapopulation variation. However, estimating per-capita food resources is difficult because guppies are omnivorous and feed on food items such as detritus for which the per-capita availability is very difficult to estimate reliably.

The reduced effect of density on intrapopulation variation can also be related to the fact that guppies observed in this study are invasive species (Rocha *et al.*, 2011). Rapid changes to traits of invasive species can enhance the tolerance to intraspecific competition (Warren *et al.*, 2019), with consequences to intrapopulation variation. This can help explain why a relatively minor difference in guppy density between the native HP and LP Trinidadian populations (mean difference = 3 ind/m^2, Chapter 4) affects the frequency of resource-use phenotypes, while major differences in guppy density between Brazilian urban and non-urban populations (mean difference = 75 ind/m^2, Chapter 3) do not affect the frequency of resource-use phenotypes. This contrast suggests that although urban dwellers often attain large densities (Møller *et al.*, 2012; Feng & Himsworth, 2014), this is not necessarily linked to increased intrapopulation variation in resource use, as previously observed in some terrestrial species (Newsome *et al.*, 2015a). Experiments that manipulate population density and closely track intrapopulation diet variation, such as performed by Svanback and Bolnick (2007), can be useful to clarify the extent to which the traits of urban invaders dampen the effect of intraspecific competition in regulating intrapopulation variation.

My data also suggest that urbanization can increase feeding efficiency of guppies through changes in feeding morphology. In Chapter 4, I concluded that carnivory is associated with variation in gut ratio, while previous work on Trinidadian guppies has shown that carnivory is associated with variation to cranium structure (Palkovacs et al. 2011). Although I find that gut ratio is similar among urban and non-urban guppies, my data show that urban populations have more guppies with short/wide crania that feed more on chironomids than non-urban populations (Fig. 5.5). This effect is enhanced by the large size of guppies in urban streams: larger guppies have shorter/wider crania (Fig. 5.4). Guppies with shorter and wider crania more efficiently feed on chironomids (Palkovacs *et al.*, 2011). Thus, urbanization not only increases chironomid availability (which reduces search time) but also sharpens guppy feeding efficiency through its effects on cranium shape and body size. Increased feeding efficiency in urban dwellers has also been shown in doves, *Zenaida asiatica* and *Zenaida macroura*, and gray squirrels, *Sciurus carolinensis* (Bowers & Breland, 1996; Shochat, 2004). This mechanism has been hypothesized to contribute broadly to the success of urban dwellers (Shochat *et al.*, 2010), and to the best of my knowledge this is the first supporting evidence from urban streams. However, the link between head shape and feeding efficiency needs to be verified in feeding experiments. Also, studies from native, non-urban populations in Trinidad suggest female guppy morphology has a heritable component, but the influence of phenotypic plasticity is not yet discarded (Robinson & Wilson, 1995; Palkovacs *et al.*, 2011). A next step is to assess whether variation in morphology of urban guppies is heritable and whether evolutionary processes contribute to the success of guppies in urban streams.

Exposing the environmental and genetic drivers of dietary change between urban and non-urban populations is fundamental to better understand the persistence of urban dwellers because nutrition is linked to survival and reproductive success (Lowe, Wilder & Hochuli, 2016; Pollock *et al.*, 2017). This information can help move urban ecology towards understanding the mechanisms responsible for controlling biodiversity in cities (McDonnell & Hahs, 2013).

5.6 Coauthor contributions

Rana El-Sabaawi[1] and Rosana Mazzoni[2] helped with experimental design; Luisa R. Manna[2] assisted with data collection; Rana El-Sabaawi[1] helped with analyzing the data and writing the manuscript.

[1] University of Victoria, Victoria, BC, Canada;

[2] Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Chapter 6 – Conclusions

6.1 Intraspecific trait-based approaches are important for exposing the mechanisms shaping urban stream biota

In this thesis I used an intraspecific trait approach to help understand how urban dwellers succeed in urban streams. I began by reviewing the literature and developing an intraspecific trait-based framework that links the effects of urbanization in streams to traits of the biota. I showed that such a framework could provide mechanistic hypotheses and predictions about ecological change in urban streams. The use of this framework can help future research to deliver the much-needed information on the factors shaping the structure and functioning of urban ecosystems (McDonnell & Hahs, 2013; Hahs & Evans, 2015). To the best of my knowledge, this was the first study to offer a mechanistic framework to investigate the effects of urbanization on the intraspecific traits of stream biota.

My review highlighted two sets of traits that would be interesting to study in the urban environment: life history and feeding related traits. I applied the proposed framework to study intraspecific trait variation in urban guppies, important globally distributed urban dwellers. I characterized intraspecific variation on two different levels: comparing population means between urban and non-urban guppies, and also looking at the trait distributions within each population. Both levels of intraspecific trait variation suggest that the consumption of chironomids is important for the success of guppies in urban streams. When considering the trait variation between urban and non-urban populations, I have shown that guppies take advantage of the high food availability in urban streams by consuming more chironomids (midge larvae) which are highly nutritious food. This allowed urban guppies to invest more towards life history traits that enhanced their success as seen by their high population density. Thus, the increased consumption of chironomids appeared to be key for the success of urban guppies. To further understand the diet differences between urban and non-urban guppies I looked at differences on diet and feeding related traits within populations. I found that urban populations have more individuals with short/wide crania which is thought to be more efficient to feed on chironomids. This helped explain that the high consumption of chironomids by urban guppies is not only an opportunistic response to the increased chironomid availability, but it also involved traits that enhanced feeding efficiency. Studies have shown that urbanization has an enormous potential to drive trait changes (Alberti *et al.*, 2017a,b; Brans *et al.*, 2017), but the specific mechanisms relating urbanization to trait change of the stream biota are largely unknown and have only recently started to be explored (e.g. Kern & Langerhans, 2019). The trophic mechanism suggested in my thesis is likely widespread because chironomids are often found in large densities in urban streams and not only guppies but also other urban dwellers have been shown to take advantage of this food resource (Moreyra & Padovesi-Fonseca, 2015; Sterling, Rosemond & Wenger, 2016; Kelly, Cuevas & Ramírez, 2019).

6.2 Factors affecting trait change in urban streams can widely differ from non-urban streams

Studies from non-urban systems suggest that population density and biodiversity can determine intraspecific trait changes. Density affects the amount of food available per-capita, thus can regulate the strength of intraspecific competition. This has been shown to determine changes to life history traits, individual diet specialization, and feeding related traits in a number of species (e.g. Mueller, Guo & Ayala, 1991; Svanback & Bolnick, 2007; Araújo *et al.*, 2011). Biodiversity, either through interspecific competition or predation risk has also been shown to drive changes to traits such as life history, diet and feeding morphology (Peckarsky *et al.*, 2002; Eklov & Svanback, 2006; Bolnick *et al.*, 2010). However, how density and biodiversity affect traits of the urban biota is largely unknown.

In my thesis I used guppies, which are an extensively used model to study life history evolution in their non-urban native range. Throughout my study I used this information to create hypotheses to test on guppies in urban systems. I relied on extensive published data to create the hypotheses I tested in Chapter 3 and conducted my own study of guppy diet variation in Trinidad (Chapter 4) in order to develop a better mechanistic understanding prior to conducting a dietary study on urban guppies. I have shown on Chapters 3 and 5 that density and biodiversity had low to moderate effects on guppy diet, life history and feeding related traits in urban systems. This suggests the drivers of trait change in urban streams differ from non-urban streams. However, my findings are based on correlation analyses which do not necessarily indicate a causal relationship, thus laboratory testing is necessary to confirm that urbanization (i.e. urban drivers of trait change such as high food availability) reduces the effect of density and biodiversity on guppy diet and traits. Moreover, guppies are an invasive species in the urban streams considered for this study and this can be a potential confounding factor. Invasive species often show changes to diet and traits during the invasion process (Whitney & Gabler, 2008; Tonella *et al.*, 2018). Thus, changes to urban guppies can be a combination of traits that were selected during the invasion process and further influenced by urbanization. This is likely the case for the majority of urban guppies throughout the globe.

6.3 Studying urban systems can help us better understand contemporary evolution

Urbanization has a homogenizing effect on ecosystems, which means that cities around the world have similar climate, hydrology, soils and biodiversity (Walsh *et al.*, 2005; McKinney, 2006; Groffman *et al.*, 2014). This similarity suggests that selective pressure for trait change and evolution are repeated among cities worldwide. The extent to which urbanization causes evolution of stream biota is generally unknown, but recent studies have shown it is likely (Kern & Langerhans, 2018). Future research can take advantage of the repeatability of the urban conditions and focus on widespread urban dwellers, such as guppies, to design a highly replicated global experiment of urban evolution (Donihue & Lambert, 2015; Johnson & Munshi-South, 2017). Such studies are already on-going for urban plants (www.globalurbanevoluti.wixsite.com). This can be used to test the pace and repeatability of urban evolution of the stream biota at a global scale, a topic of interest to ecologists and evolutionary biologists because it can help us understand convergent patterns of selection, adaptation and speciation in cities (Johnson & Munshi-South, 2017; Thompson, Rieseberg & Schluter, 2018). Such information can help managers and practitioners to improve urban design, conservation, pest management and education (Rivkin *et al.*, 2019). Guppies are good candidate species for a global scale evolutionary experiment because they are a widespread urban dweller in the Tropics and because they have been successfully used to test contemporary evolution in non-urban systems (Reznick *et al.*, 1997; O'Steen, Cullum & Bennett, 2002; Deacon *et al.*, 2011). However, other urban dwellers such as the western Blacknose Dace, *Rhinichthys obtusus*, can be a better choice for experiments in Temperate areas where they are widely distributed and have also been successfully used to test contemporary evolution (Yates & Bailey, 2009; Wallace, Croft-White & Moryk, 2013; Kern & Langerhans, 2018).

A next step is to assess the effect of trait change (either evolutionary or plastic) on the role of organisms in the urban ecosystem. Studies have long shown that changes to traits can be important for ecosystem processes such as nutrient dynamics and primary productivity (Vanni, 2002; Bassar *et al.*, 2010). Theory suggests that urban mediated changes to traits can affect ecosystem processes, but empirical evidence is rare (Alberti *et al.*, 2017b). This information can be important to assess urban mediated eco-evolutionary feedbacks which have already been hypothesized but never tested (Alberti, 2015).

6.4 Better understanding evolutionary and ecological concepts in an increasingly urbanized world is pressing

Urban ecosystems integrate social, ecological and technical infrastructure in a complex system of interactions that widely differ from non-urban systems (McPhearson *et al.*, 2016). These novel ecosystems provide us with the opportunity to test current evolutionary and ecological concepts under unique conditions. Recent studies have hypothesized that some evolutionary and ecological concepts have to be refined to be suitable for studying human altered systems (Alberti, 2015; Snell-Rood *et al.*, 2015; Grimm *et al.*, 2017). For example, my research suggests that predictions based on current concepts such as intraspecific competition and life history trait evolution theory which are based on assumptions of strict resource limitation, require careful consideration before being applied to urban stream systems. Refining ecological concepts and evolutionary theories can enhance our capacity to predict and manage ecological change in an increasingly urbanized world (Barot *et al.*, 2019).

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Appendix A – Supplementary material of Chapter 2

Table A1. Examples of trait change from non-urban systems. The link between agents and trait change is known for non-urban systems with species that inhabit urban streams. Agents of trait change are defined based on the Urban Stream Syndrome (USS) symptoms (Walsh *et al.*, 2005).

Agent of change based on USS symptoms	**Species**	**Driver of change**	**Trait**	**Reference supporting trait change**	**Reference supporting presence in urban area**
Reduced biotic richness					
	Gammarus pulex	Predation	Behavior	Ahlgren, J., K. Åbjörnsson, and C. Brönmark. 2011. The influence of predator regime on the behaviour and mortality of a freshwater amphipod, *Gammarus pulex*. Hydrobiologia 671:39–49.	Gücker, B., M. Brauns, A. G. Solimini, M. Voss, N. Walz, and M. T. Pusch. 2011. Urban stressors alter the trophic basis of secondary production in an agricultural stream. Canadian Journal of Fisheries and Aquatic Sciences 68:74–88.
	Hyla chrysoscelis	Predation	Morphology	McCollum, S. A., and J. D. Leimberger. 1997. Predator-induced morphological changes in an amphibian: Predation by dragonflies affects tadpole shape and color. Oecologia 109:615–621.	Birx-Raybuck, D. A., S. J. Price, and M. E. Dorcas. 2010. Pond age and riparian zone proximity influence anuran occupancy of urban retention ponds. Urban Ecosystems 13:181–190.

Hyla squirella	Predation	Behavior	McCoy, M. W., and B. M. Bolker. 2008. Trait-mediated interactions: influence of prey size, density and experience. Journal of Animal Ecology 77:478–486.	Pham, L., S. Boudreaux, S. Karhbet, B. Price, A. S. Ackleh, J. Carter, and N. Pal. 2007. Population estimates of *Hyla cinerea* (Schneider)(Green Tree Frog) in an urban environment. Southeastern Naturalist 6:203–216.
Helisoma trivolvis, H.campanulata and *H.anceps*	Predation	Morphology	Hoverman, J. T., R. D. Cothran, and R. A. Relyea. 2014. Generalist versus specialist strategies of plasticity: snail responses to predators with different foraging modes. Freshwater Biology 59:1101–1112.	Goulet, R. R., E. N. Leclair, and F. R. Pick. 2001. The evaluation of metal retention by a constructed wetland using the pulmonate gastropod *Helisoma trivolvis* (Say). Archives of Environmental Contamination and Toxicology 40:303–310.
				Scoggins, M., N. L. McClintock, L. Gosselink, and P. Bryer. 2007. Occurrence of polycyclic aromatic hydrocarbons below coal-tar-sealed parking lots and effects on stream benthic macroinvertebrate communities. Journal of the North American Benthological Society 26:694–707.
Rana temporaria	Predation	Life history	Laurila, A., J. Kujasalo, and E. Ranta. 1998. Different antipredator behaviour in two anuran tadpoles: effects of predator diet. Behavioral Ecology and Sociobiology 40:329–336.	Hitchings, S. P., and T. J. Beebee. 1997. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. Heredity 79:117–127.

Altered hydrography and hydrological processes	*Poecilia reticulata*	Predation	Life history	Reznick, D. N. 2001. The population ecology of contemporary adaptations: what empirical. Genetica 112:183–198.	Alexandre, C. V., K. E. Esteves, and M. A. M. de Moura e Mello. 2010. Analysis of fish communities along a rural-urban gradient in a neotropical stream (Piracicaba River Basin, São Paulo, Brazil). Hydrobiologia 641:97–114.
	Rana catesbeiana	Competition	Life history	Kupferberg, S. J. 1997. Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. Ecology 78:1736–1751.	Pillsbury, F. C., and J. R. Miller. 2008. Habitat and landscape characteristics underlying anuran community structure along an urban-rural gradient. Ecological Aplications 18:1107–1118.
	Cyprinella venusta	High flow	Life history	Machado, M. D., D. C. Heins, and H. L. Bart. 2002. Microgeographical variation in ovum size of the blacktail shiner, *Cyprinella venusta* Girard, in relation to streamflow. Ecology of Freshwater Fish 11:11–19.	Albanese, B., and G. Matlack. 1999. Utilization of parking lots in Hattiesburg, Mississippi, USA, and impacts on local streams. Environmental Management 24:265–271.
	Notropis atherinoides, Labidesthes sicculus, Lepomis macrochirus	Low flow	Morphology	Franssen, N. R., J. Harris, S. R. Clark, J. F. Schaefer, and L. K. Stewart. 2013. Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. Proceedings of the Royal Society B-Biological Sciences 280:20122715.	Koryak, M., R. H. Hoskin, R. J. Reilly, and L. J. Stafford. 2001. The impact of above grade sewerline crossings on the distribution and abundance of fishes in recovering small urban streams of the Upper Ohio River Valley. Journal of Freshwater Ecology 16:591–598.

					Walker, R. P., A. C. O’Toole, Z. Whynot, K. C. Hanson, and S. J. Cooke. 2010. Evaluation of the aquatic habitat and fish assemblage in an urban reach of the historic Rideau Canal, Ottawa, Canada: Implications for management in an engineered system. Urban Ecosystems 13:563–582.
					Otter, R. R., J. Meier, K. M. Kubach, J. M. Lazorchak, and S. J. Klaine. 2012. The effects of urbanization on *Lepomis macrochirus* using the comet assay. Ecotoxicology and Environmental Safety 84:299–303.
	Physa acuta	Low flow	Morphology	Gustafson, K. D., B. J. Kensinger, M. G. Bolek, and B. Luttbeg. 2014. Distinct snail (Physa) morphotypes from different habitats converge in shell shape and size under common garden conditions. Evolutionary Ecology Research 16:77–89.	Blakely, T. J., and J. S. Harding. 2005. Longitudinal patterns in benthic communities in an urban stream under restoration. New Zealand Journal of Marine and Freshwater Research 39:17–28.
Elevated nutrient concentration	Diatoms	Nutrient depletion	Lipid content	Fields, F. J., and J. P. Kociolek. 2015. An evolutionary perspective on selecting high-lipid-content diatoms (Bacillariophyta). Journal of Applied Phycology 27:2209–2220.	Walker, R. P., A. C. O’Toole, Z. Whynot, K. C. Hanson, and S. J. Cooke. 2010. Evaluation of the aquatic habitat and fish assemblage in an urban reach of the historic Rideau Canal, Ottawa, Canada: Implications for management in an engineered system. Urban Ecosystems 13:563–582.

Daphnia spp	Food availability/ quality	Life history	Snell-Rood, E., R. Cothran, A. Espeset, P. Jeyasingh, S. Hobbie, and N. I. Morehouse. 2015. Life-history evolution in the anthropocene: effects of increasing nutrients on traits and trade-offs. Evolutionary Applications 8:635–649.	Moore, M., S. Pierce, H. Walsh, S. K. Kvalvik, and J. D. Lim. 2000. Urban light pollution alters the diel vertical migration of Daphnia. Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen 27:779–782.
Pomacea canaliculata	Food availability	Life history	Tamburi, N. E., and P. R. Martín. 2011. Effects of food availability on reproductive output, offspring quality and reproductive efficiency in the apple snail *Pomacea canaliculata*. Biological Invasions 13:2351–2360.	Yamanishi, Y., K. Yoshida, N. Fujimori, and Y. Yusa. 2012. Predator-driven biotic resistance and propagule pressure regulate the invasive apple snail *Pomacea canaliculata* in Japan. Biological Invasions 14:1343–1352.
Spea spp.	Food quality	Morphology	Ledón-Rettig, C. C., and D. W. Pfennig. 2011. Emerging model systems in eco-evo-devo: the environmentally responsive spadefoot toad. Evolution and Development 13:391–400.	Jansen, K. P., A. P. Summers, and P. R. Delis. 2001. Spadefoot toads (*Scaphiopus holbrookii holbrookii*) in an urban landscape: effects of nonnatural substrates on burrowing in adults and juveniles. Journal of herpetology 35:141–145.
Poecilia reticulata	Food availability	Life history / Morphology	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. Robinson, B. W., and D. S. Wilson. 1995. Experimentally induced morphological diversity in Trinidadian guppies (*Poecilia reticulata*). Copeia 2:294–305.	Zanatta, N., L. H. R. Pazianoto, V. M. Cionek, P. A. Sacramento, and E. Benedito. 2017. Population structure of fishes from an urban stream. Acta Scientiarum 39:27.

	Gammarus fasciatus	Food quality	Life history	Delong, M. D., B. R. Summers, and J. H. Thorp. 1993. Influence of food type on the growth of a riverine amphipod, *Gammarus fasciatus*. Canadian Journal of Fisheries and Aquatic Sciences 50:1891–1896.	Debruyni, A. M. H., and J. B. Rasmussen. 2002. Quantifying assimilation of sewage-derived organic matter by riverine benthos. Ecological Aplications 12:511–520.
	Salmo Salar	Food quality	Life history	Jonsson, B., N. Jonsson, and A. G. Finstad. 2013. Effects of temperature and food quality on age and size at maturity in ectotherms: an experimental test with Atlantic salmon. Journal of Animal Ecology 82:201–210.	Riley, W. D., P. I. Davison, D. L. Maxwell, and B. Bendall. 2013. Street lighting delays and disrupts the dispersal of Atlantic salmon (*Salmo salar*) fry. Biological Conservation 158:140–146.
	Amphilophus citrinellus	Food quality	Morphology	Muschick, M., M. Barluenga, W. Salzburger, and A. Meyer. 2011. Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. BMC evolutionary biology 11:11:116.	Kwik, J. T. B., Z. Y. Kho, B. S. Quek, H. H. Tan, and D. C. J. Yeo. 2013. Urban stormwater ponds in Singapore: potential pathways for spread of alien freshwater fishes. BioInvasions Records 2:239–245.
Contaminants					
	Daphnia spp.	Heavy metal	Life history	Knops, M., R. Altenburger, and H. Segner. 2001. Alterations of physiological energetics, growth and reproduction of *Daphnia magna* under toxicant stress. Aquatic Toxicology 53:79–90.	Brans, K. I., M. Jansen, J. Vanoverbeke, N. Tuzun, R. Stoks, and L. De-Meester. 2017. The heat is on: genetic adaptation to urbanization mediated by thermal tolerance and body size. Global Change:DOI: 10.1111/gcb.13784.
	Dreissena polymorpha	Pharmaceutical	Life history	Fong, P. P., and N. Molnar. 2008. Norfluoxetine induces spawning and parturition in estuarine and freshwater bivalves. Bulletin of Environmental Contamination and Toxicology 81:535–538.	Contardo-Jara, V., and C. Wiegand. 2008. Molecular biomarkers of *Dreissena polymorpha* for evaluation of renaturation success of a formerly sewage polluted stream. Environmental Pollution 155:182–189.

Gambusia affinis	Heavy metal	Life history	Franssen, C. M. 2009. The effects of heavy metal mine drainage on population size structure, reproduction, and condition of western mosquitofish, *Gambusia affinis*. Archives of Environmental Contamination and Toxicology 57:145–56.	Batty, J., and R. Lim. 1999. Morphological and reproductive characteristics of male mosquitofish (*Gambusia affinis holbrooki*) inhabiting sewage-contaminated waters in New South Wales, Australia. Archives of Environmental Contamination and Toxicology 36:301–307.
Gambusia affinis, Heterandria formosa	Sterol hormones	Morphology	Bortone, S. A., and W. P. Davis. 1994. Fish intersexuality as indicator of environmental stress. BioScience 44:165–172.	Bielmyer-Fraser, G. K., M. N. Waters, C. G. Duckworth, P. P. Patel, B. C. Webster, A. Blocker, C. H. Crummey, A. N. Duncan, S. N. Nwokike, C. R. Picariello, J. T. Ragan, E. L. Schumacher, R. L. Tucker, E. A. Tuttle, and C. R. Wiggins. 2017. Assessment of metal contamination in the biota of four rivers experiencing varying degrees of human impact. Environmental Monitoring and Assessment 189.

Table A2. List of peer reviewed papers that used an interspecific trait approach in urban stream ecosystems. This list of studies was derived from a literature search on Web of Science™ based on the following key words: “urban AND stream AND trait”, “urban AND biomonitoring” and “functional AND trait AND urban AND stream”.

Paper full citation
Bagatini, Y. M., R. L. Delariva, and J. Higuti. 2012. Benthic macroinvertebrate community structure in a stream of the north-west region of Paraná State, Brazil. Biota Neotropica 12:307–317.
Bere, T., T. Mangadze, and T. Mwedzi. 2014. The application and testing of diatom-based indices of stream water quality in Chinhoyi Town, Zimbabwe. Water SA 40:503–512.
Carlisle, D. M., and C. P. Hawkins. 2008. Land use and the structure of western US stream invertebrate assemblages: predictive models and ecological traits. Journal of the North American Benthological Society 27:986–999.
Cunico, A. M., J. D. Allan, and A. A. Agostinho. 2011. Functional convergence of fish assemblages in urban streams of Brazil and the United States. Ecological Indicators 11:1354–1359.
Deborde, D. D., M. B. Hernandez, and F. S. Magnabua. 2016. Benthic macroinvertebrate community as an indicator of stream health: the effects of land use on stream benthic macroinvertebrates. Science Diliman 28:5–26.
Díaz, A. M., M. L. S. Alonso, and M. R. V. A. Gutiérrez. 2008. Biological traits of stream macroinvertebrates from a semi-arid catchment: Patterns along complex environmental gradients. Freshwater Biology 53:1–21.
Feld, C. K., and D. Hering. 2007. Community structure or function: Effects of environmental stress on benthic macroinvertebrates at different spatial scales. Freshwater Biology 52:1380–1399.
Gadawski, P., H. W. Riss, M. Plóciennik, and E. Meyer. 2016. City channel chironomids-benthic diversity in urban conditions. River Research and Applications 32:1978–1988.
Hawley, R. J., M. S. Wooten, K. R. Macmannis, and E. V Fet. 2016. When do macroinvertebrate communities of reference streams resemble urban streams? The biological relevance of Q critical. Freshwater Science 35:778–794.
Johnson, R. C., M. M. Carreiro, H. S. Jin, and J. D. Jack. 2012. Within-year temporal variation and life-cycle seasonality affect stream macroinvertebrate community structure and biotic metrics. Ecological Indicators 13:206–214.
Kennen, J. G., D. J. Sullivan, J. T. May, A. H. Bell, K. M. Beaulieu, and D. E. Rice. 2012. Temporal changes in aquatic-invertebrate and fish assemblages in streams of the north-central and northeastern US. Ecological Indicators 18:312–329.

Kratzer, E. B., J. K. Jackson, D. B. Arscott, A. K. Aufdenkampe, C. L. Dow, L. A. Kaplan, J. D. Newbold, and B. W. Sweeney. 2006. Macroinvertebrate distribution in relation to land use and water chemistry in New York city drinking-water-supply watersheds. Journal of the North American Benthological Society 25:954–976.

Kuzmanovic, M., J. C. López-Doval, N. De Castro-Català, H. Guasch, M. Petrovic, I. Muñoz, A. Ginebreda, and D. Barceló. 2016. Ecotoxicological risk assessment of chemical pollution in four Iberian river basins and its relationship with the aquatic macroinvertebrate community status. Science of the Total Environment 540:324–333.

Lafont, M., A. Vivier, S. Nogueira, P. Namour, and P. Breil. 2006. Surface and hyporheic oligochaete assemblages in a French suburban stream. Hydrobiologia 564:183–193.

Launois, L., J. Veslot, P. Irz, and C. Argillier. 2011. Development of a fish-based index (FBI) of biotic integrity for French lakes using the hindcasting approach. Ecological Indicators 11:1572–1583.

Nichols, J., J. A. Hubbart, and B. C. Poulton. 2016. Using macroinvertebrate assemblages and multiple stressors to infer urban stream system condition: a case study in the central US. Urban Ecosystems 19:679–704.

Pavel, B., R. Alexandra, C. Fedor, and Z. Ciamporová-Zatovicová. 2016. Catchment land use as a predictor of the macroinvertebrate community changes between inlet and outlet of small water dams. Environmental Monitoring and Assessment:188–550.

Pease, A. A., J. M. Taylor, K. O. Winemiller, and R. S. King. 2015. Ecoregional, catchment, and reach-scale environmental factors shape functional-trait structure of stream fish assemblages. Hydrobiologia 753:265–283.

Peoples, B. K., and E. A. Frimpong. 2012. A snapshot analysis of age distributions of fishes in urban and forested Virginia streams. Urban Ecosystems 15:927–937.

Pérez-Quintero, J. C. 2013. Mollusc communities along upstream-downstream gradients in small coastal basins of the south-western Iberian Peninsula. Hydrobiologia 703:165–175.

Qian, S. S., T. F. Cuffney, and G. McMahon. 2012. Multinomial regression for analyzing macroinvertebrate assemblage composition data. Freshwater Science 31:681–694.

Rios-Touma, B., C. Prescott, S. Axtell, and G. M. Kondolf. 2015. Habitat restoration in the context of watershed prioritization: the ecological performance of urban stream restoration projects in Portland, Oregon. River Research and Applications 31:755–766.

Robertson, A., G. Dineen, R. Baker, B. Hancock, and P. Shaw. 2014. Microinvertebrate community colonisation and succession in a new urban river: lessons for river restoration. Fundamental and Applied Limnology / Archiv für Hydrobiologie 185:31–41.

Robinson, C. T., N. Schuwirth, S. Baumgartner, and C. Stamm. 2014. Spatial relationships between land-use, habitat, water quality and lotic macroinvertebrates in two Swiss catchments. Aquatic Sciences 76:375–392.

Stevenson, R. J., Y. Pan, K. M. Manoylov, C. A. Parker, D. P. Larsen, and A. T. Herlihy. 2008. Development of diatom indicators of ecological conditions for streams of the western US. Journal of the North American Benthological Society 27:1000–1016.

Tchakonté, S., G. A. Ajeagah, A. I. Camara, D. Diomandé, N. L. Nyamsi Tchatcho, and P. Ngassam. 2015. Impact of urbanization on aquatic insect assemblages in the coastal zone of Cameroon: the use of biotraits and indicator taxa to assess environmental pollution. Hydrobiologia 755:123–144.

Tullos, D. D., D. L. Penrose, G. D. Jennings, and W. G. Cope. 2009. Analysis of functional traits in reconfigured channels: implications for the bioassessment and disturbance of river restoration. Journal of the North American Benthological Society 28:80–92.

Usseglio-Polatera, P., M. Bournaud, P. Richoux, and H. Tachet. 2000. Biological and ecological traits of benthic freshwatermacroinvertebrates?: relationships and definition of groups withsimilar traits. Freshwater Biology 43:175–205.

Vermonden, K., K. P. Brodersen, D. Jacobsen, H. van Kleef, G. van der Velde, and R. S. E. W. Leuven. 2011. The influence of environmental factors and dredging on chironomid larval diversity in urban drainage systems in polders strongly influenced by seepage from large rivers. Journal of the North American Benthological Society 30:1074–1092.

Wang, L., and P. Kanehl. 2003. Influences of watershed urbanization and instream habitat on macroinvertebrates in cold water streams. Journal of the American Water Resources Association 39:1181–1196.

Zhang, Y., B. Wang, M. Han, and L. Wang. 2012. Relationships between the seasonal variations of macroinvertebrates, and land uses for biomonitoring in the Xitiaoxi River Watershed, China. International Review of Hydrobiology 97:184–199.

Zuellig, R. E., and T. S. Schmidt. 2012. Characterizing invertebrate traits in wadeable streams of the contiguous US: differences among ecoregions and land uses. Freshwater Science 31:1042–1056.

Appendix B – Supplementary material of Chapter 3

Table B1. List of fish species that co-occur with guppies in urban and non-urban reaches in the sites AntesComu (ANC), Floresta (FLOR), Water planet sujo (WPS), Camorim limpo (CAML), Tingui (TNG), Ubatiba (UBA) in Rio de Janeiro, Brazil. Where, Asty=Astyanax sp., Call=*Callichthys callichthys*, Char=Characidium sp., Geop=Geophagus brasiliensis, Gymn=Gymnotus sp., Hopl=*Hoplias malabaricus*, Hypo=*Hypostomus* sp., Mima=*Mimagoniates microlepis*, Paro=*Parotocinclus* sp., Phal=*Palloceros* sp., Pime=*Pimelodella lateristriga*, Rham=*Rhamdia quelen*, Scle=*Scleromystax* sp., Synb=*Synbranchus* sp., Tric=*Trichomycterus* sp., Xiph=*Xiphophorus* sp.

			Sites					
Species	**Feeding habit**	**Reference**	**urban**			**non-urban**		
			ANC	FLOR	WPS	CAML	TNG	UBA
Asty	omnivore	Vilella *et al.* (2002)				x	x	x
Call	detritivore/invertivore	Lopes *et al.* (2016)			x			
Char	invertivore	Mazzoni *et al.* (2012)					x	x
Geop	omnivore	Bastos *et al.* (2011)	x	x	x		x	
Gymn	invertivore	Bonato *et al.* (2012)	x					
Hopl	piscivore	Corrêa *et al.* (2012)					x	x
Hypo	herbivore	Delariva & Agostinho (2001)			x		x	x
Mima	invertivore	Wolff, Carniatto & Hahn (2013)						x
Paro	herbivore	Leitão *et al.* (2015)						x
Phal	detritivore/invertivore	Neves, Delariva & Wolff (2015)	x					
Pime	invertivore	Rezende *et al.* (2013)					x	x
Rham	piscivore/invertivore	Bonato *et al. (*2012)	x	x	x	x	x	x
Scle	invertivore	Gonçalves, Braga & Casatti (2018)			x			
Synb	invertivore	Teresa & Casatti (2012)		x			x	
Tric	invertivore	Chara *et al.* (2006)	x					
Xiph	omnivore	Maddern, Gill & Morgan (2011)		x				

Table B2. Environmental variables used in the PCA between urban stream reaches (ANC=Antes Comu, CAR=Carioca, CATO=Catonho, ELSU=Eldo sujo, FLOR=Floresta ,WPS= Water Planet sujo) and non-urban stream reaches (CAML=Camorim limpo, ELLI=Eldo limpo, JOA=Joana, TNG=Tingui, UBA=Ubatiba, WPL=Water Planet limpo). Where, URB=urbanization, BIO=fish biodiversity (GO=guppy only, GF=guppy co-occurring with competitors and predators), CON=Specific conductivity (Spec µS/cm), TEM=Temperature (^{o}C), DO= Dissolved oxygen (mg/L), CA=canopy cover (%), FC= fecal coliforms (E. *coli*, MPN/100mL), NH_4= Ammonium concentration (µg/L) and sampling year (YR). Values represent averages only when followed by brackets with standard error (3-4 replicates).

Site	Origin	BIO	pH	CON	TEM	DO	CAN	FC	NH4	YR
ANC	urban	GF	6.4	179.9	23.1	6.9	57.2(8.6)	913900	897.7(9.4)	2016
ANC	urban	GF	5.6(0.03)	113.1(1.2)	24.5(0.05)	3.9(0.35)	58.8(10.8)	75300	425.5(13.7)	2017
CAML	non-urban	GF	6.4	179.8	22.7	8.3	94.8(0.9)	6700	7.8(2.3)	2016
CAML	non-urban	GF	6.3(0.04)	109.6(15.7)	24.8(0)	7.8(0.64)	96.5(0.6)	727	28.3(1.8)	2017
CAR	urban	GO	6.2	518	24.9	6	99(0.1)	280900	2760.5(13.6)	2016
CAR	urban	GO	7.1(0.13)	482(6.7)	26.9(0.11)	1.4(0.39)	97.6(1.6)	280900	12829.8(59.4)	2017
CATO	urban	GO	7.2(0.02)	369.4(14.9)	27.5(0.17)	3.2(0.01)	71.5(4)	43660	3399.2(2370.1)*	2017
ELLI	non-urban	GO	7.1	460	22.7	8.1	94.2(0.7)	5910	29.4(8.2)	2016
ELLI	non-urban	GO	6.2(0.11)	188.1(0.3)	27(0.12)	6.5(1.2)	98.4(0.7)	18.7	10.3(0.6)	2017
ELSU	urban	GO	7.1	736	25.4	4.4	96.8(1.1)	5210	2548.5(61.3)	2016
ELSU	urban	GO	6.2(0.07)	251.3(13.1)	27.5(1)	1.2(0.4)	95.6(2.3)	5210	583(25.9)	2017
FLOR	urban	GF	6.4	361.5	27.2	6	84.5(3.9)	24200	1655(103.5)	2016
FLOR	urban	GF	6.4(0.11)	219.7(13.2)	26.5(0.03)	1.6(0.45)	79(4.1)	36090	1645.6(72.1)	2017
JOA	non-urban	GO	5.8	234.7	22.1	8.1(0.1)*	95(1.8)	256	14.3(4.4)	2016
TNG	non-urban	GF	7.2(0.18)	108.4(0.9)	28.5(0.04)	7.3(1.29)	95.8(0.6)	882	9.2(1)	2017
UBA	non-urban	GF	5.8(0.05)	79.2(2.5)	28.3(0.09)	7.4(0.4)	92.8(1.4)	134	11.7(0.8)	2017
WPL	non-urban	GO	5.6	105.5	22.4	7.9	85.8(4.1)	1460	7.6(6)	2016
WPL	non-urban	GO	5.9(0.12)	62.9(0.1)	25.9(0.05)	12.6(0.1)	90.8(3)	125	22(3.3)	2017
WPS	urban	GF	5.9	354	25	4.2	62.4(13.4)	3000	2699.1(23.4)	2016
WPS	urban	GF	7.1(0.08)	462.9(7.4)	28.3(0.09)	3.7(0.24)	79.4(11.6)	396800	1512.1(9.3)	2017

*value estimated on the average among all reaches with the same origin on the same year.

Table B3. Principal components analysis (PCA) showing the eigenvalues (Eig), variance (Var) and cumulative variance (C.var) that each principal component (PC1-8) explain. The contribution of each environmental variable to each component is also shown where CON=Specific conductivity (Spec µS/cm), TEM=Temperature (^{o}C), DO= Dissolved oxygen (mg/L), CA=canopy cover (%), FC= fecal coliforms (E. *coli*, MPN/100mL), NH$_4$= Ammonium concentration (µg/L) and sampling year (YR).

	Principal components			Contribution of each variable (%)							
	Eig	Var (%)	C.var (%)	Ph	CON	TEM	DO	CA	NH4	FC	YR
PC1	2.7	33.494	33.4944	20.4	16.6	13.5	21.9	0.07	21.8	1.71	4.02
PC2	1.8	23.044	56.5381	0.53	14.8	24.2	0	4.77	2.08	15.4	38.3
PC3	1.5	18.257	74.7955	5.29	10.6	0.8	5.92	50.2	0	21.3	5.96
PC4	0.8	9.5545	84.3499	26.2	1.64	2.14	28.5	2.32	3.67	33.9	1.69
PC5	0.6	7.1756	91.5255	12	6.91	4.24	2.21	14.4	50.8	6.92	2.46
PC6	0.3	3.8256	95.3512	27.2	6.46	20.7	2.89	13.3	8.65	16.7	4.11
PC7	0.2	3.1218	98.4729	2.71	3.23	26.7	32	13.9	11.8	4.03	5.73
PC8	0.1	1.5271	100	5.68	39.8	7.71	6.62	1.02	1.24	0.12	37.8

Table B4. Generalized linear models (GLMMs) testing for differences in environmental and guppy population variables between urban and non-urban sites. Separate models were built for each of the response variables: NH_4=concentration of ammonium per reach (µg/L), FC=concentration of fecal coliforms (E. *coli*) per reach (MPN/100mL), GD=guppy density (ind/ m^2), IB= biomass of all invertebrates taxa per reach (mg/m^2), CB=biomass of chironomids per reach (mg/m^2). Where UR= urbanization (urban or non-urban), YR=sampling year, GO vs GF=fish biodiversity (guppy only and guppy with other fish) are fixed effects. The variance explained by both fixed plus random factor (conditional R square, R^2_c), and the variance explained only by the fixed factors (marginal R square, R^2_m) are shown. SE represent the standard error and the collinearity test was based on variance inflation ratios (VIF).

Response variable	Model family	R^2_m	R^2_c	(n)	Fixed effects	Estimate	SE	t/z value	p-value	VIF
NH4										
	Gamma (log link)	0.96	0.96	20	UR	4.27	0.34	12.46	<0.001	1.74
					YR	-0.26	0.28	-0.93	0.35	1.36
					GOvsGF	0.04	0.31	0.12	0.90	1.20
					GD	0.59	0.17	3.46	<0.001	1.70
					int	2.29	0.40	5.68	<0.001	
FC										
	Gamma (log link)	0.88	0.88	18	UR	5.39	0.67	8.05	<0.001	1.30
					YR	-1.69	0.70	-2.43	<0.01	1.33
					GOvsGF	-0.34	0.61	-0.55	0.58	1.03
					int	8.04	0.57	14.03	<0.001	
GD										
	Gamma (log link)	0.84	0.92	19	UR	3.11	0.46	6.75	<0.001	1.01
					YR	0.53	0.22	2.37	0.02	1.01
					GOvsGF	0.64	0.46	1.39	0.16	1.00
					int	0.15	0.42	0.36	0.72	

IB										
	Gamma (log link)	0.48	0.48	19	UR	1.39	0.48	2.90	<0.01	1.60
					YR	-0.29	0.41	-0.71	0.48	1.17
					GOvsGF	-0.43	0.42	-1.02	0.31	1.25
					GD	-0.004	0.004	-1.17	0.24	1.65
					int	4.01	0.44	9.08	<0.001	
CB										
	Gamma (log link)	0.57	0.57	19	UR	1.97	0.53	3.69	<0.001	1.60
					YR	-0.42	0.46	-0.90	0.37	1.20
					GOvsGF	-0.06	0.47	-0.12	0.90	1.24
					GD	-0.01	0.00	-1.38	0.17	1.64
					int	3.26	0.52	6.32	<0.001	

Table B5. Generalized linear models (GLMMs) testing for differences in guppy diets and traits between urban and non-urban sites. Separate models were built for each of the response variables: NO=number of offspring per female, CO=female condition (HSI), PC=proportion of chironomids consumed, PO= proportion of other invertebrates consumed. Where UR= urbanization (urban or non-urban), YR=sampling year, GO vs GF=fish biodiversity (guppy only and guppy with other fish), SL= standard guppy length (mm) and GD=guppy density (ind/m^2) are the fixed effects. SE represents the standard error. The variance explained by both fixed plus random factor (conditional R square, R^2_c), and the variance explained only by the fixed factors (marginal R square, R^2_m) is shown. SE represent the standard error and the collinearity test was based on variance inflation ratios (VIF).

Response variable	Model family	R^2_m	R^2_c	(n)	Fixed effects	Estimate	SE	t/z value	p-value	VIF
NO	Poisson	0.63	0.81	735	UR	0.35	0.17	2.10	0.04	1.01
					YR	0.01	0.03	0.48	0.63	1.24
					GOvsGF	0.06	0.17	0.33	0.74	1
					SL	0.53	0.01	49.85	<0.001	1.04
					GD	-0.14	0.02	-6.02	<0.001	1.24
					int	1.98	0.15	13.27	<0.001	
CO	Gamma (log link)	0.39	0.44	735	UR	0.40	0.15	2.70	<0.01	1.03
					YR	-0.39	0.04	-9.37	<0.001	1.27
					GOvsGF	0.22	0.14	1.49	0.14	1.01
					SL	-0.03	0.02	-1.68	0.09	1.02
					GD	0.16	0.04	4.38	<0.01	1.30
					int	0.84	0.13	6.48	<0.001	
PC	Binomial	0.55	0.62	230	UR	3.94	1.04	3.78	<0.001	1.23
					YR	-0.49	0.54	-0.91	0.37	1.07
					GOvsGF	1.47	0.83	1.77	0.08	1.29
					SL	-0.20	0.25	-0.78	0.43	1.07
					GD	-1.01	0.36	-2.79	0.01	1.29
					int	-5.46	1.15	-4.74	<0.001	

PO	Binomial	0.17	0.26	230	UR	-1.10	0.57	-1.92	0.05	1.05
					YR	-1.17	0.45	-2.59	<0.01	1.33
					GOvsGF	-0.09	0.61	-0.15	0.88	1.22
					SL	-0.30	0.22	-1.34	0.18	1.19
					GD	-0.18	0.26	-0.67	0.50	1.27
					int	-0.18	0.59	-0.74	0.46	
					int	1.98	0.15	13.27	<0.001	

Table B6. Full model selection with all candidate LMM models (C.model) testing the relationship between guppy traits and diet. We built separate models using number of offspring (NO) and guppy condition (CO, HSI) as response variables and sampling year (YR), fish biodiversity (guppy only and guppy and other fish, GO vs GF), body length (SL, mm), guppy density (GD, ind/m^2) and the proportion of chironomid consumed (PC, %) as fixed factors. We used reach identity as random factor. Following model selection, the coefficients of the best models (ΔAICc <2) were estimated and averages were calculated when necessary. AICc show the corrected Akaike information criterion values, while ΔAICc shows the difference in AICc between the current and the most appropriate model, and weight show the Akaike weights.

Fixed factor	C.model	df	logLik	AICc	ΔAICc	W	YR	GOvsGF	SL	GD	PC	Intercept
NO	1	6	-40.40	99.80	0.00	0.35	+	–	1.50	–	20.26	-27.67
	2	5	-42.96	100.54	0.74	0.24	–	–	1.74	–	17.52	-31.91
	3	7	-38.39	100.96	1.16	0.20	+	+	1.45	–	21.36	-25.69
	4	6	-41.06	101.12	1.33	0.18	–	+	1.68	–	18.59	-29.97
	5	6	-44.21	107.42	7.63	0.01	+	+	–	–	24.13	8.14
	6	5	-46.63	107.88	8.09	0.01	+	–	–	–	22.36	7.03
	7	5	-48.07	110.76	10.96	0.00	–	+	–	–	20.10	10.48

8	4	-50.45	111.76	11.96	0.00	–	–	–	–	18.04	9.39
9	6	-46.62	112.24	12.44	0.00	–	–	1.79	0.00	17.52	-33.04
10	7	-44.06	112.30	12.50	0.00	+	–	1.56	-0.01	20.04	-28.96
11	4	-50.85	112.55	12.75	0.00	–	–	1.93	–	–	-34.42
12	5	-49.19	113.00	13.20	0.00	–	+	1.89	–	–	-33.48
13	5	-49.37	113.36	13.56	0.00	+	–	1.93	–	–	-34.66
14	7	-44.76	113.70	13.90	0.00	–	+	1.70	0.00	18.51	-30.41
15	6	-47.70	114.41	14.61	0.00	+	+	1.90	–	–	-33.72
16	8	-42.13	114.65	14.86	0.00	+	+	1.47	0.00	21.21	-26.24
17	7	-47.41	119.00	19.21	0.00	+	+	–	0.01	24.41	8.11
18	6	-50.02	119.05	19.25	0.00	+	–	–	0.01	22.45	6.91
19	5	-52.27	119.15	19.36	0.00	+	+	–	–	–	11.16
20	4	-54.16	119.17	19.38	0.00	–	+	–	–	–	12.09
21	4	-54.36	119.58	19.79	0.00	+	–	–	–	–	10.70
22	3	-56.28	120.16	20.36	0.00	–	–	–	–	–	11.62
23	6	-50.95	120.90	21.11	0.00	–	+	–	0.01	20.44	10.23
24	5	-53.59	121.79	21.99	0.00	–	–	–	0.01	18.19	9.01
25	5	-54.28	123.17	23.37	0.00	–	–	1.94	0.00	–	-34.52
26	6	-52.53	124.05	24.26	0.00	–	+	1.92	-0.01	–	-33.98
27	6	-52.79	124.57	24.78	0.00	+	–	1.94	0.00	–	-34.62
28	7	-51.02	126.22	26.43	0.00	+	+	1.91	0.00	–	-34.02
29	5	-57.33	129.27	29.47	0.00	–	+	–	0.00	–	12.02
30	5	-57.57	129.75	29.95	0.00	+	–	–	0.00	–	10.68
31	6	-55.42	129.83	30.04	0.00	+	+	–	0.00	–	11.15
32	4	-59.50	129.86	30.06	0.00	–	–	–	0.00	–	11.49

CO

1	4	-23.96	58.78	0.00	0.40	−	−	−	−	3.56	2.31
2	5	-22.87	60.35	1.57	0.18	+	−	−	−	2.89	2.70
3	4	-25.27	61.40	2.62	0.11	+	−	−	−	−	3.14
4	5	-23.50	61.62	2.84	0.10	−	+	−	−	3.30	2.14
5	3	-27.55	62.71	3.93	0.06	−	−	−	−	−	2.70
6	5	-24.40	63.41	4.63	0.04	+	+	−	−	−	2.83
7	6	-22.38	63.76	4.98	0.03	+	+	−	−	2.39	2.55
8	4	-26.63	64.11	5.33	0.03	−	+	−	−	−	2.39
9	5	-25.15	64.92	6.13	0.02	−	−	0.04	−	3.43	1.28
10	6	-23.66	66.33	7.54	0.01	+	−	0.11	−	2.44	0.24
11	5	-25.88	66.37	7.58	0.01	+	−	0.13	−	−	0.01
12	4	-28.55	67.95	9.17	0.00	−	−	0.07	−	−	0.93
13	6	-24.58	68.16	9.37	0.00	−	+	0.07	−	3.08	0.57
14	6	-24.83	68.65	9.87	0.00	+	+	0.15	−	−	-0.77
15	5	-27.45	69.51	10.73	0.00	−	−	−	0.00	2.94	2.18
16	5	-27.52	69.65	10.87	0.00	−	+	0.10	−	−	0.07
17	6	-25.43	69.86	11.07	0.00	+	−	−	0.01	2.01	2.65
18	5	-27.66	69.93	11.15	0.00	+	−	−	0.01	−	2.96
19	7	-22.95	70.09	11.31	0.00	+	+	0.13	−	1.89	-0.53
20	4	-30.66	72.17	13.39	0.00	−	−	−	0.01	−	2.48
21	6	-27.26	73.53	14.74	0.00	−	+	−	0.00	2.82	2.10
22	6	-27.30	73.59	14.81	0.00	+	+	−	0.01	−	2.82
23	7	-25.27	74.72	15.94	0.00	+	+	−	0.01	1.85	2.60
24	5	-30.25	75.12	16.34	0.00	−	+	−	0.00	−	2.32
25	6	-28.68	76.36	17.58	0.00	+	−	0.08	0.01	−	1.03
26	6	-28.74	76.48	17.70	0.00	−	−	0.00	0.00	2.93	2.26
27	7	-26.59	77.37	18.59	0.00	+	−	0.06	0.01	1.86	1.29
28	5	-31.85	78.31	19.53	0.00	−	−	0.03	0.01	−	1.79

	29	7	-28.20	80.59	21.81	0.00	+	+	0.10	0.01	–	0.51
	30	7	-28.48	81.14	22.36	0.00	–	+	0.01	0.00	2.79	1.89
	31	6	-31.34	81.69	22.91	0.00	–	+	0.05	0.00	–	1.20
	32	8	-26.32	83.04	24.26	0.00	+	+	0.07	0.01	1.65	0.88
SL												
	1	4	-36.76	84.39	0.00	0.23	–	–	NA	–	1.13	23.66
	2	5	-35.12	84.86	0.47	0.18	+	–	NA	–	2.78	22.83
	3	3	-38.84	85.28	0.90	0.15	–	–	NA	–	–	23.81
	4	5	-35.56	85.74	1.36	0.12	–	+	NA	–	1.53	23.95
	5	4	-37.50	85.85	1.47	0.11	+	–	NA	–	–	23.31
	6	4	-37.71	86.28	1.89	0.09	–	+	NA	–	–	24.09
	7	6	-33.93	86.87	2.48	0.07	+	+	NA	–	3.22	23.15
	8	5	-36.42	87.45	3.06	0.05	+	+	NA	–	–	23.59
	9	5	-40.34	95.30	10.91	0.00	–	–	NA	0.01	0.99	23.41
	10	4	-42.41	95.67	11.29	0.00	–	–	NA	0.01	–	23.54
	11	5	-41.05	96.71	12.33	0.00	–	+	NA	0.01	–	23.96
	12	6	-38.86	96.73	12.34	0.00	–	+	NA	0.01	1.69	23.81
	13	6	-38.95	96.91	12.52	0.00	+	–	NA	0.01	2.33	22.76
	14	5	-41.27	97.15	12.77	0.00	+	–	NA	0.01	–	23.16
	15	6	-39.98	98.97	14.58	0.00	+	+	NA	0.01	–	23.56
	16	7	-37.51	99.21	14.82	0.00	+	+	NA	0.01	2.91	23.18

(-) indicates a factor not included in the model
(+) indicates a categorical factor included in the model

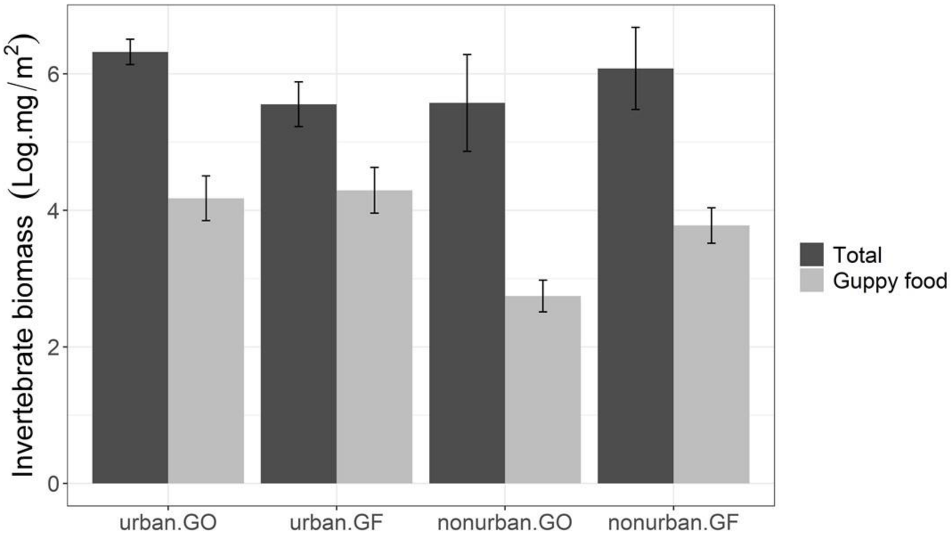

Figure B1. Total invertebrate biomass (dark grey) and invertebrate biomass available as food for guppies (light grey) found in urban and non-urban reaches. Biomass was estimated as dry weight of invertebrate per reach area (Log mg/m^2). Bars indicate the mean and lines are the standard error of the mean. Data shown includes both sampling years 2016 and 2017.

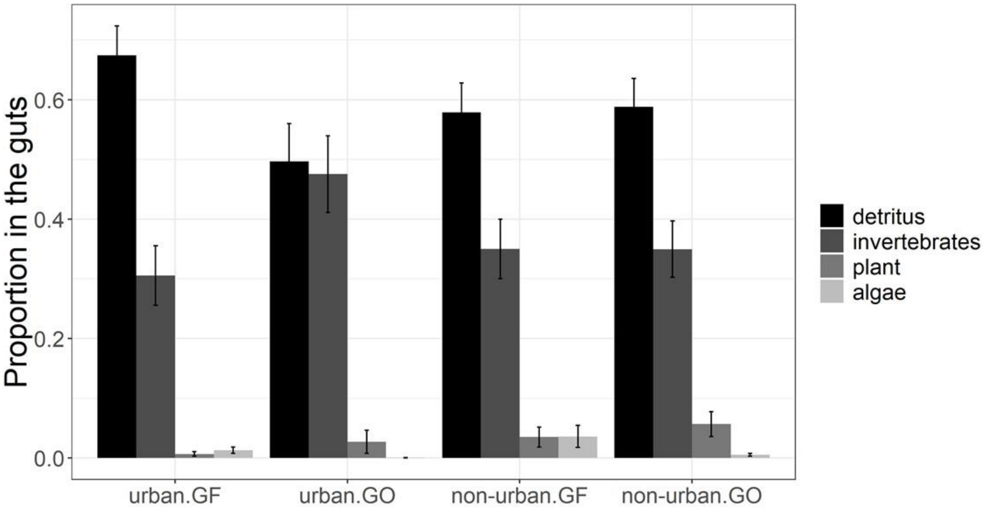

Figure B2. Proportion of each food item found in the guts of guppies in urban reaches where guppies occur with other fish species (urban.GF), urban reaches where guppies are the only fish species (urban.GO), non-urban reaches where guppies occur with other fish species (non-urban.GF) and non-urban reaches where guppies are the only fish species (non-urban.GO). Each shade of grey represents one food item, bars represent the mean and lines are the standard error of the mean. Data shown includes both sampling years 2016 and 2017.

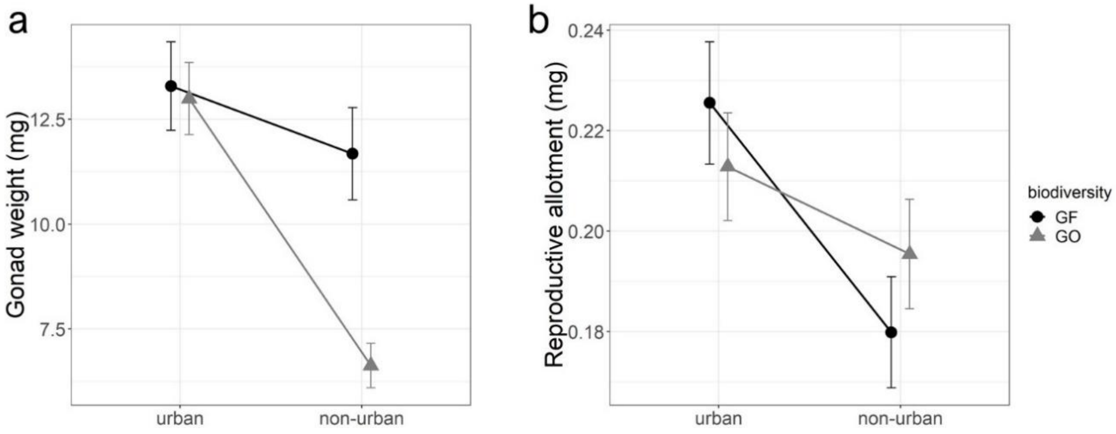

Figure B3. Guppy life history traits. Gonad weight (panel a) and reproductive allotment (panel b) of urban and non-urban guppies. In both conditions guppies occur in stream reaches with other fish species (GF, black circles) and in reaches where guppies are the only fish species (GO, grey triangle). Symbols represent the mean for all the reaches, while bars are the standard error of the mean. Data shown includes both sampling years 2016 and 2017.

Appendix C – Supplementary material of Chapter 4

Table C1. Environmental variables: canopy (%, CA), dissolved oxygen (mg/L, DO), temperature (^{o}C, TEMP), specific conductivity (μS/cm, COND) measured at each sampling pool in Aripo (ARI), Quare (QUA), El-Cedro (CED), Upper la lajas (UPL), Lower la lajas (LOL), Caigual (CAI), and Taylor (TAY) streams in Trinidad. In each stream guppies were in populations (POP) under high predation (HP), low predation (LP) or transplanted (TR). Pools with less than 3 guppies used for gut content were excluded. Canopy cover was estimated using a hemispherical densiometer.

Reach	Pool number	POP	CA	DO	TEMP	COND
ARI	1	HP	77.8	5.9	24	316.6
ARI	3	HP	87	6.21	23.8	316.3
QUA	1	HP	55.5	7.01	26	287.1
QUA	2	HP	63.8	7.25	25.8	288.2
QUA	3	HP	62.3	5.93	28.3	285.3
CED	1	HP	92	7.82	23	321.3
CED	2	HP	82.5	7.24	23.2	325.8
CED	3	HP	69	7.88	23.3	319.6
QUA	1	LP	98.8	5.81	24	315.5
QUA	2	LP	97.8	7.33	24	314.2
QUA	3	LP	98	6.63	24.1	321.1
CED	1	LP	93.3	8.48	22.6	288.2
CED	2	LP	97.8	7.79	22.7	290.1
ARI	1	LP	85	7.43	24.2	399
ARI	2	LP	88.8	5.33	24.4	394.2
ARI	3	LP	86.3	7.15	24.1	395.7
UPL	2	TR	97.3	8.32	22.1	100.1
UPL	3	TR	97.3	8.43	22.1	109.9
LOL	2	TR	93.8	7.67	22.2	117.1
LOL	3	TR	99.3	7.4	22.2	121.7
CAI	2	TR	94.5	7.48	22.7	172.9
CAI	3	TR	95	7.41	22.7	171.6
TAY	1	TR	98	7.02	23.1	234.2
TAY	2	TR	99	6.84	23.1	233.7

Table C2. Total invertebrate biomass at each reach (dry mass, mg/m^2, TB) and invertebrate biomass available as food for guppies (dry mass, mg/m^2, IB) in Aripo (ARI), Quare (QUA), El-Cedro (CED), Upper la lajas (UPL), Lower la lajas (LOL), Caigual (CAI), and Taylor (TAY) rivers in Trinidad. In each stream guppies were in populations (POP) under high predation (HP), low predation (LP) or reaches where HP guppies were transplanted into previously guppy free and predator free reaches (TR). Values in brackets indicate standard errors.

Reach	POP	TB	IB
ARI	HP	1260.5(202.4)	132.6(55.2)
QUA	HP	1274.4(172.3)	189.4(75.7)
CED	HP	1333.2(985.6)	44.8(13.9)
QUA	LP	829.4(898.5)	94.8(28.5)
CED	LP	658.6(145.9)	120.6(71)
ARI	LP	1078.3(502.3)	135.9(30.7)
UPL	TR	500(269)	92.5(10.1)
LOL	TR	515.1(62.3)	133.9(21.4)
CAI	TR	571.3(185.8)	99.7(21.3)
TAY	TR	1340.1(906)	46.6(23.1)

Table C3. Mean gut length (GL, mm), proportion of carnivorous guppies (PC), total invertebrate biomass per pool (IB, dry mass mg/m^2), algae biomass per pool (AB, µg Chla/m^2), total guppy density per pool (GD, ind/m^2), average guppy length per pool (SL, mm) and number of analyzed guts (NG) for each pool in streams reaches where guppies where . Reaches were sampled at the Aripo (ARI), Quare (QUA), El-Cedro (CED), Upper la lajas (UPL), Lower la lajas (LOL), Caigual (CAI), and Taylor (TAI) streams in up to 3 different pools (PN). In each stream guppies were in populations (POP) under high predation (HP), low predation (LP) or reaches where HP guppies were transplanted into previously guppy free and predator free reaches (TR). Values in brackets indicate standard errors.

Reach	PN	POP	GL	PC	IB	AB	GD	SL	NG
ARI	1	HP	1.7(0.54)	0.4	193.3	0.00023	10(0.05)	19.5(1.37)	7
ARI	3	HP	1.5(0.48)	0.6	59.7	0.00078	3(0.03)	17.5(1.8)	5
QUA	1	HP	2.1(0.56)	0	217.7	0.00080	4(1)	18.2(2.05)	5
QUA	2	HP	1.8(0.35)	1	85.8	0.00096	5(0.01)	17.2(0.51)	3
QUA	3	HP	2.3(0.75)	1	264.7	0.00055	2(2)	20.2(3.15)	3
CED	1	HP	1.9(0.39)	0.7	30.1	0.00048	7(-)	16.5(2.47)	4
CED	2	HP	2.2(0.33)	0.5	63.5	0.00004	20(0.8)	18(1.41)	6
CED	3	HP	2.3(0.38)	1	40.7	0.00014	11(0.02)	18.9(2.24)	7
QUA	1	LP	3.1(0.72)	0	67.3	0.00016	12(0.02)	21(2.23)	4
QUA	2	LP	3.1(1.06)	0.4	83	0.00069	9(-)	20.3(2.77)	5
QUA	3	LP	2.8(0.19)	0	134	0.00027	9(0.4)	18.7(1.89)	4
CED	1	LP	2.7(0.73)	1	186.4	0.00066	4(0.6)	20.7(3.29)	5
CED	2	LP	2.7(0.78)	0.9	21.9	0.00065	14(0.8)	21.7(2.25)	8
ARI	1	LP	3.4(1.38)	0.8	103.6	0.00019	1(0.6)	21(2.32)	5
ARI	2	LP	4.1(0.77)	0.2	177.2	0.00014	28(22)	23.2(2.48)	5
ARI	3	LP	4.1(1.02)	0	126.9	0.00007	9(4)	22.2(3.27)	4
UPL	2	TR	3.3(0.57)	0.7	81.7	0.00037	8(4)	22.4(2.69)	3
UPL	3	TR	3.2(1.01)	0.7	105.9	0.00026	25(10)	22.8(6.1)	6
LOL	2	TR	2.8(0.28)	0.4	154.7	0.00038	13(0.3)	19.2(1.04)	5
LOL	3	TR	2.8(0.68)	1	142.5	0.00021	7(0.3)	23.5(2.78)	5
CAI	2	TR	3.2(0.71)	0	116.1	0.00023	12(0.8)	20.2(1.42)	9
CAI	3	TR	4.9(1.07)	0	113.4	0.00019	17(3)	24.5(2.55)	7
TAY	1	TR	3.4(0.95)	0.2	79.1	0.00033	13(-)	21.5(1.61)	4
TAY	2	TR	3(0.64)	0	32.8	0.00018	22(0.4)	18.2(2.86)	3

(-) no *se* because all fish were caught on the first pass

Table C4. Full model selection. High predation (HP) and low predation (LP) populations were modeled together. While transplanted (TR) populations (populations originated from HP guppies that were transplanted into previously guppy free and predator free reaches) were modeled in separate. We provide a list of all possible models derived from the global model, where the proportion of carnivorous guppies (PC) per pool is the response variable and total invertebrate biomass per pool (IB, dry mass mg/m^2), algae biomass per pool (AB, Chla µg/m^2), total guppy density per pool (GD, ind/m^2), predation regime (reaches with or without predators, HPvsLP) and mean guppy length (SL) are fixed factors, and reach identity (RI) is a random factor. Models are ranked by AICc scores. Only the model with ΔAICc<2 was considered as the best models. Akaike weights (W) show the weight of evidence in favor of each model.

Population	Model	df	logLik	AICc	delta	weight	IB	AB	GD	HPvsLP	SL	intercept
HP+LP	1	3	-30.15	68.30	0.00	0.27	–	–	-0.737	–	–	0.059
	2	4	-29.22	70.09	1.79	0.11	-0.415	–	-0.702	–	–	0.075
	3	2	-32.79	70.51	2.21	0.09	–	–	–	–	–	0.220
	4	3	-31.38	70.76	2.46	0.08	–	0.527	–	–	–	0.112
	5	3	-31.59	71.18	2.88	0.06	-0.438	–	–	–	–	0.261
	6	4	-29.79	71.22	2.92	0.06	–	0.303	-0.611	–	–	0.030
	7	4	-29.83	71.29	2.99	0.06	–	–	-0.853	–	0.487	0.170
	8	4	-30.11	71.87	3.57	0.05	–	–	-0.727	+	–	0.205
	9	4	-30.73	73.10	4.80	0.02	-0.347	0.416	–	–	–	0.167
	10	3	-32.64	73.28	4.98	0.02	–	–	–	+	–	0.485
	11	3	-32.76	73.52	5.22	0.02	–	–	–	–	-0.121	0.188
	12	5	-28.83	73.67	5.37	0.02	-0.439	–	-0.815	–	0.525	0.196
	13	5	-29.09	74.17	5.88	0.01	-0.441	–	-0.688	+	–	0.334
	14	4	-31.29	74.23	5.93	0.01	–	0.513	–	+	–	0.315
	15	5	-29.14	74.27	5.98	0.01	-0.378	0.154	-0.628	–	–	0.064
	16	4	-31.33	74.30	6.00	0.01	–	0.563	–	–	0.164	0.147
	17	4	-31.34	74.32	6.02	0.01	-0.447	–	–	+	–	0.586
	18	5	-29.28	74.57	6.27	0.01	–	0.394	-0.734	–	0.649	0.161

	19	5	-29.30	74.59	6.30	0.01	–	–	-0.913	+	1.016	1.094
	20	4	-31.59	74.82	6.52	0.01	-0.438	–	–	–	-0.003	0.261
	21	5	-29.77	75.53	7.24	0.01	–	0.299	-0.604	+	–	0.156
	22	6	-27.43	76.20	7.90	0.01	-0.627	–	-0.939	+	1.489	1.682
	23	4	-32.63	76.89	8.59	0.00	–	–	–	+	0.100	0.578
	24	5	-30.53	77.06	8.77	0.00	-0.373	0.395	–	+	–	0.449
	25	5	-30.67	77.34	9.04	0.00	-0.355	0.449	–	–	0.180	0.209
	26	5	-30.99	77.97	9.67	0.00	–	0.601	–	+	0.554	0.792
	27	5	-31.11	78.22	9.93	0.00	-0.502	–	–	+	0.461	1.024
	28	6	-28.61	78.55	10.25	0.00	–	0.441	-0.792	+	1.270	1.222
	29	6	-28.67	78.66	10.37	0.00	-0.381	0.225	-0.731	–	0.595	0.191
	30	6	-29.01	79.36	11.06	0.00	-0.409	0.140	-0.619	+	–	0.310
	31	6	-29.79	80.92	12.62	0.00	-0.487	0.513	–	+	0.894	1.259
	32	7	-27.16	82.32	14.03	0.00	-0.600	0.260	-0.828	+	1.610	1.745
TR												
	1	2	-12.86	32.12	0.00	0.50	–	–	–	NA	–	-1.089
	2	3	-10.40	32.80	0.68	0.36	–	–	–	NA	1.538	-2.295
	3	3	-12.34	36.68	4.56	0.05	–	–	-0.583	NA	–	-0.766
	4	3	-12.50	37.00	4.88	0.04	–	-1.454	–	NA	–	-1.739
	5	3	-12.72	37.43	5.31	0.04	0.875	–	–	NA	–	-0.969
	6	4	-10.26	41.86	9.74	0.00	–	–	-0.322	NA	1.460	-2.019
	7	4	-10.34	42.00	9.88	0.00	–	1.121	NA	NA	1.839	-2.168
	8	4	-10.36	42.05	9.93	0.00	0.660	–	–	NA	1.529	-2.246
	9	4	-11.56	44.45	12.33	0.00	–	-2.471	-0.854	NA	–	-1.653
	10	4	-12.13	45.60	13.48	0.00	1.143	–	-0.623	NA	–	-0.667
	11	4	-12.26	45.86	13.74	0.00	1.165	-1.684	–	NA	–	-1.666
	12	5	-10.07	60.14	28.02	0.00	1.672	–	-0.533	NA	1.517	-1.894
	13	5	-10.19	60.37	28.25	0.00	1.858	2.997	–	NA	2.557	-2.120

14	5	-10.26	60.53	28.41	0.00	–	0.112	-0.307	NA	1.489	-2.013
15	5	-10.95	61.91	29.79	0.00	2.371	-3.560	-1.151	NA	–	-1.753
16	6	-10.06	116.12	84.00	0.00	1.804	0.766	-0.459	NA	1.753	-1.876

(–) show factors that were not included in the model.
(+) show factors that were included in the model.

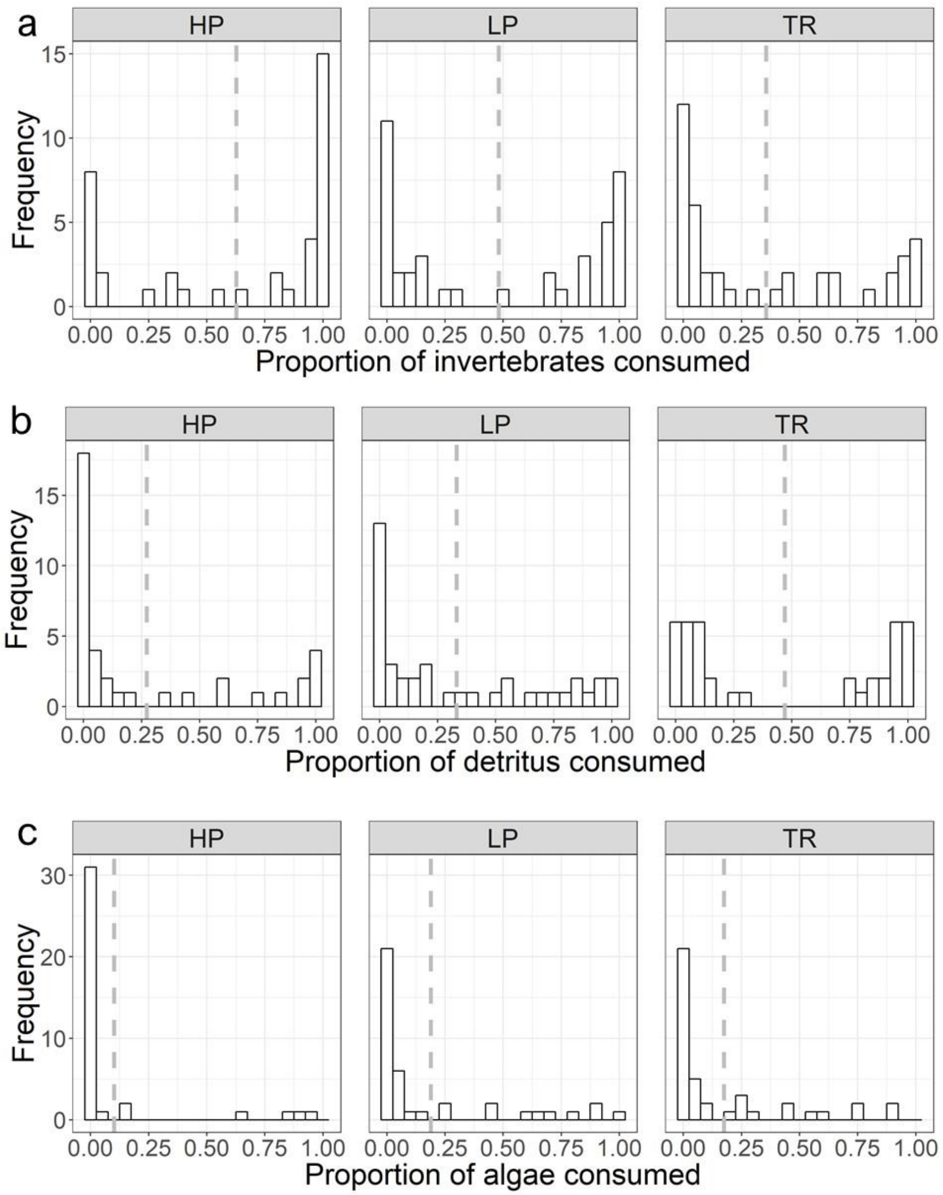

Figure C1. Distribution of all resources consumed by guppies. Histograms show the frequency of consumption for all individuals within populations occurring in reaches with predators (HP), without predators (LP) and populations originated from HP reaches that were transplanted into guppy free, predator free reaches (TR). Panel (a) show the proportion of invertebrates consumed. Panel (b) show the proportion of detritus consumed. Panel (c) show the proportion of algae consumed. Dashed grey lines indicate population mean.

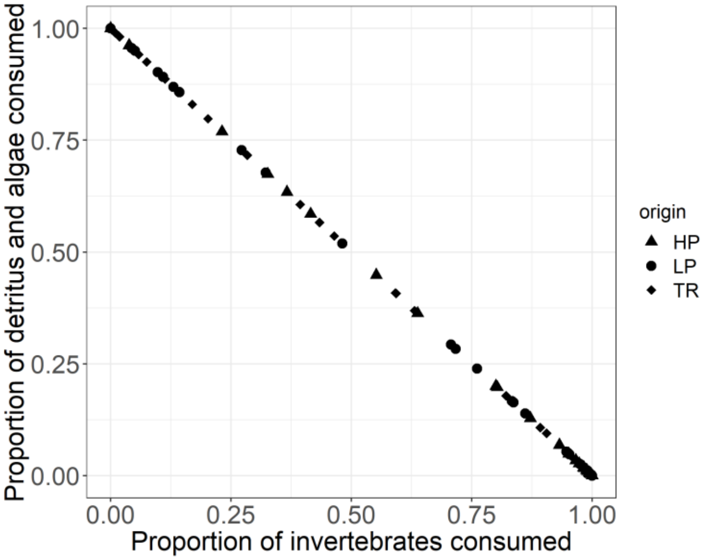

Figure C2. Correlation between the proportion of invertebrates consumed and the proportion of detritus and algae consumed. Each symbol represents one female guppy within populations occurring in reaches with predators (HP), without predators (LP) and populations originated from HP reaches that were transplanted into guppy free, predator free reaches (TR).

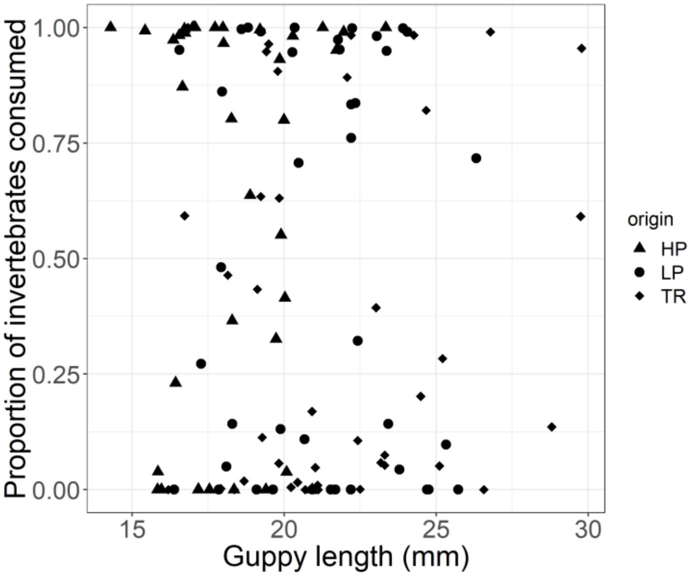

Figure C3. Correlation between resource use (proportion of invertebrates consumed) and body length. Each dot represents is one female guppy within populations occurring in reaches with predators (HP), without predators (LP) and populations originated from HP reaches that were transplanted into guppy free, predator free reaches (TR).

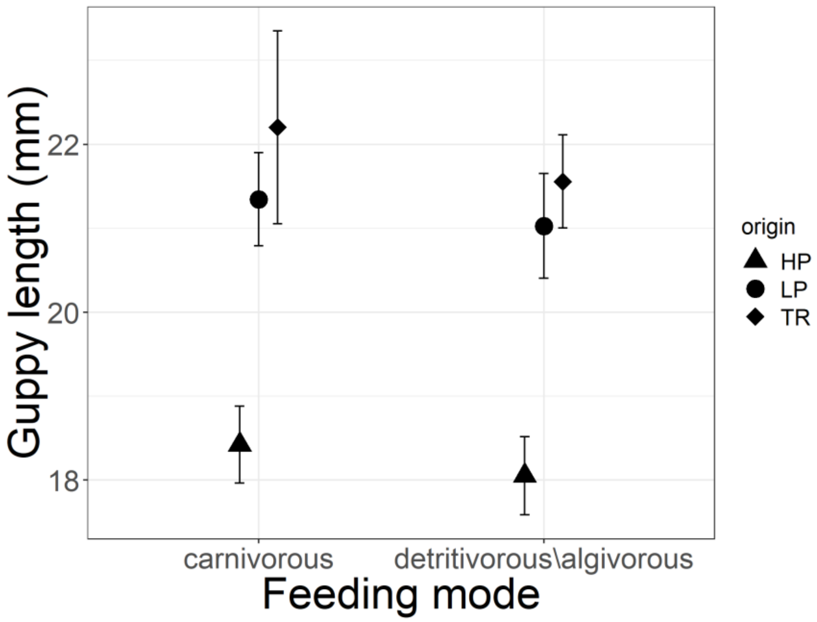

Figure C4. Average body length for each feeding phenotype within populations occurring in reaches with predators (HP), without predators (LP) and populations originated from HP reaches that were transplanted into guppy free, predator free reaches (TR). Symbols represents the average for all the sampled individual pools within stream reaches.

Appendix D – Supplementary material of Chapter 5

Table D1. List of food items consumed. Food items were identified to the lowest taxonomic level possible, often family. Items that could not be identified are shown as NI and items not consumed are indicated by (-). The bulk proportion of each consumed in all urban and non-urban populations is shown.

Food item	Urban	Non-urban
Collembola		
NI	–	0.0005
Diptera		
Ceratopongonidae	0.0023	0.0076
Chironomidae	0.2328	0.0324
Emphididae	–	0.0004
Psychodidae	0.0013	–
Simulidae	–	0.0148
Stratiomidae	–	0.0054
Tipulidae	–	–
NI	–	0.0010
Trichoptera		
Hydropschidae	–	0.0020
Hydroptilidae	–	0.0027
Polycentropodidae	–	0.0065
Ephemeroptera		
Leptohyphidae	–	0.0030
Leptoplhebiidae	–	0.0177
NI	–	0.0118
Odonata		
Gomphidae	–	0.0006
Coleoptera		
Elmidae	–	0.0021
Psephenidae	–	0.0067
NI	–	0.0042
Hydracarina		
NI	–	0.0008
Hemiptera		
Velidae	–	0.0006
NI	–	0.0006
Lepidoptera		
NI	–	0.0315
Gastropod		

NI	–	0.0028
Opisthopora		
NI	–	0.0269
Green Algae		
Diatoms	0.0007	0.0027
Filamentous algae	0.0082	0.0049
Other algae	0.0002	0.0238
Others		
Aquatic insect debris	0.0612	0.1760
Terrestrial insect	0.0052	0.0092
Detritus	0.6882	0.6007

Table D2. Linear mixed effect models for assessing the effect of guppy density and food availability on resource use phenotype and individual specialization. I built separated models for urban and non-urban populations, using as response variables the proportion of carnivorous phenotypes (PC) and gut ratio (GUT). The fixed effect used in all models were fish biodiversity (guppy only (GO) or guppy and other fish (GF)), chironomid biomass (CB, mg/m^2) and guppy density (ind/ m^2). Guppy length (SL) was not included in GUT model, because gut ratio already account for the effect of body size. All models had reach identity nested within year as random effects (not shown).

Response variable	Fixed effects	Urban population					Non-urban population				
PC		value	STE	DF	t-value	p-value	value	STE	DF	t-value	p-value
	int	0.134	0.839	4	0.160	0.881	0.761	1.212	3	0.628	0.575
	SL	-0.003	0.035	4	-0.085	0.936	-0.004	0.046	3	-0.080	0.942
	GOvsGF	0.299	0.237	4	1.260	0.276	0.181	0.193	3	0.938	0.417
	CB	0.001	0.001	4	1.451	0.220	-0.007	0.008	3	-0.904	0.433
	GD	-0.0003	0.001	4	-0.020	0.985	-0.080	0.043	3	-1.880	0.157
GUT											
	int	388.614	34.425	98	11.289	0.000	422.343	34.890	110	12.105	0.000
	GOvsGF	9.451	24.028	5	0.393	0.710	-46.858	31.482	4	-1.488	0.211
	CB	0.120	0.092	5	1.299	0.251	-2.453	1.060	4	-2.314	0.082
	GD	-0.080	0.125	5	-0.637	0.552	6.442	6.834	4	0.943	0.399

Table D3. Principal Component Analysis (PCA) loadings for the cranium shape variables obtained from a set of 8 anatomical landmarks. The landmarks were converted into 16 shape variables following a Generalized Procrustes Analysis. The 16 vectors of shape were used in the a PCA analysis. The eigenvalues (Eig), variance (Var) and cumulative variance (C.var) that each principal component (PC1-16) explain are shown. Data were combined across years (2016-2017) and across biodiversity treatments (streams where guppies are the only fish species or streams where guppies co-occur with other fish).

PCs	Eig.	Var (%)	C.var (%)
PC1	0.00090	39.8	39.8
PC2	0.00035	15.4	55.2
PC3	0.00023	10.2	65.3
PC4	0.00017	7.7	73.1
PC5	0.00013	5.7	78.8
PC6	0.00012	5.2	83.9
PC7	9.19x10-5	4.1	88.0
PC8	7.9x10-5	3.5	91.5
PC9	7.17x10-5	3.2	94.7
PC10	4.44x10-5	2.0	96.6
PC11	4.34x10-5	1.9	98.5
PC12	3.3x10-5	1.5	100
PC13	9.87x10-32	4.36x10-27	100
PC14	8.89x10-32	3.93x10-27	100
PC15	2.15x10-33	9.52x10-29	100
PC16	9.61x10-34	4.25x10-29	100

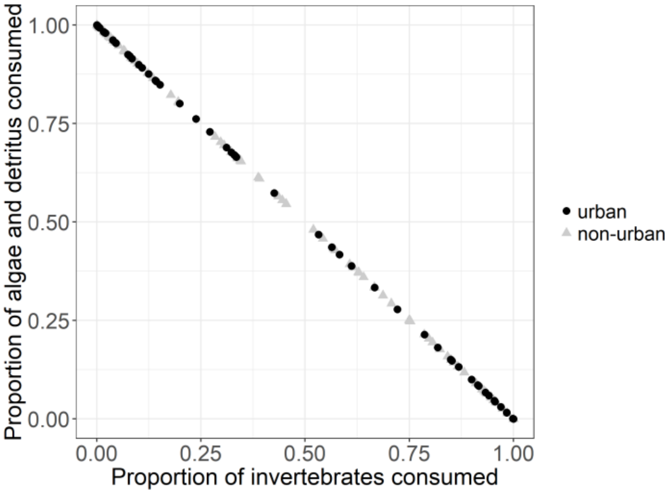

Figure D1. Correlation between the proportion of invertebrates and the pooled proportion of algae plus detritus consumed by guppies in urban (black circle) and non-urban (grey triangle) stream reaches. Each point represents one individual. Data are shown for both years 2016 and 2017.

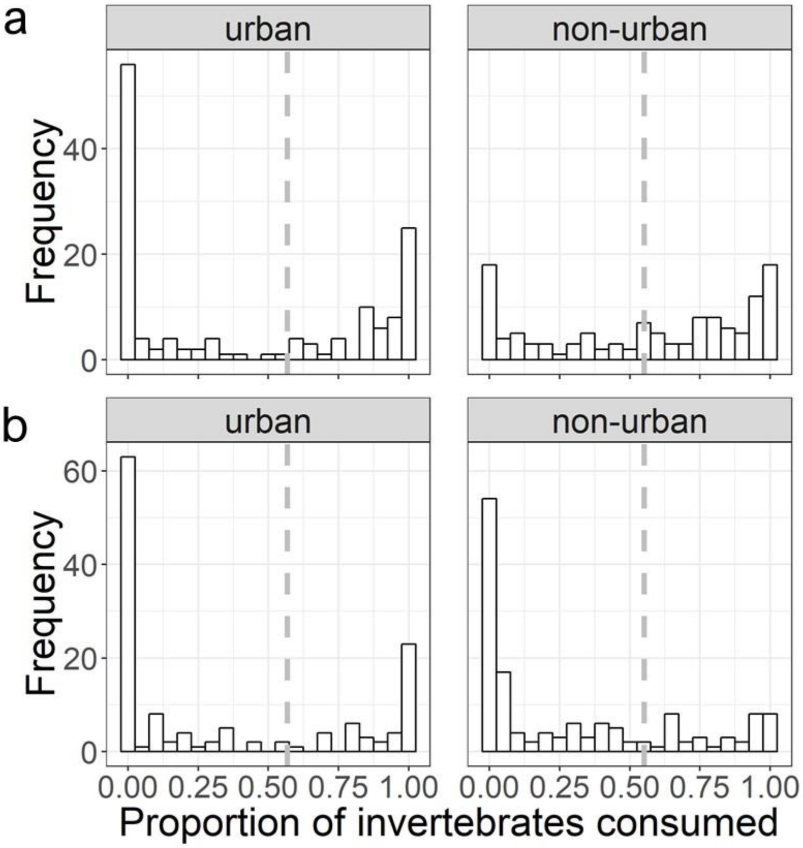

Figure D2. Distribution plots of the proportion of invertebrates consumed by guppies in urban and non-urban populations sampled in both sampling years (a) 2016 and (b) 2017. Dashed line represents the population mean.

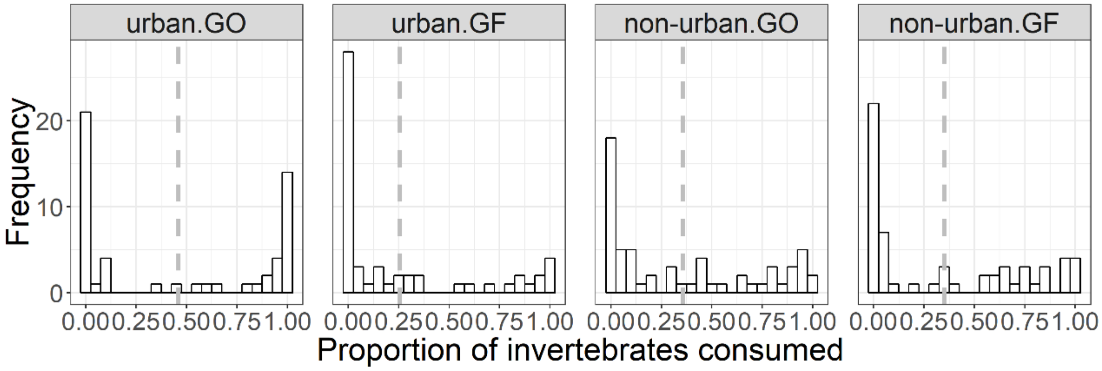

Figure D3. Distribution plots of the proportion of invertebrates consumed by guppies in urban and non-urban reaches where guppies are the only fish species (GO) and in reaches where guppies co-occur with other fish species (GF). Dashed line represents the population mean.

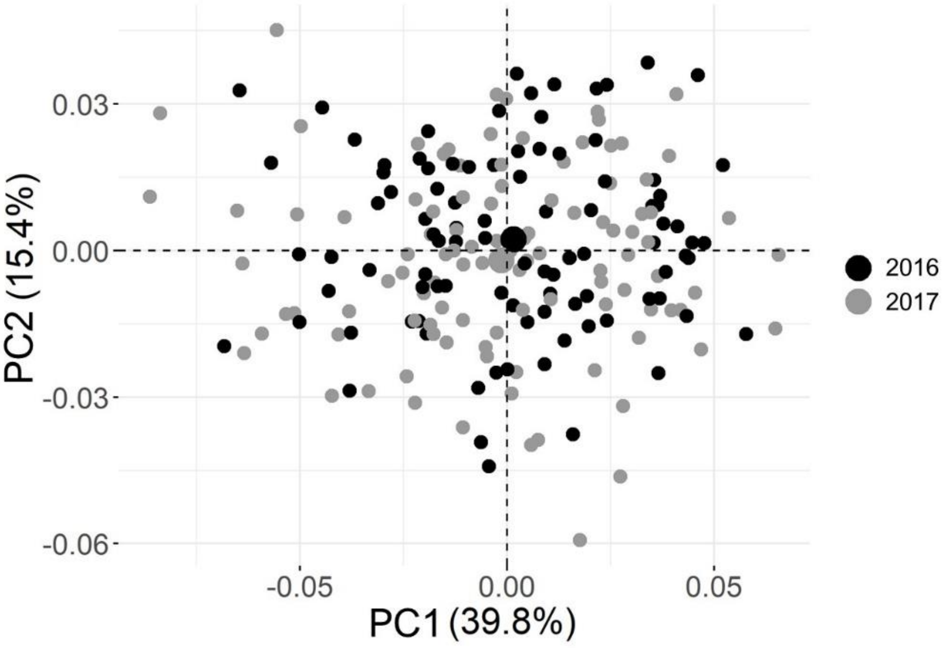

Figure D4. Principal Component Analysis (PCA) of cranium shape by year. Cranium shape for each individual was obtained from a set of 8 anatomical landmarks. The landmarks were converted into 16 shape variables following a Generalized Procrustes Analysis. The 16 vectors of shape were used in the a PCA analysis. Each point represents the cranium shape of each individual in both urban and non-urban populations in 2016 (black circle) and 2017 (grey circle). Large symbols represent the mean of each year.