DISSERTATION

INTRA- AND INTERSPECIFIC VARIATION ALONG ENVIRONMENTAL GRADIENTS: ADAPTATION, PLASTICITY, AND RANGE LIMITS

Submitted by

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ABSTRACT

PHENOTYPIC DIFFERENTIATION AND DISTRIBUTION LIMITS ALONG ECOLOGICAL GRADIENTS

Understanding the processes underlying patterns of intraspecific variation, and how these processes in turn shape the distributional limits of species is a fundamental goal of evolutionary ecology. The study of species distributed along environmental gradients provides a framework for testing how changing conditions lead to local adaptation, phenotypic plasticity, and ultimately shape distributional limits. Yet, environmental gradients are complex, being composed of a diversity of abiotic and biotic factors that act on individual species and shape the interactions between them. Thus, empirical studies aimed to understand patterns of intraspecific divergence and interspecific diversity need to evaluate the effects of both abiotic and biotic factors varying along gradients.

Evolutionary ecologists have become increasingly interested in trying to understand the costs and limits to trait variation along environmental gradients and what factors prevent species from evolving larger geographic ranges. Theory predicts that species distributed along environmental gradients should track conditions through local adaptation or adaptive phenotypic plasticity, and that a disruptive factor along the gradient (e.g. increase in the steepness of the gradient, the presence of a competitor, etc.) could result in the formation of distribution limits as conditions become unsuitable for populations persistence. Empirical studies analyzing large-scale patterns of phenotypic variation have provided support for the formation of clines in response to environmental gradients. However, less evidence has accumulated for the formation

of such patterns at local scales and clear disruptive factors leading to distributional limits remain elusive.

My dissertation takes an evolutionary ecological perspective to understand how environmental gradients shape patterns of variation within and between species. Here, I attempt to understand how abiotic and biotic factors interact to drive patterns of phenotypic variation. To approach this question, I used as a study system two closely related, ecologically similar, and parapatric species of poeciliids distributed along rivers on the island of Trinidad, West Indies.

In the first part of this dissertation, I focus on the patterns of intraspecific variation in the Trinidadian guppy (*Poecilia reticulata*) along a predation risk gradient. I used this species to explore the spatial scale at which local adaptation occurs (Chapter 1), and to investigate the role of adaptive phenotypic plasticity in allowing species to track local optima (Chapter 2). I found that local adaptation in Trinidadian guppies occurs at a smaller spatial scale than previously shown. My results also suggest that adaptive plasticity plays an important role in allowing Trinidadian guppies to track local optima along a gradient of predation risk. Furthermore, I found divergence in patterns of plasticity between Trinidadian guppy populations adapted to low- or to high-levels of predation pressure. My results suggest that this difference in adaptive phenotypic plasticity evolved as a by-product of adaptation to local environmental conditions.

In the second part of my dissertation I change my focus from patterns of intraspecific variation to patterns of interspecific variation along environmental gradients. I examine how the Trinidadian guppy and its sister species, the swamp guppy (*P. picta*), are distributed along a complex environmental gradient in lowland rivers of Trinidad (Chapter 3), and performed a series of experiments aimed to determine what factors drive their distributions (Chapter 4). As

Trinidadian rivers approach the ocean, several factors change in comparison to upstream localities, including changes in productivity, physicochemical conditions, and community composition. I found that the Trinidadian guppy and the swamp guppy show an overlapping parapatric distribution along the interface between brackish-freshwater in the lowland rivers of Trinidad. The swamp guppy is usually found in downstream sections of the rivers, both in fresh- and brackish water. On the other hand, the Trinidadian guppy is only found in freshwater, dropping off abruptly at the brackish-freshwater interface. Field and laboratory experiments suggest that brackish water environments are physiologically stressful for the two study species, as survival and growth rate in this environment were lower compared to that observed in freshwater. Also, these experiments indicate that the Trinidadian guppy is competitively dominant over the swamp guppy across all salinity conditions. Thus, I showed that asymmetric competition limits the competitively subordinate swamp guppy to the harshest end of the salinity gradient, and that stressful salinity conditions limits the dominant Trinidadian guppy to the less stressful freshwater end of the gradient.

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INTRODUCTION

INTRA AND INTERSPECIFIC VARIATION ALONG ENVIRONMENTAL GRADIENTS: ADAPTATION, PLASTICITY, AND RANGE LIMITS

Understanding the processes underlying patterns of intraspecific variation, and how these processes in turn shape the distributional limits of species along environmental gradients is a fundamental goal of evolutionary ecology. Within species, populations often exhibit phenotypic differences along environmental gradients, the magnitude of which reflect the opposing influences of natural selection and gene flow, as well as the plastic responses of individuals to environmental cues. Yet, environmental gradients are complex, being composed of a diversity of abiotic and biotic factors that act on individual species and shape the interactions between them. Thus, understanding how species are distributed along an environmental gradient provides a framework for testing how changing abiotic and biotic factors lead to local adaptation, phenotypic plasticity, and ultimately shape distributional limits. Below I briefly review the historical ecological arguments for how environmental gradients have shaped the structure of communities and the evolutionary arguments for how such gradients shape variation between populations. I then review recent arguments that have merged from these ecological and evolutionary perspectives to explain range limits.

Traditionally, environmental gradients were seen as stable systems along which communities predictably organized. However, ecologists disagreed on the major processes shaping communities, with most discussion being centered around the relative importance of interspecies relations versus the way individual species responded to intrinsic environmental requirements (Whittaker 1956). These points of view are exemplified by the opposite views of

Frederic Clements and Henry Gleason about plant succession. Clements (1916) emphasized the importance of biotic interactions in community structure, proposing that species within communities were highly interrelated and communities were discrete, self-regulated entities. On the other hand, Gleason (1926) emphasized the individual characteristics of species, suggesting that communities organized based on the intrinsic environmental requirement of each of the composing species. On the other hand, animal ecologists, like Charles Elton (1927), emphasized the complexity of environmental gradients. Elton suggested that the physiological tolerance of a species to abiotic factors could determine its range, but that the interaction with other species could also limit a species distribution. Furthermore, he suggested that abiotic and biotic factors could interact to affect species distributions (Elton 1927).

Later, Evelyn Hutchinson synthesized some of these ideas in his influential exposition about ecological niches. Hutchinson (1957) suggested that the set of abiotic conditions under which a species could maintain viable populations was the “fundamental niche” of the species. However, he noted that in reality species usually maintained viable populations in only a subset of the area predicted based on their fundamental niches. He called this the “realized niche” of the species, and proposed that it was the result of the interaction with other species, including competitors and predators. Current conceptual discussions about the Hutchinsonian niche have pointed out that Hutchinson ignored several factors affecting population dynamics that could result in the area occupied by a species being different from its fundamental niche. In particular, dispersal has important consequences for species distributions (Pulliam 2000; Soberón 2007). For example, some species could be predictively found occupying areas outside of their fundamental niche as the result of immigration (i.e. sink populations; Pulliam 1988). Nonetheless, Hutchinson’s ideas were highly influential and with the distinction between realized and fundamental niches it became evident that both abiotic and biotic factors needed to be considered in ecological studies that attempted to explain patterns of distribution.

The niche concept became dominant in ecology and resulted in a renewed interest in the role of environmental gradients in determining community turnover (MacArthur 1972). The study of parapatric distributions became important as a model to understand community organization and differences between fundamental and realized niches. Several empirical studies focused on parapatric distributions in different taxa and at different spatial scales. Examples of this are the classical work by Joseph Connell (1961) on the distribution of barnacles, by John Terborgh (1971) on the altitudinal distribution of passerine birds in the Andes hills of Ecuador, and by Craig Heller (1971, 1972) and Mark Chappell (1978) on altitudinal distribution of chipmunks in the mountains of California.

At the same time, there was parallel discussion in evolutionary biology about the role of environmental gradients in the determining clinal variation in traits, and in turn, the potential for speciation in the absence of barriers to gene flow. Although the dominant idea at that time was that allopatry was necessary for differentiation and speciation to occur (Mayr 1963), there was an accumulation of studies showing clinal variation in species characters along environmental gradients (Antonovics and Bradshaw 1970; Endler 1977). With the subsequent development of theoretical models that predicted both sympatric divergence and speciation along gradients (Endler 1977), evolutionary biologists started to question why there were limits to adaptation (Antonovics 1976). The study of species distributions and range borders provided a unique opportunity to address this question (Gaston 2003).

The integration of ecological and evolutionary perspectives brought new attention to the study of environmental gradients and the study of processes that occur at range margins. This renewed interest in the study of range margins is reflected by the increased number of publications dedicated to the subject and the appearance of several special issues dedicated to it (e.g. Oikos, Holt and Keitt 2005; The Proceedings of the Royal Society, Gaston 2009; and The American Naturalist, Geber 2011). Evolutionary ecologists have become particularly interested in trying to understand why there are limits to trait variation along environmental gradients and what factors prevent species from evolving larger geographic ranges. Several mechanisms are proposed to limit adaptation at range borders, some of which focus only on genetic constraints. For example, a frequently evoked mechanism is a lack of genetic variation in marginal populations for selection to act upon (Blows and Hoffmann 2005; Kellermann et al. 2009). Although this pattern is often found in nature, there are several exceptions that suggest that other mechanisms might come into play (Eckert et al. 2008). A second mechanism often proposed to limit adaptations to range margins is the presence of genetic trade-offs among fitness related traits (Levins 1969; Antonovics 1976; Jenkins and Hoffmann 1999; Holt 2003). For example, theory predicts that if adaptation to a habitat beyond the range margin comes at the expense of fitness within the existing habitat of a species, fitness-trade offs should prevent evolution and expansion into the new habitat (e.g. Holt 2003). Yet another hypothesized mechanisms for the limit of adaptation to conditions outside the distribution is the diluting effect of gene flow (Haldane 1956; Mayr 1963; Kirkpatrick and Barton 1997). Such models argue that gene flow from central populations to peripheral populations at the range boundaries introduces maladaptive alleles that prevent adaptive evolution (e.g. Kirkpatrick and Barton 1997). Indeed, some empirical work has shown that gene flow can reduce local adaptation, but that this effect is not necessarily stronger in marginal versus central populations (Paul et al. 2011).

All the above-mentioned models assume that species are maladapted to conditions at, and beyond, the distribution margin. More recently, some models suggested that stable ranges limits could arise due to the effects of interspecific interactions, and that adaptation to conditions outside of the range would occur in the absence of competition. For example, Case and Taper (2000) extended the gene flow model by Kirkpatrick and Barton (1997) by allowing two species to compete along the gradient. This model showed that the presence of competing species reduces the degree of gene flow and the steepness of the gradient needed to create stable distribution limits. Later, Price and Kirkpatrick (2009) relaxed the requirement of gene swamping for the formation of parapatric distributions along environmental gradients. Their model predicts the formation of evolutionary stable parapatric distributions if two ecologically similar species specialize on different resources, and one, or both, of such resources gradually decrease along the gradient. Furthermore, Doebeli and Dieckmann (2003) hypothesized that the same conditions that predict the formation of parapatric distributions (i.e. environmental gradients, gene flow, resource competition), also could predict ecological speciation, which in turn result in the formation of parapatric distributions. Doebeli and Dieckmann (2003) used a spatially explicit model, whose behavior was dependent on the amount of gene flow, the steepness of the gradient, and the evolution of assortative mating. They found that speciation and parapatric distributions of resulting sister taxa are expected at low levels of gene flow and medium gradient slopes. However, higher levels of gene flow, or random mating, will result in the formation of intraspecific trait clines without speciation occurring.

A common prediction of all the models mentioned above is the evolution of intraspecific clinal variation of traits along environmental gradients. In other words, species are predicted to track the optimum phenotype imposed by environmental gradient through local adaptation or adaptive phenotypic plasticity. Distributional limits arise when a disruptive factor (e.g. increase in the steepness of the gradient, the presence of a competitor, etc.) results in conditions beyond which populations are unable to persist. Empirical studies analyzing large-scale patterns of phenotypic variation have provided support for the formation of clines in response to environmental gradients (i.e. Kawakami et al. 2011; Mariac et al. 2011; Hangartner et al. 2012). However, less evidence has accumulated for the formation of such patterns at local scales (i.e. Whitehead et al. 2011) and clear disruptive factors leading to distributional limits remain elusive (Sexton et al. 2009). Furthermore, because organisms can track local environmental changes via adaptive phenotypic plasticity, species can increase local fitness without showing genetic differentiation (Relyea 2004). Furthermore, adaptive plasticity can play a fundamental role in range expansions by increasing a species probability of persistence after colonization of environments outside of its range (e.g. West-Eberhard 2003; Price et al. 2003; Crispo 2007; Ghalambor et al. 2007; Lande 2009). In turn, adaptation to new environments could result in the evolution of plasticity (Via and Lande 1985; Lande 2009). However, empirical studies of natural populations supporting such theory are generally absent because of the challenges of capturing the role of plasticity during the initial stages of colonization.

My dissertation takes an evolutionary ecological perspective to understand how environmental gradients shape patterns of variation within and between species. I am particularly interested in the roles of abiotic and biotic factors, and the interaction between them, in the formation of distribution limits. Furthermore, I strive to understand how these factors drive patterns of phenotypic variation. To approach these questions, I used a model study system of two closely related, ecologically similar species of livebearer fishes distributed along a freshwater-brackish water gradient in rivers on the island of Trinidad, West Indies. Below, I describe the study system in more detail, with emphasis on the factors that vary along rivers of Trinidad, including predation, productivity, and salinity. Later, I provide a brief introduction to the four chapters that compose this dissertation.

Study System

Environmental gradients along rivers of Trinidad provide a model system for testing how biotic and abiotic factors jointly shape the distributional limits of fish. This system has been extensively studied in the context of adaptive divergence between populations of the Trinidadian guppy (*Poecilia reticulata*; Endler 1995; Magurran 2005). Therefore, there is a good understanding on how factors change along Trinidadian rivers (Reznick et al. 2001; Magurran 2005). The variation in predation risk along the rivers draining from the Northern Range Mountains of Trinidad is one the most studied gradients in the system (Endler 1978; Gilliam et al. 1993; Reznick et al. 1996). Fish communities in the lowland are diverse, including multiple predatory fish species such as the Pike Cichlid (*Crenicichla frenata*), the Wolf Fish (*Hoplias malabaricus*), and several other species of cichlids and characins. Waterfalls serve as barriers to the upstream distribution of some of these species, resulting in a stepwise deletion of species as one moves from downstream sections of the rivers to the headwater streams. These upstream localities contain very simple fish communities, usually being composed by the Trinidadian guppy and an opportunistic, but gape-limited predator, killifish (*Rivulus hartii*). This gradient in community composition is reflected in gradual change in predation risk for small fish like the guppy (Endler 1978; Reznick et al. 1996).

In addition to differences in fish community composition, up- and downstream localities differ in a number of physical and chemical characteristics that affect primary productivity and thus influence resource availability for fish (Reznick et al. 2001). For example, downstream localities tend to have open canopies that increase primary productivity, causing predation risk to co-vary with reduced competition (Reznick et al. 2001). In addition, physicochemical variables, like temperature, pH, dissolved oxygen, and channel width and depth show a pattern of variation from up- to downstream localities paralleling that observed for risk of predation (Magurran 2005; Torres Dowdall et al. 2012).

In **Chapter 1**, my coauthors and I analyze the scale at which adaptation occurs along these environmental gradients. To achieve this goal we studied the evolution of life-history traits along the above-mentioned gradient of predation pressure in the Trinidadian guppy. Trinidadian guppies have served as a model system to understand how spatial environmental variation leads to divergent selection pressures and adaptive divergence between populations (Endler 1995; Magurran 2005). However, despite the continuous nature of spatial environmental variation throughout the guppies' distribution, most of the phenotypic variation has been repeatedly studied under a dichotomy of high-versus low-predation localities. Although this approach has been very successful in identifying the signature of local adaptation, it might limit our ability to identify the causes of underlying patterns of phenotypic variation. By studying variation in life-history traits along continuous gradients we can gain better insight into the diversity of adaptations exhibited by natural populations.

We selected six localities along the Guanapo-Caroni River drainage with respect to their predator community, going from upstream localities where guppies only coexist with a single gape-limited fish predator, to lowland sites where guppies coexist with a complex fish community (Gilliam et al. 1993). Along this gradient we characterized the field pattern of phenotypic variation in life-history traits. Further, to determine the genetic basis of this variation, we measured these traits in second-generation laboratory-born fish from the same localities sampled in the wild. Our results imply that local adaptation in guppies occurs at a finer scale than has previously been shown. Furthermore, while our results are consistent with predator-driven life-history variation, we also find patterns of plasticity that would not be apparent in the traditional dichotomous approach.

In **Chapter 2** my coauthors and I explore the potential role of phenotypic plasticity in allowing species to track environment gradients and in facilitating the colonization of new environments. The upstream limit to the distribution of Trinidadian guppies is known to be caused by dispersal limitation along waterfall barriers. These barriers are infrequently crossed, resulting in an increase of the area occupy by guppies at the drainage scale. This colonization of new upstream habitat often results in new selection pressures, as both predation pressure and resource availability are reduced in these upstream locations compared to downstream sources habitat (see above). Natural surveys and translocation experiments have shown that local adaptation to these new environmental conditions has independently evolved numerous times (Reznick 1982; Reznick et al. 1997; Magurran 2005). However, less clear is the role that phenotypic plasticity might have played in facilitating colonization and persistence in these new environments and the degree to which predator-induced plasticity contributes to population differentiation. We conducted common garden experiments on guppies obtained from two drainages containing populations adapted to high- and low-predation environments. We reared full-sibs from all populations in treatments simulating the presumed ancestral (predator-cues present) and derived (predator-cues absent) conditions and measured water column use, head morphology, and size at maturity. When reared in presence of predator cues, all populations had phenotypes that were typical of a high-predation ecotype. However, when reared in the absence of predator cues, guppies from high- and low-predation regimes differed in head morphology and size at maturity; the qualitative nature of these differences corresponded to those that characterize adaptive phenotypes in high- versus low-predation environments. Thus, divergence in plasticity is due to phenotypic differences between high- and low-predation populations when reared in the absence of predator cues. These results suggest that plasticity might play an important role during adaptation to novel environments, and that the evolution of plasticity occurred as a by-product of adaptation to the derived environment.

Although we have advanced in our understanding of the factors limiting the upstream distribution of Trinidadian guppies and the selection pressures at such limits, we know a lot less about the factors limiting the downstream distribution of this species. As Trinidadian rivers approach the ocean, several factors change in comparison to upstream localities, including changes in productivity, physicochemical conditions, and community composition. The lowland rivers of Trinidad have a strong anthropogenic influence, suffering a high reduction of canopy cover and often are highly eutrophied (WRA 2001). Also, as the lowlands of Trinidad are really flat, there is a strong influence of the ocean, resulting in salt-water intrusion into the rivers forming estuaries and swamps. This creates a gradient of salinity, from freshwater (<0.5 part per thousand), to brackish water (0.5-30 ppt), to saline water (30-50 ppt). Occasionally, salinity at inland sites reaches values as high as 60 ppt (personal observation, sea water≈34 ppt). Finally, there are large changes in the fish community between lowland rivers and upstream localities. These include an increase on diversity and abundance of predatory fish (Magurran and Phillip 2001) that would suggest increased predation pressure. In addition, a phenotypically similar close relative of guppies, the Swamp guppy (*Poecilia picta*), co-occurs in the lowland section of Trinidadian rivers with the Trinidadian guppy. The Trinidadian guppy and the Swamp guppy are phenotypically similar in morphology and life history traits and have a similar ecological role (Reznick et al. 1992). In Trinidad, these species show contiguous distributions with a zone of overlap where both species can be found in mixed shoals (Magurran and Ramnarine 2004; Russell et al. 2006). However, each species often uses distinct habitat, with the Swamp guppy being more common in brackish water and the Trinidadian guppy in freshwater (Reznick et al. 1992; Reznick and Endler 1982; Wischnath 1993). Despite these differences in habitat use, there is no clear understanding of the distribution limits of these two species across the environmental gradient, nor there is any knowledge of what factors drive such limits.

In **Chapter 3** of this dissertation, my coauthors and I describe the limits to the distribution of Trinidadian guppy and it close relative the Swamp guppy. Furthermore, we used the pattern of phenotypic variation along the gradient to make predictions about potential factors affecting the distribution limits of these two species. We find that salinity appears to limit the downstream distribution of Trinidadian guppies, as their populations stop abruptly at the freshwater-brackish water boundary. In contrast, the Swamp guppy appears to be limited by the productivity of the streams and competition with Trinidadian guppies.

Finally, in **Chapter 4**, I explore potential mechanisms creating the distribution pattern described in the previous chapter. I combined a series of laboratory and field experiments to explore how salinity and interspecific competition interact to determine the parapatric distribution of the Trinidadian guppy and the Swamp guppy along the brackish-freshwater ecocline in the lowland rivers of Trinidad. The results of these experiments suggest that brackish water is an osmotically challenging environment for both studied species. However, the effect of salinity in individual growth rate was more marked in the Trinidadian guppy than in the Swamp guppy. Furthermore, the Trinidadian guppy had reduced survival in brackish water compared to freshwater, whereas the Swamp guppy had similar survival across both brackish and freshwater. It appears that these stressful conditions have selected for behavioral avoidance in the Trinidadian guppy, as this species exhibited a strong preference for freshwater and avoidance of brackish water. On the other hand, we found asymmetric competition as the Trinidadian guppy is competitively dominant over the Swamp guppy, independently of the salinity level at which competition was tested. Thus, I suggest that asymmetric competition limits the competitively subordinate, but more salinity tolerant, Swamp guppy to the harshest end of the salinity gradient, and behavioral avoidance of stressful salinity conditions limits the dominant competitor, the Trinidadian guppy, to the mildest end of the gradient.

Collectively, these dissertation chapters capture the abiotic and biotic factors that lead to patterns of local adaptation, phenotypic plasticity, and range limits along an environmental gradient.

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CHAPTER 1:

FINE-SCALE LOCAL ADAPTATION IN LIFE HISTORIES ALONG A CONTINUOUS ENVIRONMENTAL GRADIENT IN TRINIDADIAN GUPPIES

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SUMMARY

Theoretical models of life-history evolution predict a continuum of fast to slow life histories, yet most of empirical support for this theory comes from studies that have considered dichotomous environments (i.e. high versus low food, presence or absence of major predators, etc.). Although this approach has been very successful in identifying the signature of local adaptation, it might limit our ability to identify the causes of underlying patterns of phenotypic variation. By studying variation in life-history traits along continuous gradients we can gain better insight into the diversity of adaptations exhibited by natural populations.

We studied the evolution of life-history traits along a gradient of predation pressure in the Trinidadian guppy (*Poecilia reticulata*). Six localities along the Guanapo-Caroni River drainage were selected with respect to their predator community, going from upstream localities where guppies only coexist with a single gape-limited fish predator, to lowland sites where guppies coexist with a complex fish community. Along this gradient we characterized the field pattern of phenotypic variation in age and size at maturity and reproductive effort. Further, to determine the genetic basis of this variation, we measured these traits in second-generation laboratory-born fish from the same localities sampled in the wild.

In nature, we found a fine-scale pattern of phenotypic variation in most life-history traits that paralleled the continuous predation gradient. In the laboratory, we observed that reproductive allocation and brood size progressively decrease while age at maturity and inter-brood interval progressively increase with a reduction in the predator community, suggesting a genetic basis to the parallel patterns observed in the field for reproductive allocation and offspring number.

However, there were some exceptions to the observed pattern of variation. Females from one low-predation locality matured younger and reproduced more frequently than expected based upon the simple nature of the fish community. We also found significant differences between our field and lab results for embryo size, suggesting that this trait is highly plastic.

Our results imply that local adaptation in guppies occurs at a finer scale than has previously been shown. Furthermore, while our results are consistent with predator-driven life-history variation, we also find patterns of plasticity that would not be apparent in the traditional dichotomous approach.

INTRODUCTION

Divergent natural selection can drive adaptive divergence in conspecific populations exploiting heterogeneous habitats (Mazer and Damuth 2001). In particular, the proliferation of life-history strategies that evolve under divergent ecological conditions has been of central interest to evolutionary biologists (Roff 1992; Stearns 1992; Charlesworth 1994). A great body of theory has been developed around the role of age-specific mortality in shaping life histories (Gadgil and Bossert 1970; Law 1979; Charlesworth 1994). High ratios of adult to juvenile extrinsic mortality are predicted to select for faster life histories, where individuals mature younger and smaller, have increased allocation to reproduction, reproduce more frequently, have larger litters, and have accelerated rates of senescence (Gadgil and Bossert 1970; Law 1979; Charlesworth 1994; Roff 1992; Stearns 1992). Extensive empirical support exists for these predictions (Roff 1992; Stearns 1992). For example, differences in adult to juvenile survival result in variation in the age at maturity and reproductive effort in brook trout, *Salvelinus fontinalis* (Hutchings 1993) and freshwater amphipods, *Hyalella azteca* (Wellborn 1994), and reduced adult mortality results in lower reproductive investment and reduced rates of senescence in Virginia opossums, *Didelphis virginiana* (Austad 1993), and in water fleas *Daphnia pulex* (Dudycha 2001).

However, despite the theoretical emphasis on a continuum of fast to slow life histories (Stearns 1992), most empirical work has focused on population comparisons at the extreme ends of an ecological gradient. For example, few studies have compared life-history traits of populations along an environmental gradient to test if traits diverge to produce a continuous variation that tracks the environmental gradient or if traits show a threshold response (Piché et al. 2008; Walsh and Reznick 2008, 2010; Kawakami et al. 2011; Mariac et al. 2011; Tomkins et al.

2011). Yet, understanding how continuous variation across environmental gradients is generated and maintained has important implications to our understanding of adaptive evolution and the processes that lead to speciation (Endler 1977).

Populations of Trinidadian guppies (*Poecilia reticulata*) have served as a model system to understand how spatial environmental variation leads to divergent selection pressures and adaptive divergence between populations (Endler 1995; Magurran 2005). In particular, Trinidadian guppies show a repeated pattern of life-history divergence in response to differences in predation risk (Endler 1995). Guppies that co-occur with predators are younger and smaller at maturity (Reznick 1982a; Reznick et al. 2001), produce more offspring per litter (Reznick and Endler 1982), produce smaller offspring (Reznick 1982b), and allocate more resources to each reproductive event (reproductive allocation) (Reznick 1982a; Reznick et al. 1996a) compared to guppies in depauperate communities that experience reduced predation pressure. These patterns are consistent with predictions from theory that models how life histories evolve in response to mortality risk (Gadgil and Bossert 1970; Law 1979; Charlesworth 1994). Similarly, guppies have been an important study system for other traits thought to be under divergent selection from predators, including various anti-predator behavior (Seghers 1974; Magurran et al. 1992; Godin and Davis 1995; Ghalambor et al. 2004; Magurran 2005; Walker et al. 2005; Botham et al. 2006; Huizinga et al. 2009), morphology (Langerhans and DeWitt 2004; Alexander et al. 2006), and coloration (Endler 1991; Magurran and Seghers 1994; Endler and Houde 1995; Houde 1997). Because many of these phenotypic differences have been shown to have a genetic basis, these results collectively provide strong evidence for local adaption to high- and low-predation communities (Magurran 2005).

Despite the continuous nature of spatial environmental variation throughout the guppies' distribution, the aforementioned traits have been repeatedly studied under a dichotomy of high- versus low-predation localities. High-predation localities tend to be higher-order, low-elevation streams, containing a diverse fish community that includes multiple potential predators of guppies. On the other hand, low-predation localities often are headwater streams, with a depauperate fish community, usually including only a killifish, *Rivulus hartii*, which is considered an occasional predator of small guppies (Endler 1978; Reznick and Endler 1982). In addition to differences in fish community composition, high- and low-predation localities differ in a number of physical and chemical characteristics that affect primary productivity and thus influence food availability for guppies (Reznick et al. 2001). For example, high-predation localities tend to have open canopies that increase primary productivity, causing predation risk to co-vary with reduced competition (Reznick et al. 2001). Thus, the inability to disentangle the effect of predation from the effects of correlated environmental variables, such as resource availability, complicates simple causal relationships between predation and divergent traits (Johnson 2002).

Several reasons exist for why past research has focused on the simple dichotomy between high- and low-predation communities and dominated the study of adaptation in guppies. First, these paired comparisons match the natural distribution of guppy populations in some drainages, where the transition from high- to low-predation habitat occurs abruptly among adjacent communities separated by a single waterfall barrier (e.g. Aripo River; Reznick 1982a). Second, variation in life-history traits is consistent and repeatable between guppies collected from localities that are easily characterized by the fish community (i.e. only *Rivulus* in low-predation localities and the presence of *Crenicichla* in high-predation localities; e.g. Reznick and Bryga

1996). Thus these contrasts have proved an effective way to assess the effects of predation on local adaptation. Finally, the focus on this dichotomy is in part the consequence of an emphasis on statistical power and efficiency. The dichotomy approach has been very productive and virtually every attribute studied in guppies has suggested local adaptation (Endler 1995). However, this approach also risks falling short of revealing the diversity of adaptations displayed by natural populations of guppies.

In his first comprehensive exposition on the role of predation in shaping the evolution of male coloration in guppies, Endler (1978) described a gradient of fish communities that ranged from diverse communities downstream to those that contained only a single species of fish, *R. hartii,* in the headwater streams. Between these extremes lay a fairly regular sequence of communities in which there is a stepwise deletion of some species as one moves from the most diverse communities downstream to the least diverse upstream. Corresponding with this stepwise deletion of predators, Endler described a parallel sequence of change in male coloration, which implies that the individual species of predators had unique effects in shaping male coloration and that the difference between high- and low-predation populations was the cumulative effect of the predator community (Endler 1978). This gradient in male coloration in guppies suggests that local adaptation in this species occurs at a much finer scale than has been traditionally addressed in the guppy system. Thus, while the high- and low-predation dichotomy has served well as a basis for studying adaptation in guppies, it does not represent the full diversity of communities experienced by Trinidadian guppies, and may not represent the full spectrum of local adaptation exhibited by guppies. More importantly, if natural selection occurs at smaller spatial scales along this habitat gradient, we might be under-exploiting the opportunities that are available for using guppies as a model for studying how and why certain aspects of the phenotype evolve.

Here we report how guppy life histories vary along a gradient defined by changes in the assemblage of fish species, with stepwise deletions of potential predators from higher to lower order streams. We examined life-history traits of guppies from six sites along the Guanapo River in the Northern Range Mountains of Trinidad (Fig. 1.1). The two end points along this river represent typical high- and low-predation localities previously considered in the bulk of the existing literature on guppy evolution. In addition to these localities, we examined guppies from three other fish communities that lie between the traditional high- and low-predation localities both in terms of location and in terms of the complexity of the predator community. We also incorporated a locality from the Caroni River, downstream of the confluence of the Guanapo and Caroni Rivers, which extends the gradient to incorporate a community with additional predators not typically included in past studies of high-predation communities.

MATERIALS AND METHODS

Characterization of sampling localities

The Guanapo River is part of the larger Caroni River Drainage on the south slope of the Northern Range Mountains of Trinidad, West Indies. Gilliam et al. (1993) conducted a detailed survey of the fish communities along the Guanapo River, beginning near its confluence with the Caroni River and ending at its headwater tributaries (Fig. 1.1). At each sampling locale, Gilliam et al. (1993) conducted a detailed survey of the fish community and characterized several environmental characteristics. In doing so, they have provided the most detailed description of the gradient of fish communities for any river drainage in Trinidad. This gradient is defined by changes in the assemblage of fish species, with stepwise deletions of potential predators from higher to lower order stream, and by correlated changes in the physical and biotic environment. To confirm the gradient in potential predation pressure on guppies initially reported by Gilliam et al. (1993), we consulted other published surveys (Kenny 1995; Phillip 1998) and carried out visual surveys. In addition to the predator community, we also characterized the abiotic environment by establishing six stations, one every 50 meters, along the stream at each of these six localities. At each station we measured stream channel width and took three measures of stream depth (at one fourth, one half, and three fourths of the stream width). We recorded temperature, dissolved oxygen, and pH at each station. We took four canopy openness readings at each station using a spherical densiometer (one reading in the direction of each cardinal point). These variables were always measured in the afternoon and taken in April 2010.

Collection sites and husbandry of laboratory stocks

Between January and April of 2008 and 2009, which corresponds with the dry season in Trinidad, we collected between 40 and 60 female guppies from each of the six localities with the goal of comparing life-history traits of guppy females along this gradient, both in the field and in common garden assays. Half of the females from each locality were immediately euthanized with an overdose of MS-222 (ethyl 3-aminobenzoate methane sulphonic acid salt, Sigma-Aldrich, St. Louis, Missouri, USA) and preserved in 5% formalin to quantify field differences among localities in life-history traits. The second half of the collected females were transported to the laboratory at Colorado State University and used in a common garden study to determine the genetic basis of the life-history variation.

We established laboratory stocks from 20-30 wild-caught adult females collected from the same six localities to characterize genetic differences among populations. Guppies store sperm, so each female carried viable sperm from one or more males with whom she had mated before capture. Guppy females were individually housed in either 10-liter glass tanks (Taylor, Guanapo Low, Middle and High, Caroni) or in 1.5-liter tanks in recirculating systems (Taylor, Caigual, and Guanapo High). All fish were kept on a 12:12 hour light cycle and at 25 ± 1ºC at Colorado State University. There were no differences in life-history traits between fish reared in 10-liter glass tanks *versus* 1.5-liter recirculating tanks (all $P > 0.05$; although there was a significant interaction between population and rearing environment for offspring number, $P = 0.05$). Differences in offspring number among high- and low-predation fish were reduced, although qualitatively maintained, in 1.5-liter recirculating tanks compared to 10-liter glass tanks (Appendix I.I). Because this interaction would tend to mask evidence of a gradient in offspring number, we are confident that patterns of variation in offspring number among populations represent real genetic differences and, if anything, underestimate phenotypic divergence.

Throughout the experiment, all fish were kept on a quantified food diet. Each tank was fed twice daily (AM: Tertramin^{TM} tropical fish flake paste, PM: hatched *Artemia* cysts), and each week, food levels were adjusted to reflect the increase in size of fish and the number of fish in each tank following previous protocols (Reznick 1982a; Reznick et al. 2004). The maximum amount of food provided to adults during the course of this experiment (25 μL) matched the high food level used previously by Reznick (1982b) and Reznick et al. (2004).

To minimize potential maternal and environmental effects, the laboratory study was performed on the second-generation (G2) laboratory-born fish of wild-caught females. All wild-caught females gave birth after being isolated in the laboratory, however the ultimate number of second-generation families derived from each wild-caught female varied among populations, with two populations being represented by less than 20 families (Guanapo Low=16 and Guanapo Middle=18). Up to ten offspring from each G1 litter were reared together until they were old enough to be accurately identified and separated by sex (approximately 28 days). The accumulation of dark pigments in the anal area and elongation of the anal fin was used to classify females or males respectively following Reznick (1982b). Once sexed, a single female from each family was individually housed in a 10-liter glass tank or a 1.5-liter recirculating tank. When G1 females were large enough to be sexually mature (approximately 20 mm standard length), they were each paired randomly with an unrelated G1 male from the same population. The same protocol was repeated for G2 fish. However, as soon as they were separated by sex, G2 females were crossed weekly to determine their age at first parturition (Reznick 1982a). Thereafter, females were re-mated after each reproductive event to estimate the reproductive frequency and effort of each population. Males were added to the female tank in the evening after the PM feeding and removed the following morning before the AM feeding so that they never interfered with the food rations given to the females.

We contrasted the life histories of the field-collected and lab-reared individuals from each locality to gain insight into the environmental and genetic basis of variation among populations. Phenotypic values of the laboratory-reared G2 generation, propagated from wild-caught females, are interpretable as reflecting genetic differences among localities, whereas the phenotypic values of field-collected individuals should reflect both genetic and environmental effects. Thus, by contrasting these two data sets we gain insight into the degree to which any differences between localities are due to environmentally induced plastic responses or fixed genetic differences.

Life history characterization

Field data.

We characterized phenotypic variation in life-history traits among wild populations of guppies following previously published methods (Reznick and Endler 1982; Reznick et al. 1996a). Briefly, each formalin-preserved female from our field collection was dissected and scored for the number of embryos and their stage of development following Haynes (1995). We then removed the gastrointestinal tract from each female, and dried the somatic and reproductive tissues, including developing embryos, in a drying oven at 80°C until mass was stable. From these females we measured: 1) number of developing embryos, 2) mean dry weight of developing embryos, and 3) reproductive allocation, while controlling for stage of development. Mean dry weight of developing embryos was estimated by dividing the total weight of the brood by the number of embryos. Reproductive allocation (RA) was determined as the dry mass of the brood divided by the sum of the dry masses of the somatic tissue of the female and the brood. RA is thus the proportion of the total dry mass of the mother that consisted of developing embryos.

Laboratory data.

In the laboratory life-history assay, we quantified: 1) age and weight at first parturition in females, 2) interbrood interval, 3) number of offspring in the first three broods, 4) dry weight of offspring in the third brood, and 5) reproductive allocation (proportion of total dry mass that consists of developing embryos, calculated from the dry mass of the third brood). Interbrood interval was calculated as the average time between the first three reproductive events. Upon the third parturition, females and their offspring were euthanized using an overdose of MS-222 and preserved in 200-proof ethanol. Females were then dissected and the somatic tissue, the reproductive tissue and the preserved offspring were dried at 80 ºC until mass was stable as described above for wild caught fish. Mean lean offspring weight and reproductive allocation were calculated based on the third brood of each female.

Statistical Design and Analysis.

We analyzed field and laboratory data separately using an analysis of covariance (ANCOVA) design, including locality of origin as a categorical fixed effect. We log-transformed dry offspring mass so the data more closely approximated a normal distribution. Fecundity in guppies has been shown to increase with female size, so we included female dry mass as a covariate to model patterns of reproductive allocation, offspring number, and offspring size (Reznick et al. 1996a). We also included stage of development as a covariate when analyzing reproductive allocation and embryo mass from wild-caught females, because offspring mass decreases as development progresses (Reznick and Endler 1982). We observed heterogeneity of slopes for reproductive allocation among wild-caught populations when we included developmental stage as a covariate (Table 1.2). Reproductive allocation declined as development progressed in all populations except for Guanapo High, where RA increased with development. In order to make inferences about the difference among populations in RA, we conducted two *ad-hoc* analyses. First, by eliminating Guanapo High from the dataset we determined that the interaction of reproductive allocation with developmental stage was no longer significant, and that the rank order of the remaining populations was not affected by excluding the Guanapo High population. Second, as developmental stage had a significant but small negative effect in reproductive allocation (parameter estimate = -0.006), we removed stage of development as a covariate, which did not affect the rank order of reproductive investment among populations. In Table 1.2, we present the result of all three analyses, but we only discuss the results of the model that included all populations without stage of development in the analysis, as results from the other models would not change our interpretation. Female size was the only covariate included when analyzing offspring mass and reproductive allocation from laboratory-reared females.

We tested the effect of the deletion of predators along the environmental gradient using an *a posteriori* Tukey's HSD pairwise comparison test. By doing this analysis, we gained greater statistical power to determine not just if there was variation among the study localities, but also if this variation paralleled the predation gradient.

RESULTS

Stream characteristics

The six localities sampled in this study show a gradient of potential predators along the Guanapo River, with downstream sites containing increasingly diverse predator assemblages relative to upstream sites (Table 1.1). Beginning at the headwaters, guppies at the Taylor locality co-occur with a second species of fish (*R. hartii*). The Caigual and Guanapo Low localities have two more species (*Rhamdia sebae* and *Synbranchus marmoratus*) in addition to *R. hartii*. Community complexity increases with the addition of four potential predators of guppies at the Guanapo Middle locality (*Aequidens pulcher, Astyanax bimaculatus, Hemibrycon taeniurus* and *Hoplias malabaricus*). *Crenicichla spp.*, which often preys on guppies, is first found in the Guanapo High location. The Caroni River site presents a more complex community with other potential predators (*Eleotris pisonis, Dormitator maculatus, Polycentrus schomburgkii*, plus avian predation by several species of egrets, *Bubulcus ibis*, *Egretta alba, E. thula*; Table 1.1).

Several physical attributes of the environment also vary along the Guanapo-Caroni Drainage. For instance, there is a gradual increase in stream width from upstream to downstream locales (Table 1.1, Appendix I.II). There is also a steep change in canopy cover from the Guanapo Low to the Guanapo Middle site, shifting from primarily closed canopy to largely open canopy sites. This step in canopy cover is correlated with the presence or absence of major guppy predators (i.e. Taylor, Caigual, and Guanapo Low *versus* Guanapo Middle and High, and

Caroni). We found a shift in water temperature that tracked canopy cover along the drainage, where closed canopy sites where similar in temperature and cooler than the open canopy sites (Table 1.1). A sharp increase in water temperature was found between Guanapo Low and Guanapo middle and water temperature continued to increase downstream to the Caroni site (Table 1.1). No clear trends were observed for dissolved oxygen or pH (Table 1.1).

Guppy life-history traits

Life-history traits showed significant phenotypic (Table 1.2) and genotypic variation (Table 1.3) among the studied localities. Except for size at maturity and offspring size, all other traits showed continuous changes along the studied gradient (Fig. 1.2 to 1.6). We expand on these patterns below.

Age and size at maturity.

Field estimates of age and size at maturity can only be approximated from the smallest size at which females begin to reproduce, and was not possible here because of the poor representation of small females in the samples from most localities. However, in the laboratory, age at first parturition can more accurately be estimated and was found to vary significantly among populations (Table 1.3). Age at first parturition decreased along the stream gradient, being latest in the locality furthest upstream (i.e. Caigual) and becoming earlier in subsequent downstream sites (Fig. 1.2). The only locality not in line with this gradual change was the Taylor population, which had an earlier age at maturity than expected given the simple nature of the fish community (Fig. 1.2). Size at first parturition also varied among localities, but was not affected by the gradient (Table 1.3, Fig. 1.2). Females from the Guanapo Middle locality matured at the largest size, while females form the Caroni and Caigual localities matured at the smallest size.

Reproductive effort.

Reproductive effort can be separated into: a) how frequently females reproduce (i.e. interbrood interval), and b) how much energy females invest in each of their broods (i.e. reproductive allocation). Interbrood interval measured in the laboratory significantly varied among populations (Table 1.3). Females from the most downstream locality, Caroni, had the shortest interbrood interval compared to all other populations, and interbrood interval gradually increased moving upstream, from high- into low-predation localities (Fig. 1.3). This resulted in a significant gradient effect, and is consistent with predictions that high mortality rates will favor shorter interbrood intervals.

The significant interaction between population of origin and developmental stage in the analysis of reproductive allocation of wild caught fish (Table 1.2) limited our ability to make direct comparisons of this trait among localities. In all populations, reproductive allocation declined as development progressed. However, in fish from the Guanapo High locality, reproductive allocation increased with development. When analyzing a subset of the data that excluded the Guanapo High samples, developmental stage was a significant covariate, but there was no significant interaction with locality. In this case, we found significant variation among populations in reproductive allocation (Table 1.2). This result was similar to that obtained when retaining the Guanapo High samples, but ignored developmental stage as a covariate. In both cases, there was a significant gradient effect in reproductive allocation, with RA increasing in locations where piscivorous fish are present (Fig. 1.4). When this trait was analyzed in laboratory-reared females, the results were similar (Table 1.3), but the pattern of variation along the gradient was more pronounced (Fig. 1.4). Female size was a significant covariate in the analysis of the lab data, with larger females having greater reproductive allocation (Table 1.3).

Offspring size and number

The pattern of variation in offspring number was very similar in wild-caught and laboratory-reared fish. Females in the Caroni sites had the highest number of offspring, and offspring number gradually decreased along the stream gradient, resulting in a gradient in wild-caught and laboratory-reared fish (Fig. 1.5). Female dry mass was a significant covariate in both models (Table 1.2 and 1.3), and there were no significant interactions between the independent variables ($P > 0.05$ for all interactions).

There was significant variation among populations in offspring size in both wild-caught and laboratory-reared females (Tables 1.2 and 1.3). However, the significant pattern in offspring size observed in offspring of wild-caught females along the studied gradient was not observed in second-generation laboratory-reared guppies (Fig. 1.6). In the wild, offspring were larger in the upstream populations than in downstream populations, as would be predicted if there is a trade-off between the size and number of offspring. However, offspring mass in the laboratory was lower in the most upstream and downstream populations and higher in intermediate populations (Fig. 1.6). In the wild-caught females, stage of development and female size were significant covariates in the analysis of offspring size (Table 1.3), and there were no significant interactions between the independent variables ($P > 0.05$ for all interactions). As seen in previous studies, offspring size decreased with stage of development and was positively related to female body mass. In laboratory-reared fish, only female dry mass was a significant covariate (Table 1.3), and there were no significant interactions between the independent variables ($P > 0.05$ for all interactions).

DISCUSSION

Understanding how continuous phenotypic variation across environmental gradients is generated and maintained has important implications to our understanding of adaptive evolution and the processes that lead to speciation (Endler 1977). Comparisons between species occupying different ends of environmental gradients shed light into the mechanisms driving community structures and distribution limits (Case and Taper 2000; Chase and Leibold 2003; Price and Kirkpatrick 2009). For example, a growth-mortality trade-off drives the distribution pattern of the wood frog (*Rana sylvatica*) and the leopard frog (*R. pipiens*) across a gradient of resource availability (Schiesari et al. 2006). Yet, studies comparing variation among populations are critical to understand the mechanisms behind phenotypic variation and divergence (Endler 1977). Common garden comparisons or molecular approaches can be used to determine the genetic basis of phenotypic variation along environmental gradients. For example, common garden experiments have shown that variation in predation risk along a community gradient has direct and indirect effects on the evolution of life histories in the Trinidadian killifish (*Rivulus hartii*, Walsh and Reznick 2008, 2009, 2010). Similarly, molecular markers have been used to show that clinal variation in life-history traits in a perennial sunflower (*Helianthus maximiliani*) is the result of local adaptation to spatially heterogeneous environments (Kawakami et al. 2011). Moreover, the use of modern genomics has the potential to relate variation in life-history traits to clinal genetic variation (e.g. Mariac et al. 2011).

Here we combined field data and common garden experiments to show how guppy life histories vary along a continuum of predation risk and demonstrate that such variation has a genetic basis. Our results reveal a fine-scale continuous gradient in life histories in populations that appear to experience a gradient in predation risk. Previous research on guppies has focused on comparisons between low-predation localities where only guppies and *R. hartii* occur versus high-predation localities where, in addition to these two species, several other piscivorous fish are present. Comparisons of guppies from these two types of localities have shown that phenotypic and genotypic diversity in life-history traits among guppy populations are correlated with the predator communities to which guppies are exposed. Using a combination of field and common garden data, we show that there is a genetically based gradient in life histories between these two ends of the continuum for all measured traits, except for offspring size and female size at maturity. Below, we discuss these results in the context of the variation in ecological factors along the stream, how ecological factors might favor the evolution of plasticity versus the evolution of fixed genetic differences, and the range of variation expected along the whole gradient.

Ecological gradients in Trinidadian streams

Endler (1978) showed that color in male Trinidadian guppies varies gradually from downstream, high-predation localities to upstream, low-predation localities. He proposed that this gradient in coloration was related to gradual changes in the predator community that exerted a graded selective pressure on male coloration. Similar to Endler (1978), we found a graded response in most aspects of the female life history that corresponded to a gradient in predation risk as one moves from headwater tributaries to higher order streams and rivers. To the extent that it has been evaluated, an increase in predator abundance and diversity is correlated with an increase in mortality rate of guppies (Reznick et al. 1996b). Thus, we suggest that the observed gradient in life histories is correlated with a parallel gradient in predation risk. Life-history theory predicts that the differences among localities in risk of mortality should in turn select for differences among populations in life-history traits such as age and size at maturity and reproductive effort (Gadgil and Bossert 1970; Law 1979; Charlesworth 1994). While additional studies of other drainages in the Northern Range Mountains are needed to test the generality of these results, our findings are consistent with predictions from life-history theory that gradually increasing predator-induced mortality rates from headwater to lowland streams select for gradually faster life histories.

While our results are consistent with predictions from life-history theory on how increasing predation should shape the life histories, we also recognize that other ecological factors also co-vary along this gradient (Grether et al. 2001; Reznick et al. 2001). The most evident changes along this gradient are the size of the stream and the amount of light that reaches the stream (Table 1.1). These factors are known to translate into higher primary productivity and thus potentially increase food resources available for guppies (Grether et al. 2001; Reznick et al. 2001). A second notable factor is the indirect effects of predation (Abrams and Rowe 1996; Reznick et al. 1996b). Predator consumption results in a reduction of prey abundance, which could drive the evolution of life histories by altering the competitive environment (Gadgil and Bossert 1970; Law 1979). Thus, increased predation pressure could also indirectly affect the strength of density-dependent selection, as those individuals that survive predation will be left with increased resource availability and experience lower intraspecific competition (Abrams and Rowe 1996; Reznick et al. 2001; Bassar et al. 2010). It is difficult to make specific predictions of the effects of density-dependent regulation on life history evolution given that these predictions depend on the specific demographic consequences imposed by predators (Abrams and Rowe 1996). Currently, we lack the information for predicting how life histories will evolve under density-dependent regulation on guppies, but we must bear the environment and the indirect effects of predation in mind as potentially confounding factors that can also influence life-history variation.

Genetic and plastic differences in life histories

In our laboratory common garden experiment, we observed that reproductive allocation and brood size per brood progressively decrease while age at maturity and interbrood interval progressively increase with decreasing complexity of the predator community. This suggests a genetic basis to the parallel pattern observed in the field for reproductive allocation and offspring number (Figs 1.2 and 1.3). An earlier age at maturity and shorter interbrood interval co-varying with increasing predation pressure is predicted from theory (Gadgil and Bossert 1970; Law 1979; Charlesworth 1994), and is consistent with previous comparisons of high- and low-predation populations of guppies (Reznick 1982a; Reznick and Endler 1982). The one locality that did not follow this trend was the Taylor locality where females matured younger and reproduced more frequently than expected based upon the simple nature of the fish community (Figs 1.2 and 1.3). Why the Taylor deviates from the continuum will require further study. However, we suspect that other mortality sources beside predation (e.g. water pollution, disease, flash floods) may have selected for faster life histories because detailed mark-recapture studies have found unusually high mortality in the Taylor compared to other low-predation streams (SW Fitzpatrick et al. unpublished data).

A second trait that shows an interesting deviation from the observed gradient in life histories is offspring size (Fig. 1.5). The field data show that females from all low-predation populations produce large offspring, but as soon as the more effective predators appear in the gradient (e.g. *Hoplias*, characins, and cichlids), offspring size starts to decrease, reaching the smallest sizes in the Caroni River. Such patterns are expected if offspring size and number trade-off against each other. However, this pattern of variation was not observed in our common garden experiments. In the laboratory, females from the two low-predation populations, Taylor and Caigual produced the smallest but also the fewest offspring. These results for offspring size are interesting for several reasons. In prior comparisons between high- and low-predation guppies, we have consistently observed a compression of the differences in offspring size between laboratory-reared (Reznick 1982a,b; Reznick and Bryga 1996; Reznick et al. 2004) and wild-caught fish (Reznick and Endler 1982; Reznick et al. 1996a).

However, the results here document for the first time an inversion in the pattern of variation of offspring size where low-predation guppies have smaller offspring in the laboratory than high-predation guppies. High-predation guppies usually produce more, smaller offspring than low-predation guppies (Reznick 1982a), whereas, in our laboratory data, we see that high-predation guppies produce more and larger offspring. Thus, the combination of our laboratory and field data suggests a high degree of phenotypic plasticity in offspring size in the two low-predation populations implying genotype x environment interactions. Prior comparisons between high- and low-predation populations from other drainages did not reveal such extreme genotype x environment interactions when reared in the laboratory under high and low levels of food (Reznick 1982a; Reznick et al. 2004). Thus, these results are also evidence of a genetically based decoupling between offspring size and number, such that the expected trade-off between the size and number of offspring is observed in the field but not in the laboratory where resources were controlled.

While we observed that offspring number is similar between the laboratory and the wild, offspring mass tends to be smaller in the laboratory, which, in turn, drives a significant reduction in reproductive allocation in laboratory females compared to wild females. It is difficult to determine what specifically drives the decrease in offspring size and reproductive allocation in the laboratory, as several factors, including resource availability, predation risk, competition, water quality, etc., change between the laboratory and nature. Specifically, food quantity and quality are the most likely causes of this difference, as our restricted food levels could have led to sub-optimal energy allocation to reproduction. Evaluating this hypothesis requires replicating the current experiment under different food levels. Indeed, previous common garden experiments testing the effect of food level on life-history traits in guppies found that, while food level did not significantly affect reproductive allocation, it did affect offspring mass (Reznick 1982; Reznick and Bryga 1996). However, the effect of restricting food was the production of larger offspring (Reznick 1982; Reznick and Bryga 1996). It is possible that the food level we used was higher than typically found by low-predation guppies in the wild and lower than what is encountered by high-predation populations, producing the plasticity pattern observed in our study. However, as no food by predation regime interaction has previously been recorded (Reznick 1982; Reznick and Bryga 1996), further experiments will be needed to determine the cause of these patterns.

What type of selective environment would favor such plasticity in offspring size and number? Life-history theory predicts an increase in reproductive effort as adult mortality increases (Charlesworth 1994), but does not make specific predictions about how resources should be distributed among offspring or patterns of plasticity in resource allocation. In contrast, other theory suggests that the relationship between offspring size and number is determined by offspring fitness, which in turn should influence how parents distribute resources among offspring (Smith and Fretwell 1974; Wilbur 1977). Guppies occurring in low-predation sites are thought to experience reduced resource availability due to high intra-specific competition and lower primary productivity, such that large offspring have a competitive advantage over smaller offspring (Reznick 1982b; Jørgensen et al. 2011). However, the advantage of large offspring disappears under high resource availability (Bashey 2008). If the low-predation populations studied here experience predictable fluctuations in population density and resource availability, theory predicts such variable environments could favour plasticity in offspring size (Levins 1968; Via and Lande 1985; Moran 1992). We have observed large fluctuations in the population densities of these two low-predation sites that correspond with seasonal flash floods during the Trinidadian wet season (Torres-Dowdall personal observation). Thus, one hypothesis for why plasticity in offspring size has evolved in these populations, but not other previously examined low-predation populations, is that these populations experience more variable environments. For example, the marine bryozoan *Bugula neritina* experience a high degree of environmental variation in the field, which selects for increased plasticity in offspring size (Monro et al. 2010). Similarly, environmental variation in habitat quality (i.e. seed size and resistance) has selected for high levels of plasticity in egg size on the seed beetle *Stator limbatus* (Fox 2000; Fox et al. 2001). Comparative studies of the population dynamics of low-predation populations in combination with laboratory experiments raising guppies under high and low food availability might shed further light on this issue.

Our results strongly suggest that the genetic variation observed along this predation gradient is the result of local adaptation. However, the observed pattern of genetic variation among populations could also be influenced by gene flow (Garcia-Ramos and Kirkpatrick 1997; Barton 2001). Our study system falls along a linear gradient, which potentially has a strong directional axis of dispersal and gene flow. If natural selection favors two extreme ecotypes, a high-predation and a low-predation ecotype, gene flow along the linear gradient can contribute to the observed pattern of variation. Indeed, although we lack estimates of gene flow in this drainage, we suspect there is an interaction between selection and gene flow, producing the observed pattern of genetic and phenotypic divergence between localities. However, previous studies on guppies have shown that gene flow between populations is highly reduced by physical barriers, including waterfalls and geographic distance (Crispo et al. 2006). All of our sites are separated by at least one waterfall that is at least 2 meters high, and some of our sample localities are separated by several kilometers. Therefore, the effect of gene flow is probably small. To confirm this prediction, comparing neutral genetic variation (i.e. F_{ST}) and adaptive quantitative variation (i.e. Q_{ST}) along the habitat gradient could provide insight on the relative roles of selection and gene flow in the observed pattern of life history variation (Barton 2001; Volis and Zhang 2010; Kawakami et al. 2011).

Extending the high-predation life-history continuum

In this study we sampled a population that is beyond the normal range of communities commonly included in the guppy literature. The Caroni River population showed an even faster life history than fish from Guanapo High, a typical *Crenicichla* locality (Figs 1.2 to 1.6). The Caroni life history demonstrates that guppy life-history traits can evolve beyond the range found within prior research on their life history evolution (Reznick 1982a; Reznick and Bryga 1996; Reznick et al. 2004). Although there are large changes in the fish community between the Caroni River and the Guanapo High localities that would suggest increased predation pressure (Table 1.1), there are also several other factors that vary between these sites, making it difficult to assign causality to the observed changes in life-history traits. The Caroni site is in a much larger river and has a strong anthropogenic influence (WRA 2001). It has lost most of its canopy and is highly eutrophied. In addition, a phenotypically similar close relative of guppies, the swamp guppy (*Poecilia picta*), is present at this site and can potentially compete with guppies for resources affecting the selection pressures guppies' experience. Thus, the Caroni locality, as well as other lowland guppy population, provides an opportunity to explore the high-predation and high resource limits of life history variation in guppies and how other ecological factors, like interspecific competition, affect life history evolution.

CONCLUSION

In this study we provide evidence for fine scale genetic differentiation in life-history traits that is correlated with a gradient in predation pressure. Endler (1978) studied how a similar gradient in predation affects male coloration in guppies, and in general our results are consistent with his. However, one notable difference here is that Endler found a stronger effect of *Crenicichla* on male coloration than we found for life-history traits in female guppies. *Crenicichla* is an important diurnal predator with acute vision (Kemp et al. 2009), thus it is reasonable to expect that it would play a larger role in Endler's study. In the current study, mortality rate is proposed to shape life history evolution and it appears that other predators are as effective in this regard as is *Crenicichla*. Thus, it is insightful to consider how guppies that experience variation in predation risk respond to different predators. For example, Botham et al. (2006) showed that guppies' behavioral response depends on the predator they are exposed to, being the most responsive toward *Crenicichla*. Comparisons of guppies from communities having only a subset of the predators present in typical *Crenicichla* sites could be informative in this regard. Such comparisons could also help to disentangle the effects of co-varying environmental factors that potentially confound all studies attempting to ascribe a particular agent of selection to patterns of phenotypic variation. For example, there is a clear break in life histories on the Aripo River in association with a barrier waterfall and a break in predation risk that is independent of changes in the physical environment (Reznick 1982a), potentially allowing for better separation of predation and resource availability effects. Finally, comparisons along gradients provide a better framework for interpreting the scale at which selection shapes phenotypic and genetic variation. In the current study, if we had only compared the extremes of the gradient (i.e. Taylor versus Guanapo High), we might have incorrectly concluded that predation pressure was not correlated with the evolution of life histories in guppies from the Guanapo River. However, by looking at the whole gradient we found strong evidence for predation driving life history evolution in guppies at a relatively fine scale. Finally, our results are in agreement with an increasing number of studies showing that selection along environmental gradients can produce clinal adaptive genetic variation on life-history traits (e.g. Walsh and Reznick 2008, 2009, 2010; Kawakami et al. 2011; Mariac et al. 2011; Tomkins et al. 2011), and hold promise for better estimates of the scale at which adaptive divergence occurs.

TABLES AND FIGURES

Table 1.1. Description of the localities sampled along the longitudinal gradient of the Guanapo Drainage. Predator community represents the new species founded at each locality going from the headwater stream (Taylor) to the Caroni River.

	Population	Stream order	Temperature (°C)	DO%	pH	Mean width (cm)	Mean depth (cm)	Canopy (% open)	Predator community
1	Taylor	2	23.5 (0.3)	87.5	7.48	187.54 (0.20)	6.77 (0.57)	6.56 (0.19)	*Rivulus hartii*
2	Caigual	3	23.2 (0.4)	95.1	7.81	204.47 (0.37)	24.98 (0.60)	5.28 (0.13)	*R. hartii* *Synbranchus marmoratus Rhamdia sebae*
3	Guanapo Low	4	23.7 (0.4)	91.3	7.75	403.43 (0.32)	30.73 (0.86)	9.90 (0.46)	All the same as Caigual.
4	Guanapo Middle	4	24.9 (0.9)	93.2	7.90	351.67 (0.31)	12.06 (0.40)	72.23 (0.29)	*Hoplias malabaricus* *Aequidens pulcher* *Astyanax bimaculatus* *Hemibrycon taeniurus* Plus all in the Guanapo Low
5	Guanapo High	4	27.3 (0.8)	98.4	7.92	564.40 (0.47)	20.41 (0.59)	72.43 (0.71)	*Crenicichla spp.* *Cichlasoma bimaculatum* Plus all in the Guanapo Middle
6	Caroni	5	29.4 (1.1)	69.9	7.71	1575 (19.45)	212.67 (58.34)	100.0 (0)	*Polycentrus schomburgkii* *Eliotris pisonis* *Dormitator maculates* *Gymnotus carapo* Plus all that are in the Guanapo High

Table 1.2. Statistical results (F-values) for ANCOVA on reproductive allocation, offspring mass (mg) and offspring number in wild caught females.

	df	Reproductive Allocation			Offspring Number	Offspring Size
		Full model	Excluding GH[1]	W/O DS		
Locality	5	9.41***	11.00***	8.08***	11.30***	17.06***
Female Size	1	1.82^{NS}	3.98^{NS}	0.06^{NS}	43.26***	20.55***
Interaction (LxFS)	5	0.26^{NS}	0.23^{NS}	0.51^{NS}	1.07^{NS}	1.90^{NS}
Developmental Stage	1	6.25*	13.55***	—	—	24.97***
Interaction (LxDS)	5	4.41**	1.50^{NS}	—	—	1.61^{NS}
Residual sums of squares (df)		0.26 (100)	0.26 (85)	0.34 (106)	1686.74 (106)	1.96 (100)
Total sums of squares (df)		0.52 (117)	0.46 (99)	0.52 (117)	5104.27 (117)	13.54 (117)
R^2		0.49	0.49	0.34	0.67	0.86

* 0.05> *P* >0.01; ** 0.01> *P* >0.001; *****P* <0.001

[1] Degrees of freedom for population effect in the model excluding fish from Guanapo High population is 4.

Table 1.3. Statistical results (*F*-values) for ANCOVA on the life-history traits measured in the third brood of G2 females.

	df	Age at first parturition	Size at first parturition	Interbrood interval	Reproductive Allocation	Offspring Number	Offspring Size
Locality	5	22.28***	14.15***	12.25***	29.07***	17.59***	14.64***
Female Size	1	—	—	—	8.19**	36.62***	18.64***
Interaction (LxFS)	5	—	—	—	1.14^{NS}	1.15^{NS}	0.99^{NS}
Residual sums of squares (df)		10837.22 (158)	0.37 (152)	475.06 (154)	0.07 (99)	993.69 (101)	1.04 (100)
Total sums of squares (df)		18479.49 (163)	0.54 (157)	663.97 (159)	0.22 (110)	2492.06 (112)	2.81 (111)
R^2		0.41	0.31	0.28	0.66	0.60	0.63

* 0.05> *P* >0.01; ** 0.01> *P* >0.001; ****P* <0.001

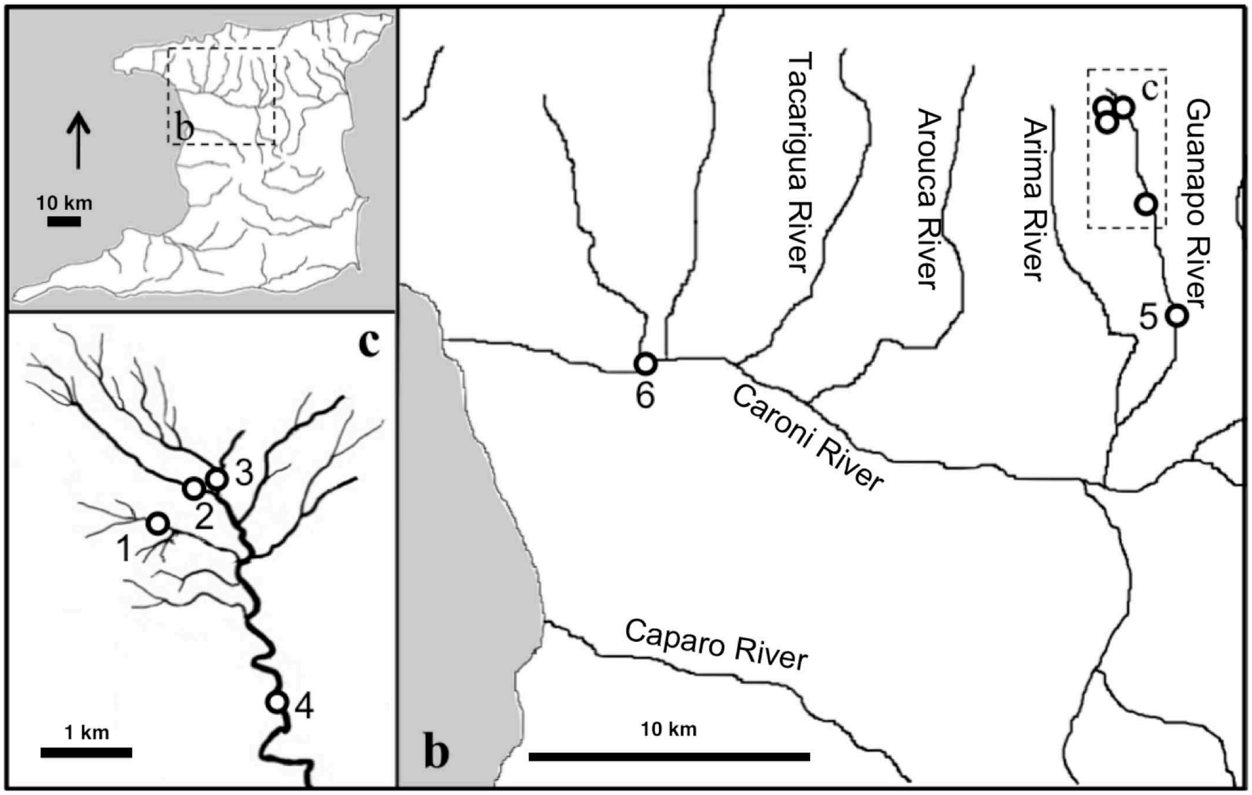

Figure 1.1. Localities sampled along the Guanapo - Caroni Drainage. a) Study area within the island of Trinidad, b) detail of the Guanapo – Caroni drainage showing the localities studied, c) detail of the headwaters of the Guanapo River. Localities sampled are 1) Taylor; 2) Caigual; 3) Guanapo Low; 4) Guanapo Middle; 5) Guanapo High; and 6) Caroni.

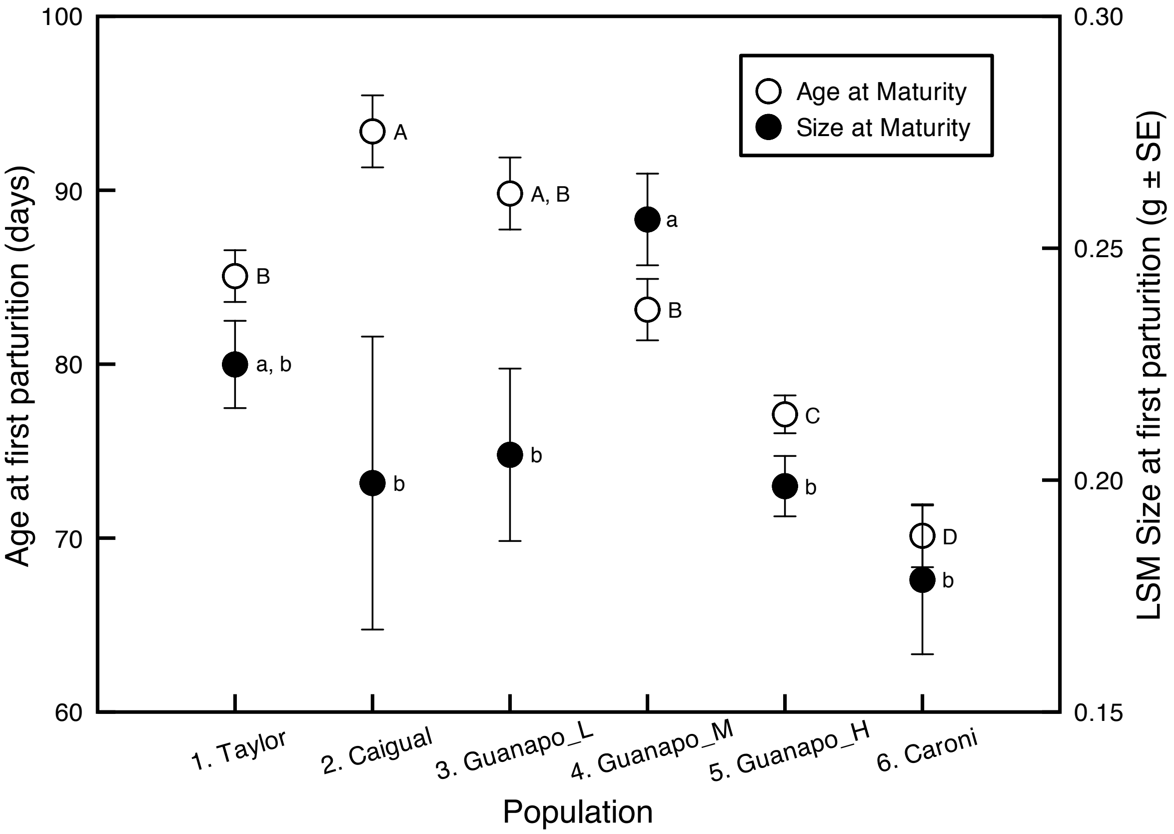

Figure 1.2. Mean (± SE) age at first parturition (white circles) and adjusted least square mean (± SE) wet mass at first parturition (black circles, corrected for female age) of G2 laboratory-reared females. A clear gradient of variation is observed for age at maturity (except for Taylor), but size at maturity does not follow this pattern. Letters by population means represent Tukey's HSD pairwise comparison. Different letters imply significant least squares mean differences between populations at a α=0.05 (upper-case letters for age at maturity, lower-case letters for size at maturity).

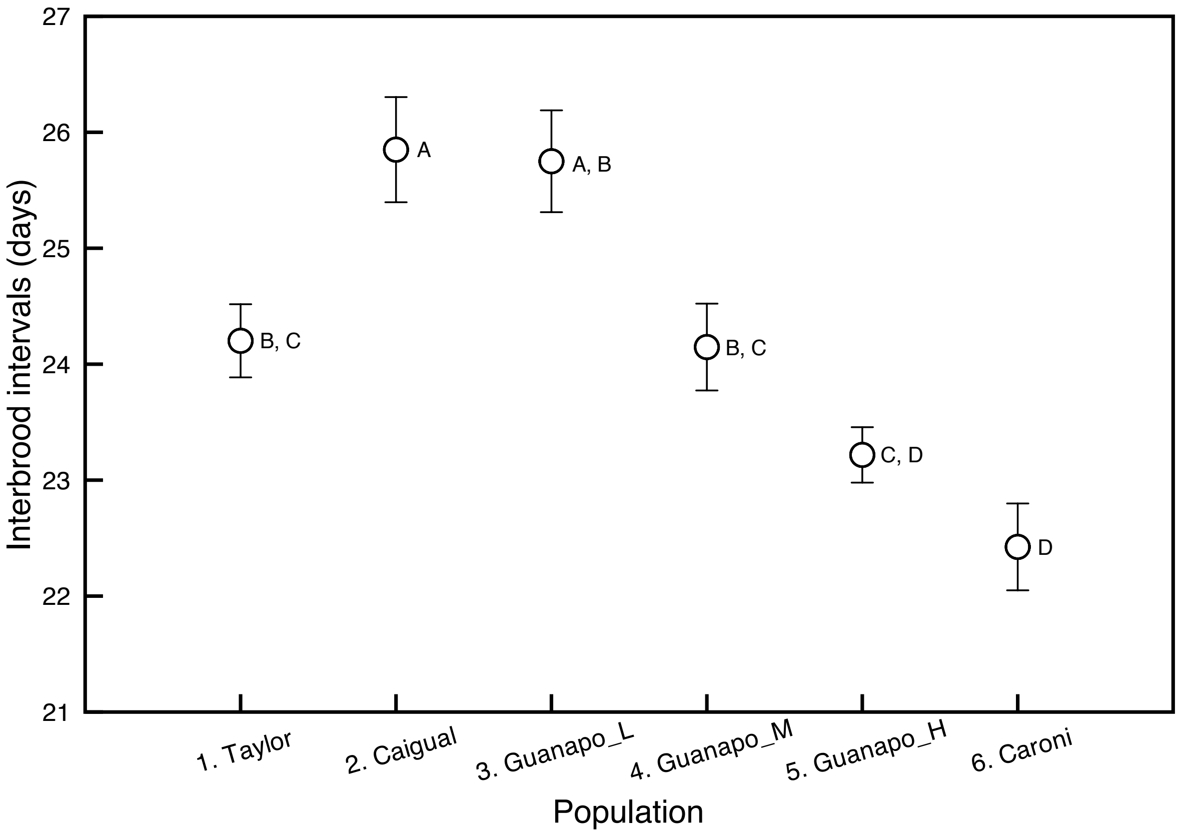

Figure 1.3. Mean (± SE) interbrood interval of G2 laboratory-reared females. A clear gradient of variation is observed in interbrood interval (except for Taylor) where increasing species richness of the predator community corresponding to more frequent reproductive events. Letters by population means represent Tukey's HSD pairwise comparison. Different letters imply significant least squares mean differences between populations at a $\alpha=0.05$.

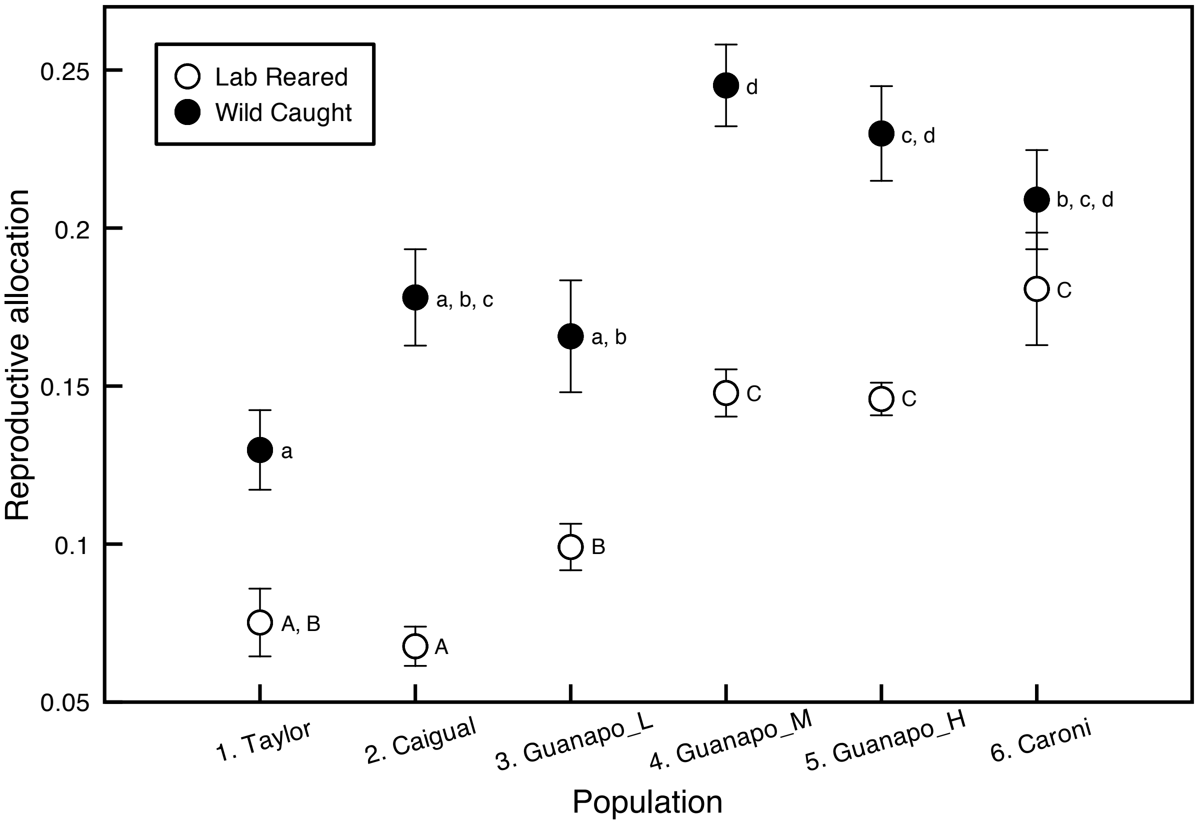

Figure 1.4. Mean (± SE) reproductive allocation for the third brood in G2 laboratory-reared females (black circles) and wild caught females (white circles). Trends in the laboratory and field data are similar; as the number of potential predatory species increases, females increase reproductive allocation. Although a gradual increase in reproductive allocation is observed from low-predation to high-predation localities was observed in both wild-caught and laboratory-reared females, the pattern is more continuous in laboratory-reared females. Letters by population means represent Tukey's HSD pairwise comparison. Different letters imply significant least squares mean differences between populations at a $\alpha=0.05$ (upper-case letters compare means for laboratory-reared females, lower-case letters compare means for wild-caught females).

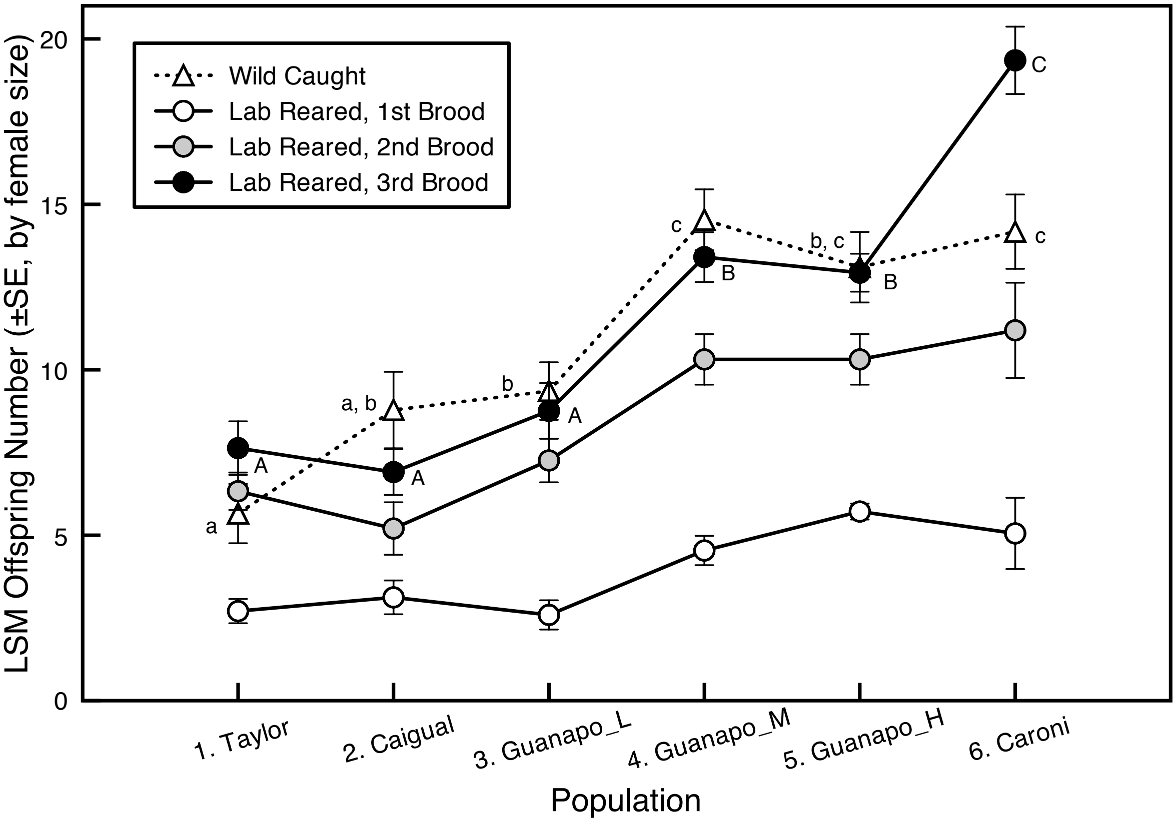

Figure 1.5. Least square mean (± SE) offspring number (corrected for female mass) produced from the third brood of G2 laboratory-reared females (black circles) and wild caught females (white circles). Lab data replicates the pattern observed in the wild, where the number of potential predatory species is positively correlated with the number of offspring produced per brood. Letters by population means represent Tukey's HSD pairwise comparison. Different letters imply significant least squares mean differences between populations at a α=0.05 (upper-case letters compare means for laboratory-reared females, lower-case letters compare means for wild-caught females).

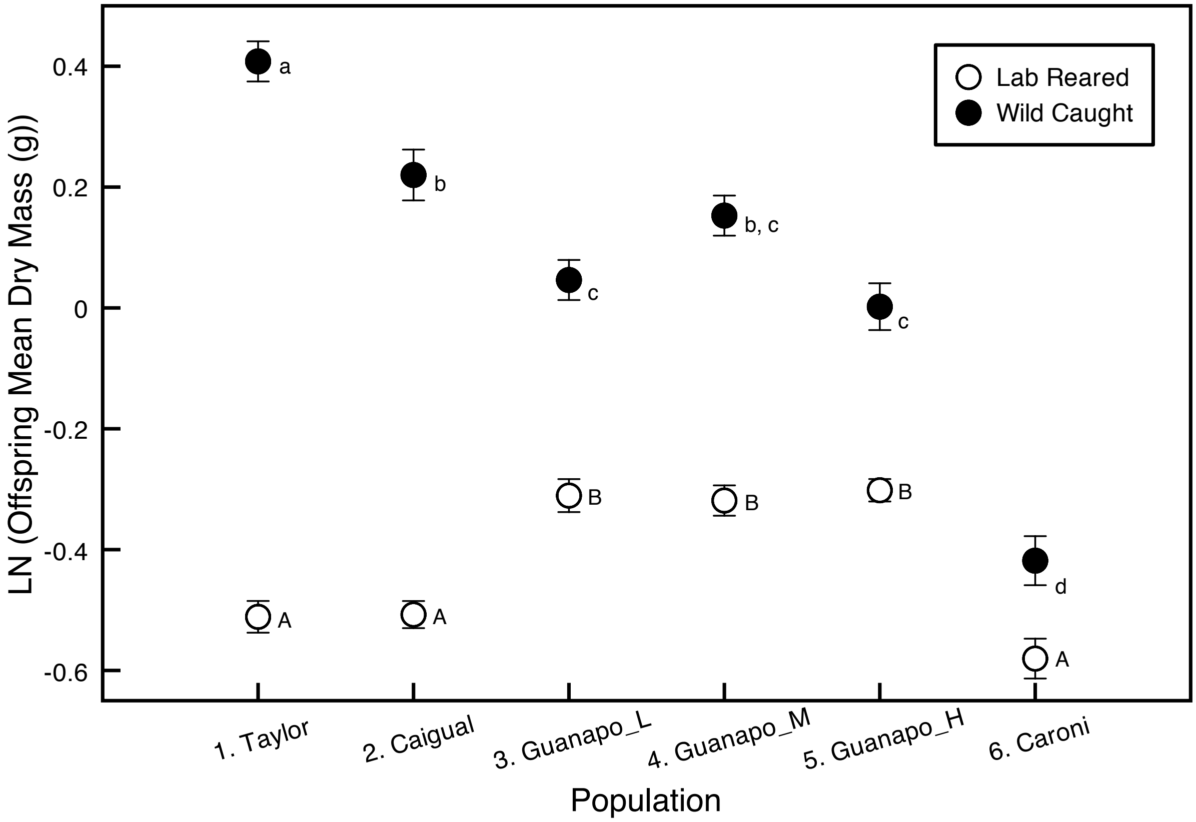

Figure 1.6. Least square mean (± SE) offspring number (corrected for female mass) produced from the third brood of G2 laboratory-reared females (black circles) and wild caught females (white circles). Offspring size in the wild follows the expected pattern of larger offspring in low-predation sites and smaller offspring in high-predation sites. However, lab data do not reflect this pattern suggesting this trait can be highly plastic. Letters by population means represent Tukey's HSD pairwise comparison. Different letters imply significant least squares mean differences between populations at a $\alpha=0.05$ (upper-case letters compare means for laboratory-reared females, lower-case letters compare means for wild-caught females).

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CHAPTER 2:

LOCAL ADAPTATION AND THE EVOLUTION OF PHENOTYPIC PLASTICITY IN TRINIDADIAN GUPPIES (*POECILIA RETICULATA*)

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SUMMARY

Divergent selection pressures across environments can result in phenotypic differentiation that is due to local adaptation, phenotypic plasticity, or both. Trinidadian guppies exhibit local adaptation to the presence or absence of predators, but the degree to which predator-induced plasticity contributes to population differentiation is less clear. We conducted common garden experiments on guppies obtained from two drainages containing populations adapted to high- and low-predation environments. We reared full-sibs from all populations in treatments simulating the presumed ancestral (predator-cues present) and derived (predator-cues absent) conditions and measured water column use, head morphology, and size at maturity. When reared in presence of predator cues, all populations had phenotypes that were typical of a high-predation ecotype. However, when reared in the absence of predator cues, guppies from high- and low-predation regimes differed in head morphology and size at maturity; the qualitative nature of these differences corresponded to those that characterize adaptive phenotypes in high- versus low-predation environments. Thus, divergence in plasticity is due to phenotypic differences between high- and low-predation populations when reared in the absence of predator cues. These results suggest that plasticity might play an important role during colonization of novel environments, and then continue to evolve as a by-product of adaptation to the derived environment.

INTRODUCTION

Understanding the causes and consequences of phenotypic divergence among natural populations is a fundamental goal of evolutionary ecology. When populations occupy different environments, divergent selection pressures can result in phenotypic differentiation in traits that confer a local fitness advantage (Kawecki and Ebert 2004). Adaptive phenotypic divergence can occur through local adaptation and/or adaptive phenotypic plasticity. In the absence of any homogenizing effects of gene flow, divergent selection can lead to the evolution of locally adapted ecotypes, where resident genotypes produce phenotypes with higher relative fitness than genotypes originating from other habitats (Kawecki and Ebert 2004). Alternatively, adaptive phenotypic plasticity in response to environmental cues can move population phenotypes toward a local optimum without any genetic differentiation (Price et al. 2003; Schlichting and Pigliucci 1998).

Empirical studies of local adaptation often use translocation or common garden experiments to test the degree to which fitness, or traits linked to fitness, persist across different environments (Gotthard and Nylin 1995; Kawecki and Ebert 2004). By measuring the plasticity or shift in trait values by a genotype across different environments (i.e. the reaction norm), these approaches can separate the genetic and environmental contributions to the phenotype (Via et al. 1995; Kawecki and Ebert 2004; Williams et al. 2008). For example, demonstrating local adaptation typically requires that adaptive phenotypic differences observed in the field be maintained under common garden conditions (Hereford 2009). Further, any genotype-environment interactions should confer higher relative fitness to a population in its native environment relative to individuals from other populations (Hereford 2009). Thus, significant evidence for genotype by environment interactions reveal how phenotypic plasticity has evolved and the expected phenotypes that would initially be produced by each population invading the opposite environment (Gotthard and Nylin 1995; Schlichting and Pigliucci 1998; Ghalambor et al. 2007).

It is challenging to identify which features of the environment most affect plasticity and select for divergent adaptations. Predation is often heterogeneous in its distribution and has been shown to impose strong evolutionary and plastic responses in the phenotypes of potential prey (Kerfoot and Sih 1987). For example, predator-induced mortality has been demonstrated to be a strong selective pressure capable of driving rapid evolution in a suite of anti-predator traits in insects (e.g. Whitman and Agrawal 2009), fish (e.g. Webb 1986; Reznick and Ghalambor 2001; O'Steen et al. 2002), and lizards (e.g. Irschick and Losos 1998; Warner and Andrews 2002). Predation can also cause strong ecological effects, inducing plastic responses in many of the same traits under direct selection from predators (e.g. Harvell 1990; Agrawal 2001; Stoks et al. 2003; Relyea and Auld 2005). For example, predators can directly reduce fitness in prey populations through increased mortality, and indirectly by eliciting stress responses that lead to changes in behavior, reductions in foraging time budgets, and reductions in growth rate (e.g. Sih 1980; Lima and Dill 1990; Sih and Moore 1993; Werner and Anholt 1993; Brown et al. 1999; Nonacs and Blumstein 2010). Thus, spatial variation in predation risk has the potential to act as both an agent of divergent selection and an environmental cue that induces phenotypic plasticity.

Guppies (*Poecilia reticulata*) show a repeated spatial pattern of local adaptation to the presence and absence of predators throughout the Northern Range Mountains of Trinidad, where small headwater tributaries generally lack piscivorous fish and downstream rivers contain a suite of piscivorous species (Haskins et al. 1961; Seghers 1973; Endler 1995). Genetic studies of natural populations of Trinidadian guppies suggest that this spatial pattern of local adaptation has independently evolved numerous times because adjacent high- and low-predation populations within a drainage are genetically similar and highly divergent from populations in neighboring drainages (Carvalho et al. 1991; Crispo et al. 2006; Alexander et al. 2006; Suk and Neff 2009; Willing et al. 2010). Thus, guppy populations experiencing relatively high predation in larger streams appear to have repeatedly colonized and successfully established populations in upstream tributaries where both predation pressure and resource availability are reduced (Reznick et al. 2001).

Variation in predation pressure has repeatedly led to population-level divergence in multiple guppy traits, including life histories (e.g. Reznick 1982; Reznick et al. 1996), morphology (e.g. Alexander and Breden 2004), behavior (e.g. Magurran and Seghers 1991, 1994; Huizinga et al. 2009), and swimming performance (e.g. Ghalambor et al. 2003). Many of these traits have a genetic basis and have been shown to evolve on contemporary time scales (reviewed in Reznick et al. 1997). Additionally, environmental cues can induce adaptive plastic responses in several of these same traits. For example, in response to visual or chemical cues from predators, guppies alter habitat use (Botham et al. 2006), age at maturity (Rodd et al. 1997; Gosline and Rodd 2008), shoaling behavior (Huizinga et al. 2009), and other life history traits (Dzikowski et al. 2004). Similarly, in response to changes in food quantity or type, guppies exhibit adaptive plasticity in head morphology (Robinson and Wilson 1995), and age and size at maturity (Reznick 1990). Thus, while we expect guppy populations to show local adaptation in behavioral, morphological, and life history traits, we also predict these same traits to exhibit some degree of plasticity in response to environmental conditions during development. What remains unclear, however, is the degree to which predator-induced plasticity explains the magnitude of population differentiation, the role of plasticity during the process of local adaptation, and the degree to which populations differ in the magnitude of plasticity they exhibit.

Here, we experimentally reared family lines of guppies derived from two high- and low-predation population pairs under conditions mimicking the ancestral, high-and derived, low-predation environments by exposing them to chemical cues from a natural predator of guppies. Specifically, we assessed whether exposure to these predator cues induces plastic changes in habitat use (measured as position in the water column), head morphology, and size at maturity to test if the magnitude and direction of plasticity has diverged between high-and low-predation populations. This suite of traits comprises phenotypes that have been shown to be correlated with fitness in high- and low-predation guppy populations through their effects on food acquisition, predator avoidance, and mate acquisition (Seghers 1973; Reznick 1982; Reznick et al. 2001; Magurran 2005; Robinson and Wilson 2005; Langerhans 2010).

MATERIALS AND METHODS

Experimental subjects

Gravid *Poecilia reticulata* females were collected from high- and low-predation localities from the Yarra and Guanapo rivers located in the Northern Range Mountains on the island of Trinidad, West Indies. The Yarra River is one of the major drainages on the Northern Slope region, and the Guanapo River is part of the larger Caroni drainage on the southern slope of the Northern Range Mountains. Previous work has identified these regions as containing two distinct genetic lineages of guppies that have independently evolved in response to the presence and absence of predators (e.g. Willing et al. 2010). Within drainages, we collected fish from two localities: 1) a high-predation locality where guppies coexist with a diversity of large piscivorous fish, and 2) a low-predation locality that lacks large piscivorous fish. Within the Yarra drainage, individuals from the high-predation population were collected approximately 2 kilometers upstream from the confluence with the ocean, while individuals from the low-predation population were collected from a small upstream tributary of the Yarra River (see Reznick et al. 1996 for details). In the Guanapo drainage, individuals from the high-predation population were collected approximately 7 kilometers upstream from the confluence of the Guanapo River with the higher order Caroni River. Individuals from the low-predation population were collected from the Taylor stream, a small tributary to the Guanapo River (see Gilliam et al. 1993 for details).

Wild caught females from each population were kept individually either in 10-liter glass tanks without recirculating water (Yarra fish) or in 1.5-liter tanks in recirculating systems (Guanapo fish) (12L: 12D, temperature 25±1°C; Reznick 1982) and fed twice daily (AM: Tertramin™ tropical fish flake paste, PM: hatched *Artemia* cysts). We propagated two lab-born generations from these wild collected females to generate distinct F2 family lines. To maximize genetic variation within populations and to minimize any confounding maternal effects, F2 family lines were generated by randomly crossing lab-born fish within each generation and population. Not all collected fish were successfully propagated to the second generation, thus our sample size varies among populations. Of the original families per population in the Yarra drainage, 11 of the low-predation and 13 of the high-predation families persisted through two lab reared generations, while 15 low-predation and 23 high-predation families persisted from the Guanapo drainage.

Experimental design

We conducted a common garden experiment where F2 families from all four populations were reared in the presence or absence of predator cues. Chemical cues are thought to play an important role in predator detection in guppies (Nordell 1998; Brown and Godin 1999; Dzikowski et al. 2004; Huizinga et al. 2009). Previous work has identified specialized epidermal cells, called club cells, which release an alarm pheromone when ruptured during a predation event (Pfeiffer 1974). We fed guppies to pike cichlids (*Crenicichla spp.*) – a common predator of guppies in the southern slope of the Northern Range of Trinidad – to mimic the chemical cues in nature and ensure that both the alarm pheromone and any predator kairomones (chemicals excreted by predators) were present in the water (see below).

Within 24 hours post parturition, families of F2 litters were evenly split between two tanks (n= 2-10 fish per tank) corresponding to one of two treatments: with or without predator cues. To simulate predator cues, fish from the Yarra populations were reared in non-recirculating 10-liter tanks, where we exchanged water from tanks housing pike cichlids maintained on a diet of 2 guppies daily. Every three days, 1L of predator-conditioned water (with predator cue treatment) or 1L of conditioned tap water (without predator cue treatment) was exchanged in each tank and all observations occurred within 24h following water exchanges. Fish from the Guanapo drainage were reared in 1.5-liter tanks that were part of a recirculating system. A pike cichlid (fed a diet of 2 guppies daily) was maintained in the sump of the system to generate the predator cue treatment, and an identical system lacking a pike cichlid was used for the without predator cue treatment. Thus, in both types of tanks, the water in the predator cue treatment contained both predator kairomones and guppy alarm cues which were expected to act in concert to induce plastic responses to perceived predation risk (Nordell 1998; Dzikowski et al. 2004; Schoeppner and Relyea 2005).

Data collection

We measured three dependent variables: vertical position in the water column (a proxy for habitat use), male size at maturity, and head morphology. The vertical position of fish in the water column was measured prior to morning feedings. Each trial consisted of recording the number of fish at the water surface (i.e. within 1 cm of the surface), and the number of fish throughout the remainder of the water column (i.e. between the bottom of the tank and 1cm of the surface) once per minute for five minutes. The average of these five observations was used to quantify the weekly proportion of fish using the surface versus the remainder of the water column. In all cases fish were not fed between 12 and 15 hours prior to observations in effort to standardize any effect of hunger on behavior. To test if there was an acclimation or habituation effect to the chemical cues, we conducted a pilot study where we measured the vertical position of fish from the Yarra drainage at three, four and five weeks old. We found no effect of age on the proportion of fish at the surface ($F_{2,102.4}$=0.560, P=0.572; P-values for all interactions were >0.5), suggesting no habituation to the treatment over time. Therefore, water column use was only measured at maturity in subsequent observations.

Once male guppies reached maturity, as determined by the development of the anal fin following Reznick (1982), they were anesthetized with tricainemethanesulfonate (MS-222; 100 mg/l) and photographed. Photographs were used to calculate size at maturity by measuring the standard length to the nearest 0.01 mm (ImageJ Software; Abramoff et al. 2004) and to measure head shape. Geometric morphometrics was used to quantify variation in head shape between individuals (Rohlf and Marcus 1993). To capture the variation in head shape, four homologous landmarks and one sliding semi-landmark (Bookstein 1997) were digitized from digital photographs with tpsDig2 (Rohlf 2003; Figure 2.3). A Generalized Procrustes Analysis (Tpsrelw software; Rohlf 2003) was performed to align the sliding semi-landmark among all specimens and to remove isometric size, rotational, and position effects on Cartesian coordinates (Bookstein 1991).

Statistical analysis

A mixed model analysis of variance (ANOVA) was used to test for differences in the proportion of fish that were found at the surface of the tank and differences in standard length at maturity. We used drainage, treatment, natural predation regime (hereafter predation regime), and their interactions as fixed factors, and family as a random effect. We square root-arcsine transformed the proportion of fish at the surface to closer approximate normality in the data.

The analysis of head morphology was performed in two steps. First, a multivariate analysis of covariance (MANCOVA, performed in R v. 2.13; http://www.r-project.org) was used to test for differences in head shape due to the effects of drainage, treatment and predation regime with centroid size (Bookstein 1991) included as a covariate. The MANCOVA model evaluates how each factor in the model explains variation in head shape (matrix of aligned landmarks coordinates). Further, we estimated the partial variance (η^2), the effect size of each model parameter. We found drainage of origin to be the dominant effect in the MANCOVA (Table 2.1), and therefore, we subsequently analyzed each drainage separately to look for qualitatively similar responses to the experimental treatments.

To visualize how head shape in each high- and low-predation population pair responded to the two rearing environments, we performed a Canonical Variate Analysis (CVA). In

MorphoJ, CVA was performed on the aligned specimen coordinates (Klingenberg 2011). Predation regime and rearing treatment were used as classifiers in the CVA to identify axes of shape that maximize the difference in head shape among populations in each treatment. Discriminant function analysis was used to show how rearing treatment corresponds to variation in head shape (MorphoJ; Klingenberg 2011). The discriminant function analysis finds the linear combination of the shape variables that best separates the groups reared with and without predator cues. The variation in head shape along the discriminant axis was depicted with wire frame outlines that capture the nature of shape change (MorphoJ; Klingenberg 2011).

Given that fish from the two drainages were reared in different systems (non-recirculating 10-liter tanks *versus* 1.5-liter tanks in the recirculating system), drainage effect and rearing conditions are confounded factors in our experimental design, thus we explicitly considered how rearing system might influence the interpretation of any effects of drainage, or its interaction with other factors, on variance observed in our study. All analyses were performed in JMP (JMP, v. 9. SAS Institute Inc., Cary, NC, 1989-2011) except where otherwise noted.

RESULTS

The presence of predator cues during development caused guppies to significantly shift their vertical position to within 1cm of the water column surface, whereas in the absence of the predator cues guppies were rarely observed at the water column surface ($F_{1,56.91}$=278.45, P<0.0001; Fig. 2.1). However, no differences between drainages or predation regime within drainages were observed (all P values, including those for interaction terms >0.05; Fig. 2.1). In the presence of predator cues, 64.9% of the fish were observed at the surface of the water, whereas only 3.1% of the fish were observed at the surface in the treatment without predator cues (Fig. 2.1). Families did not significantly differ in their use of the water column, or in how they reacted to the presence or absence of predator cues (95% confidence interval for the variance component: -0.01, 0.02 and -0.02, 0.06, respectively).

We found that in both drainages the size at maturity was significantly larger in fish from low-predation regimes than their counterparts from high-predation regimes when reared in the absence of predator cues (Fig. 2.2). However, no differences in size were observed between populations when reared in the presence of predator cues, resulting in a significant interaction between predation regime and treatment (Fig. 2.2, treatment x predation regime interaction $F_{1,48.19}$=8.046, P=0.007). Furthermore, we found a higher degree of plasticity (i.e. a steeper slope) for size at maturity in fish from the Guanapo Drainage than in fish from the Yarra Drainage across treatments, as differences in size between fish reared with and without predator cues was greater for Guanapo fish than it was for Yarra fish (Fig. 2.2, treatment x drainage regime interaction $F_{1,43.83}$=11.690, P=0.001). This higher degree of plasticity in Guanapo guppies appears to be independent of the natural predation regime (i.e. high- versus low-predation), as we did not find a significant interaction between drainage, treatment, and predation regime (P-values >0.05). All main effects were significant (drainage: $F_{1,45.35}$=6.066, P=0.018; predation regime: $F_{1,50.40}$=4.397, P=0.041; treatment: $F_{1,44.60}$=53.923, P<0.0001). Variation among families explained 24% of the observed variation in size at maturity (95% confidence interval for the variance component estimate: 0.01, 0.36). However, families did not significantly differ in their response to the presence or absence of predator cues (95% confidence interval for the variance component estimate: -0.19, 0.19).

Fish reared with predator cues also developed longer and shallower heads than fish reared without predator cues (Fig. 2.3a). A multivariate analysis of covariance (with centroid size as a covariate) revealed that head morphology differed between high- and low-predation guppies, between fish from the Yarra and Guanapo drainages, and in response to the presence/ absence of predator cues in the rearing environment (Table 2.1). However, the magnitude of the difference in head shape between fish from high and low predation regimes, independent of rearing conditions, is greater in fish from the Yarra Drainage than in fish from the Guanapo Drainage (Table 2.1; all other interactions were non significant). We used CVA to visualize trends in head shape variation as a function of treatment and predation regime. The first canonical variate axis (CV1; Fig. 2.3b), explained between 77-80% of the total variation, and similar to the results for size at maturity, reveals that fish from different predator regimes differ in the degree to which they exhibit plasticity when reared in the absence of predator cues than when reared in the presences of predator cues (Fig. 2.3b). The second canonical variate axis (CV2; Fig. 2.3b) explained an additional 15-20% of the variation, and further separates high- and low-predation populations when reared in the absence of predator cues (Fig. 2.3b). However, the high-predation populations between drainages differ in their plastic response because head shape in the Yarra high-predation is invariant to the treatment (Fig. 2.3b).

DISCUSSION

Adaptive phenotypic divergence in response to different environmental conditions can be due to local adaptation, phenotypic plasticity, or both (Reznick and Travis 1996; Schlichting and Pigliucci 1998). Common garden studies using treatments designed to simulate natural environmental variation can experimentally test whether phenotypic divergence represents evolved genetic differences or a plastic response to the environment (Gotthard and Nylin 1995; Kawecki and Ebert 2004; Williams et al. 2008; Hereford 2009).

Here, in a comparison of guppies from high- and low-predation environments from two different drainages, we found evidence for evolution in some, but not all, traits thought to be under divergent selection from predators. Yet, the evidence for evolutionary divergence was dependent on the rearing environment. When second generation lab born fish were reared in the *presence* of chemical cues produced by pike cichlids consuming guppies, no differences were found between guppies from high- and low-predation environments in any of the examined traits, regardless of their drainage of origin (Figs. 2.1, 2.2 and 2.3). However, when fish were reared in the *absence* of these predator cues, guppies from high- and low-predation environments did exhibit significant differences in size at maturity and a similar pattern was found in head shape (Figs. 2.2 and 2.3).

The observed differences between guppies from high- and low-predation regimes suggest a genetic basis to what are thought to be adaptive differences between guppy populations occurring in high- and low-predation environments, but also indicate that plasticity in two of the three analyzed traits has evolved. The divergence in the slope of the reaction norms between high- and low-predation populations within drainages occurs because guppies from low-predation regimes express a low-predation phenotype in the common garden environment that mimics the derived native environment (predator cues absent) and a high-predation phenotype in the environment that mimics their high-predation ancestral environment (predator cues present). These results are consistent with models that view the evolution of reaction norms as a by-product or indirect consequence of selection acting on mean trait values in different environments (e.g. Via and Lande 1985; Gotthard and Nylin 1995; Via et al. 1995; Czesak et al. 2006). Such models predict that when the cross-environment genetic correlation for a trait is low, selection in one environment can change the trait value independently of changes in the mean value of the same trait in a different environment. Thus any changes in the slope of the reaction norm occur as a by-product or indirect effect of the response to selection in the respective environments (Falconer 1990; Czesak et al. 2006). Here, our results suggest that adaptation to the low-predation environment results in divergence from the ancestral, high-predation populations and a change in the slope of the reaction norm for size at maturity and head morphology (Figs. 2.2 and 2.3). These results qualitatively differ from other studies of plasticity in guppies, where adaptive divergence between high- and low-predation populations has not resulted in changes to the slope of the reaction norm (e.g. Huizinga et al. 2009). However, the dichotomous predation environments examined in this study preclude the ability to evaluate other models arguing that selection acts directly on plasticity through changes in the coefficients that describe the shape of the reaction norm (e.g. Gavrilets and Scheiner 1993a, 1993b). Nevertheless, our results are similar to those found by Hairston and De Meester (2008), where the evolution of predator-induced plasticity in *Daphnia* populations was driven by selection in one environment, rather than changes in slope across all environments. Similarly, selection experiments in the seed beetle *Stator limbatus*, found that the evolution of host plant-induced plasticity in egg size was associated with the selection on trait values, but also depended on the environment where selection occurred (Czesak et al. 2006). Below, we first discuss why the phenotypic differences between guppies from the different predation regimes are likely to be adaptive in support of the argument that plasticity evolved as a by-product of adaptation to the low-predation environment. We then discuss these results in the context of the role plasticity is likely to play in the process of colonization and adaptation to novel environments.

Plasticity and Divergence in Behavior, Morphology, and Size at Maturity

We found that the magnitude of plasticity and divergence between the ancestral, high- and derived, low-predation populations varied depending on the trait being examined. In all populations individuals exhibited a significant behavioral shift towards the surface of the water column when reared in the presence of the predator cue, whereas when reared in the absence of the cue, few, if any, individuals occupied the surface of the water column (Fig. 2.1). The similarity of these behavioral responses across populations is notable for several reasons. First, the populations from the two drainages were evaluated in different rearing tanks (non-recirculating 10-liter tanks vs. 1.5-liter tanks in a recirculating system), but still exhibited indistinguishable responses. Second, the degree of plasticity (i.e. the slope of the reaction norm) has been conserved across drainages and populations. Finally, the lack of differentiation between populations within drainages suggests no evolutionary divergence has occurred in response to different predation regimes. Such predator-induced behavioral changes are presumably adaptive (Seghers 1973; Reznick et al. 2001; Botham et al. 2006), because a shift in habitat use towards the surface reduces susceptibility to a predator strike by reducing the dimensionality of the environment, which in turn may allow for earlier detection of predators and longer strike distances (Walker et al. 2005). However, it should be noted that no study to date has tested whether prey species at the water surface are more likely to survive a predator encounter compared to those lower in the water column.

In contrast to the behavioral response, when comparing populations within drainages, we found evidence for evolutionary divergence between predation regimes for size at maturity; males from the low-predation populations were significantly larger at maturity than males from the high-predation populations (Fig. 2.2). However, this result was largely driven by a significantly greater increase in the size of low-predation males when reared in the absence of the predator cue (Fig. 2.2). Past studies have emphasized the important fitness consequences of maturing at a smaller size under high predation conditions, and at a large size under low-predation conditions (e.g. Reznick 1982; Magurran 2005). In low-predation localities, sexual selection via female mate preferences favors a larger male body size (Reynolds and Gross 1992; Magellan et al. 2005). In contrast, males in high-predation localities are under selection to mature at a smaller age and size because they experience high mortality rates (Reznick 1982) and coexist with a suite of larger predators that selectively prey on larger guppies (Liley and Seghers 1975). Indeed, similar to our results, past common-garden studies carried out in the absence of any predator cues have also demonstrated a genetic basis to the smaller size at which high-predation guppies mature (Reznick 1982; Reznick et al. 1990; Reznick and Bryga 1996; Magurran 2005). Thus, the ability to mature at a larger size would be predicted to be both an adaptive plastic response in the absence of predators, and an evolutionary response driven by sexual selection and relaxed selection from predation. Yet, the ability to exhibit plasticity in size at maturity when reared in the absence of the cue appears to be a derived response, as both high-predation populations examined in this study had shallower reaction norms compared to their low-predation counterparts (Fig. 2.2). There were also differences between drainages, as both high- and low-predation regime guppies from the Guanapo drainage matured at a significantly smaller size than the Yarra populations (Fig. 2.2). A plausible explanation for this pattern is that pike cichlids, which were used to simulate predation risk in this study, are common guppy predators in the Guanapo drainage and throughout the southern slope of the Northern Range Mountains, whereas they are absent from most of the northern slope rivers. Thus, Guanapo populations may be more sensitive to the chemical cues of the pike cichlid owing to their longer history of coexistence, although evidence for this greater sensitivity was not found in the behavioral or morphological traits we measured (Figs. 2.1 and 2.3b). Alternatively, the concentration of the predator cues may have been higher in the recirculating systems the Guanapo fish were reared in, compared to the non-circulating tanks of the Yarra fish. This could explain the observed variation between drainages as there is evidence that prey responses can be sensitive to the abundance of predator kairomones (Van Buskirk et al. 2011).

Lastly, we found that head morphology differed between drainages, predation regimes, and the rearing environment (Table 2.1). Similar to our findings for size at maturity, the canonical variate analyses reveals that within drainages, high- and low-predation populations only diverged when fish were reared in the absence of predator cues (Fig. 2.3b). In the absence of the pike cichlid cue, the low-predation populations from both drainages developed a greater angle from the tip of the rostrum to the ventral and dorsal posterior terminus of the head, yielding a more stout head shape with the mouth in a more anterior position and aligned with the eye (Fig. 2.3a). However, in the presence of the pike cichlid cue, fish from all populations developed a more fusiform head with a dorsal shift in the position of the mouth, and were indistinguishable from each other (Fig. 2.3b). These results suggest a trend for an evolutionary transition from a less plastic, ancestral morphology in the high-predation environment to a more plastic morphology with respect to the shape of the head and associated trophic structures (Fig. 2.3b). Past work has shown that the position of food resources in the water column can induce plastic changes in guppy trophic morphology (Robinson and Wilson 1995), suggesting that a behavioral shift away from the surface of the water column could play a role in developing a stout head with a more terminal mouth position. Moreover, recent work on the trophic ecology of guppies found low-predation populations to have a more generalized diet compared to high-predation populations (Bassar et al. 2010; Zandonà et al. 2011). A highly specialized diet could favor a more canalized head morphology as observed in the high-predation Yarra population, but additional work is needed to relate foraging behavior and diet to trophic morphology. An alternative explanation is that a fusiform head shape may influence escape performance, as a more fusiform head shape correlates with the presence of predators across several fish taxa (Langerhans 2010). A more streamlined head could increase escape performance from predators if it reduces drag (Langerhans 2010). Collectively, as with size at maturity, the difference between high- and low-predation populations in the absence of the predator cue suggests evolutionary divergence following colonization and adaptation to the low-predation environment.

The Role of Plasticity in New Environments

There has been renewed interest in understanding the possible role phenotypic plasticity plays in the process of adaptation to new environments (e.g. West-Eberhard 2003; Price et al. 2003; Crispo 2007; Ghalambor et al. 2007; Lande 2009), and experimental studies that compare reaction norms between ancestral and derived populations and environments can empirically test whether evolutionary divergence follows the direction of plasticity. Theory predicts that when populations experience strong directional selection, due either to changes in habitat conditions or colonization of new habitats, adaptive plasticity can increase the probability of population persistence, and in turn favor the evolution of increased plasticity and peak shifts towards the new local optimum (e.g. Baldwin 1896; Price et al. 2003; Lande 2009; Chevin and Lande 2010). However, empirical studies of natural populations supporting such theory are generally absent because of the challenges of capturing the role of plasticity during the initial stages of colonization. Instead, an increasing number of studies have compared reaction norms across environments in ancestral and derived populations (e.g. Yeh and Price 2004; Wund et al. 2008) or the plastic versus evolved responses to native and novel environments (e.g. Chapman et al. 2000; Losos et al. 2000), to infer the role plasticity might play when individuals are exposed to new environments and subsequently experience evolutionary divergence. For example, the

African cichlid, *Pseudocrenilabrus multicolor*, shows adaptive plasticity by modifying its gill size in response to hypoxic conditions, likely facilitating initial colonization and subsequent local adaptation to hypoxic swamps (Chapman et al. 2000). Similarly, the marine ecotype of the threespine stickleback, *Gasterosteus aculeatus*, exhibits patterns of phenotypic plasticity in trophic morphology consistent with derived ecotypes that successfully colonized and adapted to freshwater benthic and limnetic (Day et al. 1994; Wund et al. 2008). Below, we discuss how the patterns of plasticity observed in the current study contribute to our understanding of how plasticity and selection jointly facilitate local adaptation.

We observed patterns of adaptive plasticity in behavior, morphology and life history traits that likely contribute to population persistence among high- and low-predation habitats in nature. Additionally, we found evidence for evolutionary divergence between high- and low-predation populations consistent with directional selection and local adaptation occurring in the low-predation environment following colonization. Specifically, it appears that an initial behavioral response of high-predation guppies invading a low-predation environment would be to shift habitat use away from the water column surface in effort to better exploit a limited forage base. Because this behavioral response is “complete” or identical to the response of the locally adapted, low-predation populations (Fig. 2.1), it suggests the plastic response shields the population from directional selection (e.g. Price et al. 2003; Ghalambor et al. 2007). Such adaptive plasticity may explain why we found no evidence of divergence between populations. Alternatively, there might not be sufficient genetic variation in this trait for selection to act upon, which is suggested by the non-significant family effect in our model. In contrast, when reared in the absence of the predator cues, the high-predation populations exhibit a plastic response in head shape and size at maturity that is “incomplete” compared to the low-predation populations

(Figs. 2.2 and 2.3). By incomplete, we mean the plastic responses are in the direction favored by selection, but do not result in trait values produced by the locally adapted low-predation populations. Thus, while these plastic responses may be adaptive relative to a non-plastic genotype, there is still an opportunity for directional selection to act (e.g. Price et al. 2003). In addition, there appears to be sufficient standing genetic variation in the size at which individuals mature, as almost 25% of the observed variation was due to variance among families. Indeed, the evolutionary divergence from the ancestral, high- to derived, low-predation populations likely reflects the role of selection and local adaptation following colonization of the low-predation environment (Via and Lande 1985; Lande 2009). As a consequence, the derived, low-predation populations exhibit steeper slopes in their reaction norms (Figs. 2.2 and 2.3). The evolution of increased plasticity following colonization of a new environment has been interpreted as evidence for the "Baldwin Effect" (compare Figs. 2.2 and 2.3 with Fig. 1b in Crispo 2007).

Our results are also in contrast with other theoretical models that predict that plasticity should be lost following adaptation to a new environmental through genetic assimilation (e.g. Lande 2009), because it is costly to maintain (e.g. van Tienderen 1991; Moran 1992; DeWitt et al. 1998; but also see Van Buskirk and Steiner 2009), or due to random processes in the absence of selection to retain it (e.g. Masel et al. 2007). Instead, we found that the low-predation genotypes were still able to express a high-predation phenotype when reared in the presence of a predator cue (Figs. 2.1-3). One hypothesis for the observed retention of predator-induced plasticity is that it is maintained by selection because juvenile and smaller adult guppies do suffer some predation from *Rivulus hartii*. However, if this were a size-specific response, we would have expected predator-induced changes in behavior, and perhaps other traits, to be reduced once guppies reached a size class sufficient to preclude predation from *R. hartii*. We found no age effect on the proportion of fish at the surface in fish from the Yarra drainage (see Material and Methods for details), suggesting that the observed behavior was not size- or age-specific. Thus, if low-predation genotypes invade high-predation environments, we would predict that the traits examined herein would not evolve because low-predation fish show a "complete" adaptive response to predator cues so there would be insufficient phenotypic variation for selection to act on. This asymmetry in plasticity between high- and low-predation ecotypes could have important implications for the interactions between gene flow and selection among localities (Crispo et al. 2008). For instance, introgression via migrants from upstream, low-predation populations into downstream, high-predation populations should not have negative fitness implications based on the three traits examined here. However, immigrants from high-predation locales moving into low-predation populations should have lower relative fitness and be selected against because of the incomplete plastic responses in head shape and size at maturity.

In conclusion, this study provides empirical evidence for the evolution of adaptive plasticity between ancestral and derived populations of guppies as a by-product of local adaptation to environments with reduced mortality selection via predation. The colonization and establishment of populations in the derived environment could have been facilitated by plasticity in habitat use, size at maturity and head shape that existed in the ancestral high-predation population. Heritable phenotypic divergence between high- and low-predation population in size at maturity, and, to a lesser extent head shape, could be attributed to the subsequent effects of directional selection in the new environment. Thus, these results support the view that adaptive evolution can proceed by increasing adaptive plasticity (e.g. Crispo 2007; Lande 2009).

TABLES AND FIGURES

Table 2.1. Statistical results of MANCOVA model testing treatment effects of head shape in male Trinidadian guppies. Drainage tested fixed differences among the Yarra and Guanapo drainages and their respective rearing design; Predation tested for differences between fish from high- and low-predation regime origin within drainages; and Treatment tested for the plastic response to being reared in the presence or absence of predator chemical cues. Centroid size was used as a covariate. The percentage of the partial variance (η^2) explained by each factor or interaction is in the last column.

	df	df_{den}	Wilk's test	*P*	η^2
Drainage	6	110	82.795	<0.0001	0.802
Predation	6	110	4.225	0.004	0.153
Treatment	6	110	12.957	<0.001	0.215
DxP	6	110	2.651	0.021	0.125
DxT	6	110	1.094	NS	0.061
PxT	6	110	1.052	NS	0.055
DxPxT	6	110	1.023	NS	0.053
Centroid Size	6	110	6.136	0.002	0.168

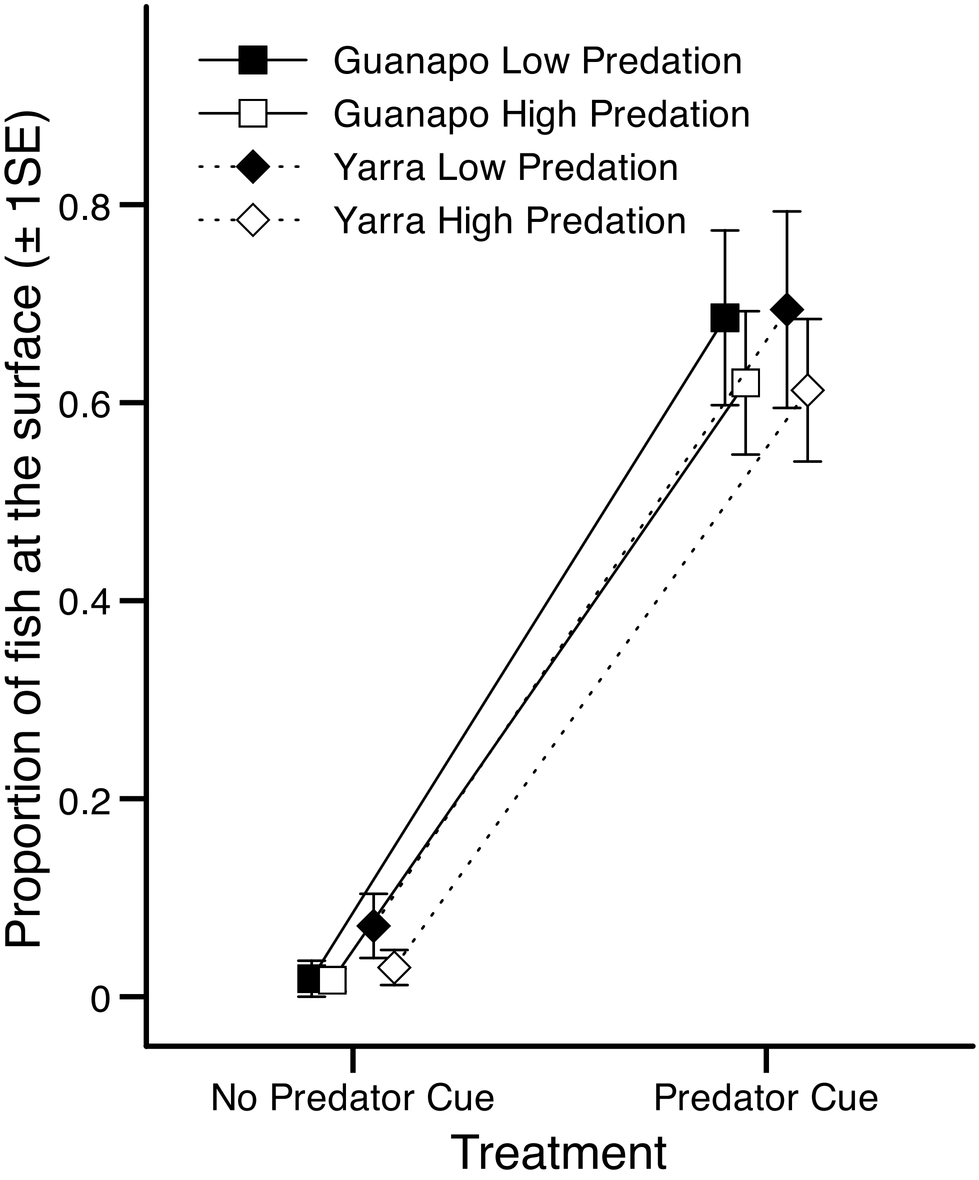

Figure 2.1. Mean and standard errors of proportion of fish at surface from low- (filled symbols) and high- (open symbols) predation regimes from the Guanapo (square symbols) and the Yarra (diamond symbol) drainages reared in the presence or absence of predator cues. Symbols are slightly displaced to facilitate visualization of the error structure.

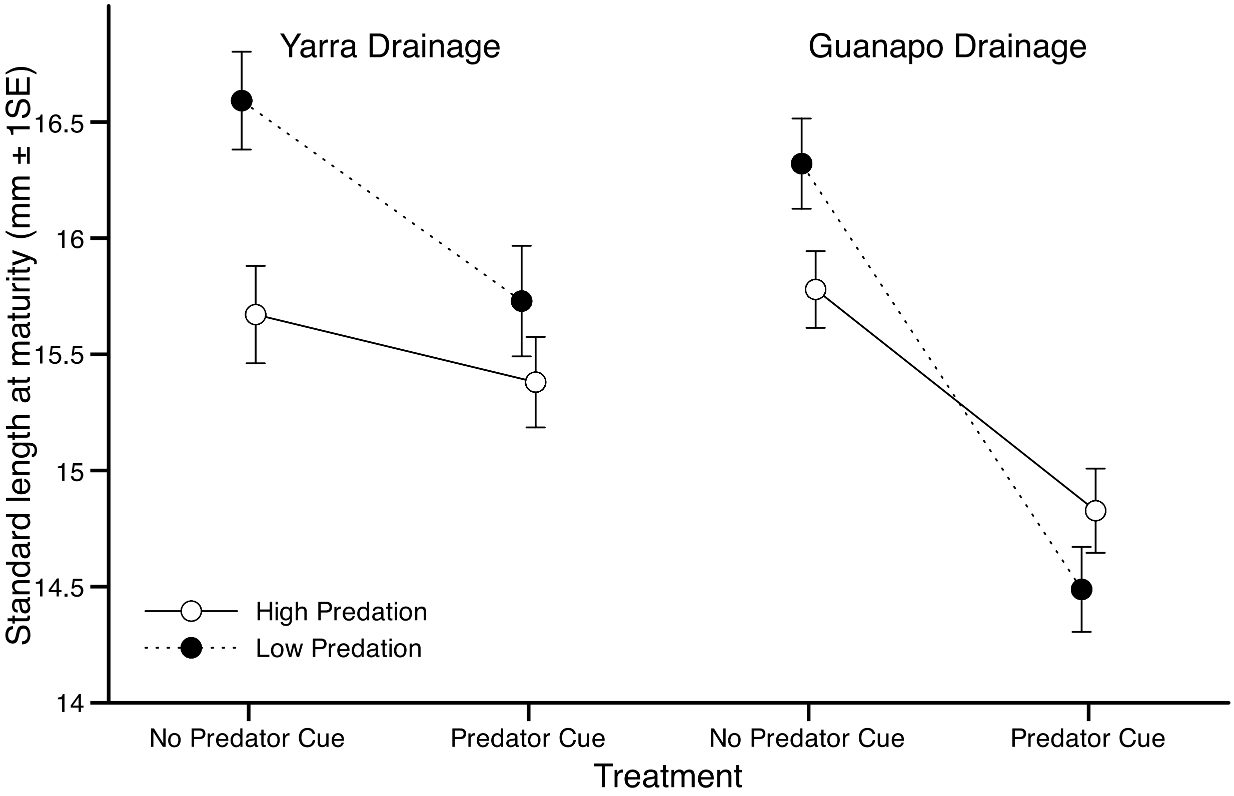

Figure 2.2. Mean and standard errors of standard length at maturity from low- (filled symbols) and high- (open symbols) predation regime guppies reared in the presence or absence of predator cues. The right panel depicts fish from the Guanapo drainage, and the left panel depicts fish from the Yarra drainage. Symbols are slightly displaced to facilitate visualization of the error structure.

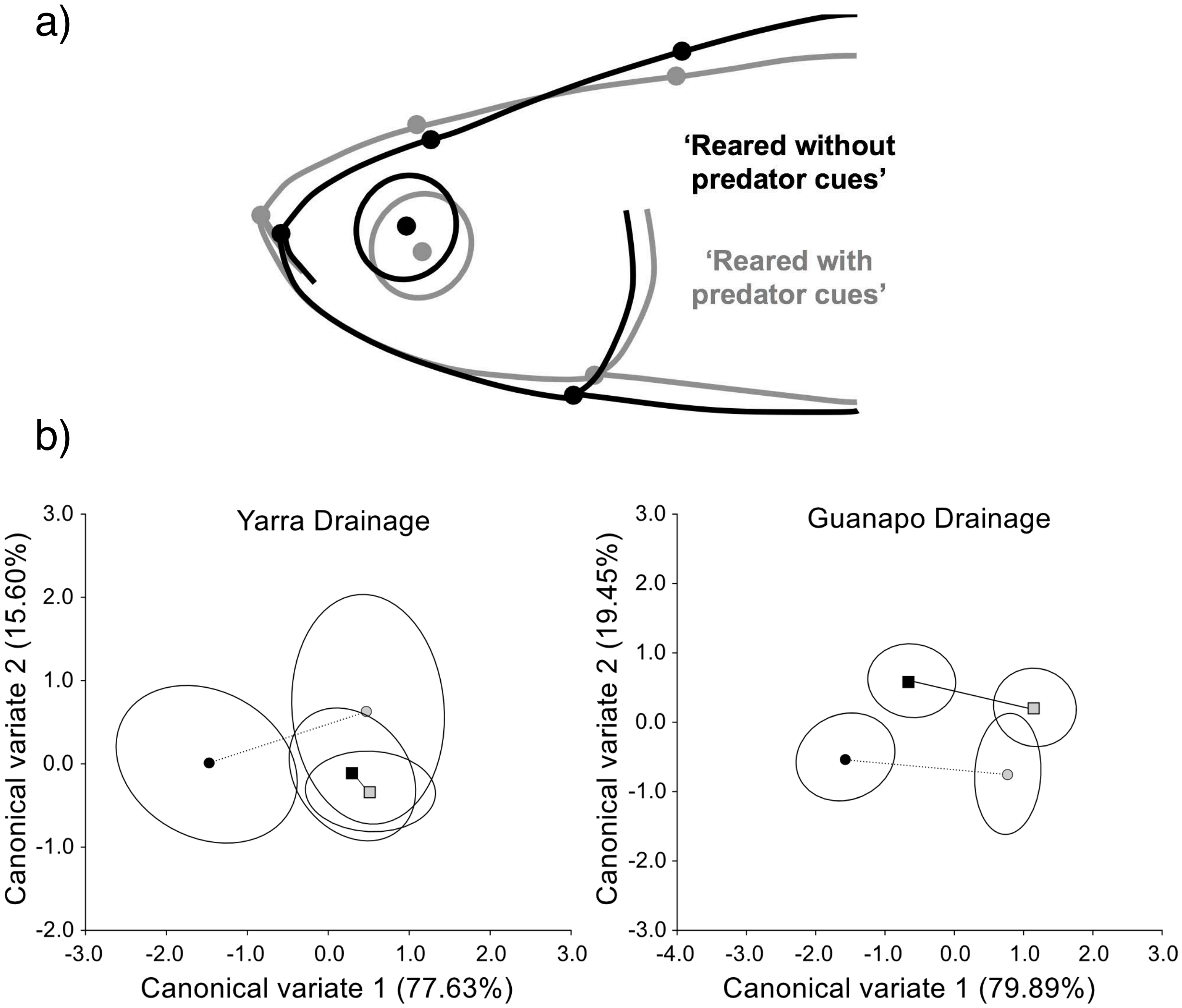

Figure 2.3. a) Changes in head shape due to the effect of rearing environment identified by discriminant function analysis (Klingenberg 2011). When reared in the presence of predator cues, guppies develop a more fusiform head shape (grey lines). In the absence of predator cues, the angle from the rostrum to the posterior terminus of the head increased (black line). Solid circles represent landmarks used for morphological analysis. b) Mean and 95% confidence ellipses for CV1 and CV2 scores from low- (circles) and high- (squares) predation regime guppies reared in the presence (grey symbols) or absence (black symbols) of predator cues. Analyses were performed for each drainage separately. Note that low-predation genotypes responded more strongly to the treatment than the high-predation genotypes, and that the 95% confidence ellipses overlap for the high- and low-predation populations in the presence of the predator cue, but not in its absence.

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CHAPTER 3:

ECOLOGICAL CORRELATES OF THE DISTRIBUTION OF TWO POECILIID SPECIES ACROSS A SALINITY GRADIENT

INTRODUCTION

The niche of a species is defined by the set of ecological conditions under which populations are maintained (Hutchinson 1957). In other words, it is the range of all ecological factors under which intrinsic population growth remains positive. This concept is central to the development of ecological research (Chase and Leibold 2003); and its importance to biogeography has also long been emphasized (Grinnell 1917), receiving heightened attention in the last decade in the context of species distribution limits (Pulliam 2000; Soberón 2007; Angert 2009; Holt 2009; Wiens 2011; Chase and Myers 2011; Peterson et al. 2011). In theory, mismatches between a species' niche and local habitat use should result in negative population growth. When this occurs along environmental gradients, one outcome is the formation of distribution limits, as the net gain of individuals at a certain point along the gradient no longer exceeds the net loss (Gaston 2003). Thus, the niche influences processes at local scales, which in turn shape biogeographic patterns at regional scales (Gaston 2003; Soberón 2007; Wiens 2011). A major challenge in ecology and biogeography is determining which of the factors that define a species' niche play the most important roles in the formation of distribution patterns (Bull 1991; Brown et al. 1996; Sexton et al. 2009). This task is particularly difficult to accomplish because environmental gradients exhibit complex interactions among multiple factors (Vannote 1980; Attrill and Rundle 2002), and because different limiting factors might act at varying spatial scales (Fausch et al. 1994; Poff 1997; Silvertown et al. 2006).

Climatic factors are often responsible for the formation of species range limits and large-scale biogeographic patterns (Gaston 2003; Lomolino et al. 2006; Sexton et al. 2009). However, at small geographical scales, evidence suggests that other abiotic factors determine species' distribution limits, though the nature of these interactions depends on the system under study

(Sexton et al. 2009; Wiens 2011). For instance, in aquatic systems, some of the most relevant factors are water salinity, flow, temperature, and dissolved oxygen (Allan and Castillo 2007). Water salinity is particularly important for both marine and freshwater organisms because ionic balance is crucial for homeostasis (Nordlie et al. 1991) and directly affects several traits related to fitness, such as survival (Shervette et al. 2007), growth rate (Bœuf and Payan 2001), behaviour (Peterson-Curtis 1997), and several life history traits (Gomes and Monteiro 2007). Thus, salinity has the potential to be an important factor affecting species distributions (Marshall and Elliott 1998). Furthermore, steep gradients of salinity are known to occur where rivers discharge into the sea forming estuarine environments (Winemiller and Leslie 1992; Attrill and Rundle 2002). Yet, the role of salinity as an important abiotic factor in shaping species distributions is more often assumed than tested empirically.

Two useful approaches to identify the relevant environmental factors responsible for the formation of distribution limits are the use of statistical niche models (Austin 2007; Holt 2009) and analysis of phenotypic variation along environmental gradients (Caughley et al. 1988). Statistical niche models establish a statistical relationship between a species distribution, usually in the form of presence-absence data, and a dataset of one or more environmental variables (e.g. Austin et al. 1990; Peterson 2001; Cadena and Loiselle 2007). These models are useful for contrasting predictions derived from niche theory. For example, niche theory predicts abrupt changes in species dominance along stressful scenopoetic abiotic gradients with little or no overlap (Chase and Leibold 2003; Peterson et al. 2011). Hutchinson (1978) defined scenopoetic variables as those that are not dynamically linked to population dynamics, in opposition to those abiotic factors that are affected by population density (Jones et al. 1994). Thus, one species would always displace the other at a certain level of the abiotic factor; because it can maintain positive intrinsic population growth either at lower food levels, higher predation, or higher parasitism pressure than the other species. However, these models are usually based on coarse-grain data and mainly represent the range of conditions that constitute the realized niche of a species instead of its fundamental niche, as biotic interactions or dispersal limitations sometimes restrict species from otherwise favourable habitat (Pulliam 2000; Soberón 2007). The combination of these approaches, statistical niche models and analysis of phenotypic variation along gradients, can help to further isolate potential factors that limit species distributions at finer-scales.

Analyzing patterns of phenotypic variation of populations along environmental gradients is used to generate inferences about the factors affecting species distribution (Caughley et al. 1988). Although this approach does not conclusively determine the specific factor limiting a species' distribution, it is a useful step to identify candidate variables that impose ecological boundaries (Caughley et al. 1988). For example, increased levels of physiological stress at the range-margin of the dogwhelk *Nucella canaliculata* suggest that gradual changes in temperature and desiccation probability determine the southern range limit of this species (Sorte and Hofmann 2004). Similarly, clinal changes in fecundity and hatching success at the southern limits of the hen harrier *Circus cyaneus* suggest that changes in temperature and rainfall might be responsible for the formation of the range limit (García and Arroyo 2001).

In particular, life history theory constitutes a useful framework to make predictions about phenotypic variation along abiotic and biotic gradients (Roff 1992; Stearns 1992; Charlesworth 1994). Life history traits (i.e. growth rate, age and size at maturity, reproductive effort, and senescence) are often correlated strongly with fitness and, therefore are determinant of population dynamics (Cole 1954). Thus, studying variation in life history traits along environmental gradients provides information about relevant selection factors (e.g. Kawamaki et al. 2011; Whitehead et al. 2011; Torres-Dowdall et al. *in press*).

The livebearer fish *Poecilia reticulata* (the Trinidadian guppy) and its phylogenetic sister species *P. picta* (the swamp guppy) are extensively distributed across the northern region of South America and the Caribbean islands (Wischnath 1993; Breden et al. 1999; Hrbek et al. 2007; Meredith et al. 2010). These species are phenotypically similar in morphology and life history traits and have a similar ecological role (Reznick et al. 1992). On the island of Trinidad, West Indies, both species can be found in mixed shoals (Magurran and Ramnarine 2004; Russell et al. 2006); but each species often uses distinct habitat, with *P. picta* being more common in brackish water and *P. reticulata* in freshwater (Reznick et al. 1992; Reznick and Endler 1982; Wischnath 1993). However, distribution limits of these two species across the salinity gradient are unclear given that they overlap in parts of their range. Furthermore, other factors, such as resource availability and community composition are expected to co-vary with the salinity gradient along lowland rivers of Trinidad (Magurran and Phillip 2001; Magurran 2005; Zandonà et al. 2011). Thus, several ecological factors could affect the distribution of *P. picta* and *P. reticulata* throughout Trinidad.

In this study we took two approaches to understand the distribution limits of *P. picta* and *P. reticulata* along longitudinal sections of rivers in Trinidad. First, we used a statistical niche modelling approach based on presence/absence data of the two species from 91 localities along the lowland rivers of Trinidad to determine the range of salinity, dissolved oxygen, pH, and elevation each species occupies. Second, we studied the pattern of phenotypic variation of specific traits along these gradients, focusing on fecundity, reproductive allocation, condition, and body size. We sampled *P. picta* and *P. reticulata* from the center and edge of their distribution in four different drainages to determine the generality of our results.

Predictions

Our primary goal was to explore the range of abiotic factors under which *P. picta* and *P. reticulata* are found and how those factors affect the distribution of these two species, paying special attention to the role of salinity. Poeciliids are generally considered euryhaline (Rosen and Bailey 1993), so we expect *P. picta* and *P. reticulata* to be present both in freshwater and brackish water environments. However, we expect *P. picta* to be less common or absent from most freshwater habitats and show an increasing presence as water salinity increases. In contrast, we expect *P. reticulata* to become uncommon in brackish environments and eventually disappear (Gibson and Hirst 1955; Zimmerer 1983; Wischnath 1993; Kenny 1995; Shikano and Fujio 1997). If salinity is an important factor shaping the distribution of these species, we expect *P. picta* and *P. reticulata* to show a sharp parapatric distribution (i.e. no co-occurrence along the gradient). However, several other factors change along the salinity gradients in the rivers of Trinidad (see Materials and Methods), and there is no *a priori* reason to expect that both species would respond equally to the gradient. For instance, each species may fail to occupy the entire gradient due to factors other than salinity. For example, niche theory predicts that species can show trade-offs in their ability to cope with biotic factors, facilitating coexistence (Chase and Leibold 2003). Thus, *P. picta* and *P. reticulata* could still be expected to co-occur along longitudinal sections of Trinidadian rivers if at least one of them is more sensitive to biotic than abiotic conditions (e.g. Taniguchi and Nakano 2000).

Finally, we expect *P. reticulata* and *P. picta* life-history traits to vary along lowland rivers in response to changes in abiotic and biotic factors gradients (Roff 1992; Stearns 1992;

Charlesworth 1994). We expect a decrease in reproductive allocation, fewer offspring, and a decrease in condition when abiotic factors result in physiological stress. On the other hand, stress associated with biotic factors could result in the opposite pattern. For example, in poeciliid fish, competition usually results in increase offspring size (Reznick 1982; Jørgensen et al. 2011). Therefore, we expect an increase in offspring size in localities where both species co-occur if competition is responsible for distribution limits. Additionally, increased mortality due to predation is expected to select for increased reproductive effort and offspring number, and a reduced size at maturity (Charlesworth 1994).

MATERIALS AND METHODS

Collection sites and environmental variables

Our study area compromises the lowland rivers of Trinidad, West Indies (Fig. 3.1). The most evident factor varying along the lowland rivers is salinity. However, several biotic factors also change predictably along the longitudinal section of rivers in Trinidad (see below). First, there appears to be an increase in resource quantity and quality from upstream to downstream localities (Zandonà et al. 2011). At the freshwater-brackish water interface, rivers are very wide and eutrophication is common compared with upstream sites where only *P. reticulata* is present (WRA 2001). In addition, there is a dramatic increase in mysid shrimp (*Metamysidopsis* spp.) abundance in the brackish waters where *P. picta* occurs (Mohammed 2005; pers. obs.), suggesting that food might not be limiting in this environment. Second, the complexity of fish communities also changes along the longitudinal profile of rivers (Winemiller and Leslie 1992). Downstream communities tend to be more diverse, which could result in an increase in interspecific competition and predation risk (Magurran and Phillip 2001; Magurran 2005). Although few detailed studies have been done across the freshwater-brackish water interface in

Trinidad, there is evidence that predation pressure increases as one moves downstream (Endler 1995) and piscivorous fish become particularly common and abundant at the freshwater-brackish water interface (Gilliam et al. 1993; Phillip and Ramnarine 2001).

Between 2008 and 2010 we sampled a total of 91 locations (75 independent locations and 4 sites sampled on multiple occasions) throughout Trinidad, representing both fresh- and brackish water habitat (Fig. 3.1). We deliberately sampled multiple locales along each river in order to determine the lowland distribution limits of *P. picta* and *P. reticulata*. Sites close to the freshwater-brackish water interface were visited on more than one occasion. In particular, we sampled two sites, one at the Guayamare River and the other at the Caroni River, in eight different occasions including four times during the wet season (June-January) and four during the dry season (February-May; Fig. 3.1). These sites showed temporal variation in salinity levels, being fresh during the wet season and brackish during the dry season (Table 3.1). We determined presence or absence for the target species at each site by actively sampling the shores of the rivers using butterfly nets. We maintained a consistent sampling effort across all sites searching fish for 30 minutes, despite the presence and absence of species being relatively easy to quantify. When these poeciliids were present, we fished a minimum of 100 fish to determine species composition. We decided to standardize our effort by capturing a constant number of fish instead of maintaining a constant time because rate of capture varied significantly among localities due to factors other than species abundance, such as stream characteristics, slope of the shore, substrate of the channel, or coastal vegetation. All fish were classified as *P. picta* or *P. reticulata*, and subsequently released at their site of capture. At all sample sites we recorded salinity, geographic coordinates and elevation. At a subsample of these sites (n=42), we also recorded dissolved oxygen, and pH using either an YSI 85 or an YSI ProPlus. Before each use, the YSI meter was calibrated for dissolved oxygen and allowed to warm-up following instruction by the manufacturer.

To quantify field differences among localities in life-history traits four different rivers (Caroni, Caparo, Nariva, and Guayamare) were sampled in April 2010. In each river an upstream, midstream and downstream site was selected and 40 – 60 fish of each species (≈1:1 sex ratio) were sampled. These three stream localities represented, a) a freshwater-upstream locality where only *P. reticulata* occurred, b) a freshwater-midstream locality where both species were present, and c) a brackish water-downstream locality where only *P. picta* occurred. However, because there was a very low density of *P. picta* in the Caparo River, we did not sample *P. picta* in this river due to worries that we may negatively impact the population. Upon capture, fish from each locality were immediately euthanized with an overdose of MS-222 and preserved in 5% formalin.

We characterized phenotypic variation in life-history traits of wild populations of both species following Reznick and Endler (1982) and Reznick et al. (1996). Briefly, each formalin-preserved female from our field collection was dissected and scored for number of embryos and stage of development following Haynes (1995). We then removed the gastrointestinal tract from each female and dried the somatic and reproductive tissues, including developing embryos, in a drying oven at 80℃ until mass was stable. From these females we measured: 1) the number of developing embryos, 2) the mean dry mass of developing embryos, and 3) reproductive allocation. Mean dry mass of developing embryos was estimated by dividing the total mass of the brood by the number of embryos. Reproductive allocation was determined as the dry mass of the brood divided by the sum of the dry masses of the somatic tissue and of the brood. Reproductive allocation is thus the proportion of the total dry mass of the mother that consisted of developing embryos, corrected for stage of development. For males, we determined male size at maturity as the average standard length of adult males, given that males have determinate growth with an asymptote around sexual maturity (Reznick 1990; pers. obs.).

To compare condition among localities we used the relative condition index (K_n; LeCren 1951). We estimated relative condition index by species and sex, as both study species are sexually dimorphic (i.e. females are larger than males). Condition is calculated as the intercept (a) and the slope (b) of the least squares regression of log-transformed individual mass (M_i) on log-transformed individual length (L_i) which is used to calculate predicted mass ($M_i{*}=aL_i^b$). The predicted mass was estimated at the drainage level. Thus, it allowed comparisons among populations within drainages but was not confounded by potential genetic differences among drainages. An individual's index is then estimated as the ratio of the observed individual mass to the predicted mass (LeCren 1951; Peig and Green 2010).

Statistical design and analysis

All the analyses were performed separately for *P. picta* and *P. reticulata*, as we do not intend to directly compare the species, but rather test the factors that determine the distribution of each species. The only exception is the comparison between the life-history traits of these two species in the three freshwater sites where they coexist (see below).

Statistical Niche Model - We constructed logistic models based on physicochemical variables (dissolved oxygen, pH, and salinity) and elevation. Elevation is usually used as an indirect variable to the study of distributions as it correlates with several other variables (Austin 2007). In out study, it was preferred as a spatial explanatory variable over distance from the mouth of the river for several reasons. Mainly because the brackish-freshwater interface changes seasonally and the magnitude of this change is associated with elevation, thus it better represents the probability of brackish water intrusion into lowland rivers. In addition, channel size, canopy cover, and community composition gradually change with elevation, potentially affecting the likelihood of finding each species. Furthermore, analyses including distance to the mouth of the river did not change our conclusions.

We considered linear, symmetrical unimodal, or skewed responses of the species to the environmental gradients by including linear, quadratic and cubic polynomial functions in our models. The effect of elevation was only considered linear for *P. picta* because it is expected to be present at sea level but to decrease upstream. Similarly, we consider elevation only linear for *P. reticulata* because it is known to occur at higher elevations than those sampled in this study, thus we are only sampling the lower tail of its distribution. We also included interaction terms between environmental variables (Guisan et al. 2006). However, for *P. reticulata* we did not consider interactions between salinity and other variables because this species occurred only at freshwater sites (see results). Thus, the combination of salinity levels higher than 0.5 ppt and other variables do not exist. Similarly, for *P. picta* we did not consider the interaction between salinity and elevation, because at higher elevation localities water is always fresh. Finally, we included a null model (intercept only) to compare to candidate models. These models were contrasted against data from the subset of sites where all the predictor variables were collected (training data, n= 35). We then tested the predictions of the best-supported models for *P. picta* and *P. reticulata* with data from the subset of sites that were not used to evaluate the models (test data, n= 56 sites).

We used Akaike's Information Criterion adjusted for small sample size (AIC_C) to evaluate the relative importance of competing candidate models for explaining *P. picta* and *P.*

reticulata presence or absence in the lowland rivers of Trinidad (Burnham and Anderson 2002). AIC_C scores decrease if the addition of new parameters increases the fit of the model enough to compensate for the penalty cost of adding these parameters (i.e. parsimony criterion; Burnham and Anderson 2002). We calculated and used Akaike weights (w_i) to rank the candidate models, and report a subset of confidence models including those with at least $1/8*w_i$ of the top model (Burnham and Anderson 2002). Because none of our models was overwhelming supported by our data we used model averaging to determine the importance of the different factors driving the distribution of *P. picta* and *P. reticulata* (Burnham and Anderson 2002; Johnson and Omland 2004). To estimate the parameters of the composite model we used the confidence subset of models. Reducing the number of models to be combined is recommended as removal of a poorly fitted model can increase estimation accuracy (Yuan and Yang 2005). However, including all the candidate models does not affect the results. We calculated the weighted average for model parameters and 95% confidence interval using averaged unconditional standard error for each parameter (Burnham and Anderson 2002). We averaged parameters over all models in the confidence set, setting the parameter *i j* equal to 0 for variable *i* if it was not included in a model *j* (Lukas et al. 2009). When performing the model averaging for the probability of occurrence of *P. picta*, we did not use the model that includes the interaction between elevation and dissolved oxygen, as interactive terms can result in inaccurate estimations of main effect parameters. However, the weight contribution of this model was small (Table 3.2). Finally, we estimated a cumulative Akaike weight to evaluate the overall importance for each parameter by adding the Akaike weights of all the models containing such parameters (Burnham and Anderson 2002).

Spatial Patterns of Intraspecific Life history Variation - We analyzed *P. picta* and *P. reticulata* life history data separately using analyses of covariance (ANCOVA), including locality of origin and drainage as categorical fixed effects. Fecundity in guppies has been shown to increase with female size so we included female standard length as a covariate for determining reproductive allocation, offspring number and offspring size (Reznick et al. 1996). We also included stage of development as a covariate when analyzing embryo weight and reproductive allocation from wild-caught females because offspring weight decreases as development progresses (Reznick and Endler 1982).

Interspecific Phenotypic Variation between P. picta and P. reticulata - We compared *P. picta* and *P. reticulata* life-history traits in sites where they co-occur using analyses of covariance (ANCOVA), including species and drainage as categorical fixed effects. As for the analyses of spatial variation, we included female standard length as a covariate for determining species differences in offspring size, and stage of development as a covariate for analyzing embryo weight.

RESULTS

Statistical Niche Model

Poecilia reticulata – This species was present in 55 of the 91 sites evaluated. We observed some temporal variation in the presence of *P. reticulata* at the two sites that we sampled multiple times (i.e. Caroni and Guayamare Rivers at interface between fresh- and brackish water). We found this species at these sites only when water was fresh, which mostly coincides with the rainy season (Table 3.1). Furthermore, the only factor that appears to differ between sites where *P. reticulata* is presence or absence is salinity (Fig. 3.2).

Water salinity had a negative effect on the probability of finding *P. reticulata*, resulting in an abrupt distribution limit at the interface of fresh- and brackish water (Fig. 3.3). Salinity was present on all the models in the confidence set (Table 3.2), and this variable had a cumulative weight across all models close to 1 (Table 3.3). There is not much evidence for the effects of altitude, dissolved oxygen and pH on the probability of presence of *P. reticulata* (Table 3.3). Therefore, we based our predictions of the presence or absence of *P. reticulata* on the set of independent localities only using the top model, as it accounted for 41% of the total weight (Table 3.2), and salinity was the only significant variable in the composite model (Table 3.3). This model predicted the presence or absence of *P. reticulata* with an overall error rate of 20%. Out of the 32 sites that were predicted to have *P. reticulata* present, it was actually absent at seven localities (Downstream Couva, two rivers on the southwest peninsula in St. Patrick, and four rivers in the northwest part of the island). The model predicted *P. reticulata* to be absent at 22 sites; however, we did find this species in three of those sites, two sites in the Nariva Drainage, and a brackish site at the Caparo River.

Poecilia picta – This species was present in 66 of the 91 sites evaluated, and no temporal variation was observed at the interface of fresh- and brackish water independently of changes on salinity. *Poecilia picta* is found over a wider range of physicochemical characteristics (Fig. 3.2). However, in Trinidad, it appears to be limited to sections of the river at low elevation (Fig. 3.3). Indeed, elevation was present in all the models in the confidence set (Table 3.2), and had a cumulative weight close to 1 (Table 3.3).

Poecilia picta was found at most sites below 10 m above sea level. However, above 10 m the probability of finding this species significantly decreased for every meter of increase in elevation (Table 3.3). There is no evidence for the effects of salinity, dissolved oxygen and pH on the probability of presence of *P. picta* (Table 3.3). As with *P. reticulata,* to predict the presence or absence of *P. picta* at the independent localities we used the top model, as elevation was the only significant variable in the composite model (Table 3.3), and the model with just elevation as an explanatory variable accounted for 35% of the total weight (Table 3.2). This model had an overall error rate of 14%. Of the 41 sites that were predicted to have *P. picta* present, it was actually absent at eight sites (Upstream Caparo, two rivers on the southwest peninsula in St. Patrick, and five rivers in the northwest part of the island). *Poecilia picta* was absent at all sites where it was predicted to be absent.

Spatial Patterns of Intraspecific Life History Variation

Poecilia reticulata – Overall, fish from upstream and downstream localities showed no differences in life history phenotypes. There was a trend for offspring size to be larger in downstream compared to upstream localities, but it was not statistically significant (Table 3.4, Fig. 3.4). Male condition also varied among localities, with fish being in better condition downstream on all rivers, except on the Caroni River where the pattern was reversed (Table 3.4, Fig. 3.5). This tendency for Caroni fish to be in poorer condition downstream was also observed in females (Fig. 3.4). Most of the phenotypic variation observed in *P. reticulata* could be explained by intrinsic differences among rivers (Table 3.4).

Poecilia picta – Intraspecific variation among localities was observed only for offspring size and male size at maturity (Table 3.4). Overall, females from upstream, freshwater sites produced larger offspring than those from downstream, brackish sites (Fig. 3.4). Males were larger in downstream brackish sites than in upstream freshwater sites (Fig. 3.5). Reproductive allocation and female condition depended on the river analyzed and the locality within rivers (Table 3.4). Female reproductive allocation did not vary among localities in the Caroni River, was higher for freshwater females in the Guayamare River, but lower for freshwater females in the Nariva River (Fig. 3.4). Females from the Caroni and the Nariva rivers were in better condition in upstream freshwater sites, but females from the Guayamare River were in poor condition at these sites (Fig. 3.4).

Interspecific Life History Variation

Poecilia picta and *P. reticulata* did not differ in reproductive allocation in sites where they co-occur ($F_{1,128}$=2.1063, P=0.1491), and both species responded similarly within each drainage (Drainage effect: $F_{2,128}$=6.1601, P=0.0028; Interaction: $F_{2,128}$=0.0097, P=0.9903). However, *P. picta* produced, on average, larger offspring than *P. reticulata* when controlling for developmental stage (least squared mean (lsm) ± SE: 0.7730 ± 0.0206 and 0.6650 ± 0.0246 mg respectively; $F_{1,128}$=11.3608, P=0.001). Although the size of the offspring vary among drainages, fish form all drainages showed a similar pattern (Drainage effect: $F_{2,128}$=6.1601, P=0.0028; main effects interaction: $F_{2,128}$=0.0097, P=0.9903; all interactions with the covariate were not significant, P>0.05). Nonetheless, *P. picta* had a lower fecundity than *P. reticulata* when controlling for female size (lsm±SE: 7.1565 ± 0.5064 and 9.4128 ± 0.6131 embryos respectively; $F_{1,126}$ 7.3972, P=0.0075). Again, there was a drainage effect but interspecific patterns were consistent within each drainage (Drainage effect: $F_{2,126}$=3.8991, P=0.0228; main effects interaction: $F_{2,126}$=2.8510, P=0.0615; all interactions with the covariate were not significant, P>0.05). Differences between *P. picta* and *P. reticulata* in the size of mature males varied among rivers. While *P. picta* males were always larger, the difference was more pronounced in the Guayamare drainage than in the other two drainages (Species effect: $F_{2,239}$=361.7872, P<0.0001; Drainage effect: $F_{2,239}$=27.1539, P<0.0001; Interaction: $F_{2,239}$=8.5371, P<0.0003).

DISCUSSION

Poecilia reticulata and *P. picta* show a contiguous distribution pattern in the rivers of Trinidad, with a zone of overlap in the lowland, freshwater sections. Both species occupy a wide range of pH and dissolved oxygen, as previously seen for *P. reticulata* (Magurran and Phillip 2001). However, the factors that appear to limit the distribution of these two species differ. On the one hand, *P. reticulata* is primarily limited to the freshwater sections of the rivers, and the probability of occurring rapidly declines as salinity departs from 0 ppt. The only exception to this pattern was observed in the Caparo River, where *P. reticulata* was found in brackish water. This site was unique because a bridge formed an artificial barrier bridge that resulted in an immediate transition from fresh (0 ppt) to brackish water (7.7 ppt). Although *P. reticulata* was found below the barrier bridge, the species dropped out within the first 50 m of brackish water. In contrast, the distribution of *P. picta* is limited to the lowlands of Trinidad, and the species is rarely found over 10 m above sea level.

The statistical niche models based on salinity and elevation performed relatively well in predicting the presence or absence of *P. reticulata* and *P. picta*, respectively. Most erroneous predictions were false positives (i.e. the species was predicted to be present at localities where it was not observed), a common error of statistical niche models in cases where the realized niche of a species is smaller than its fundamental niche due to biotic interactions or dispersal limitations (Pulliam 2000; Soberón 2007). For example, the false positive observed for *P. reticulata* were mainly associated with sites in the north-eastern part of Trinidad, where the species is uncommon (Magurran and Phillip 2001). Some of these sites might be suitable for guppies, as exemplified by the successful introduction of this species in rivers where it was previously absent (e.g. Gordon et al. 2009). This suggests that the realized niche of *P. reticulata* in Trinidad is smaller than its fundamental niche, probably due to dispersal limitations. Nonetheless, our intention was not to produce an exhaustive description of the ecological niche of either of the studied species, but to determine the range of conditions under which these species co-occur in the lowland sections of the rivers of Trinidad. Furthermore, our sample design is limited in the sense that we might have failed to detect the studied species at sites where they were indeed present. Therefore some of the false positive predicted by our models might have been caused by the limitations of our sample design. However, because the studied species are relatively easy to detect in the field, we think that this factor was unlikely to affect our results in a significant way.

Maintaining water balance is one of the major physiological challenges of aquatic species (Nordlie et al. 1991; Bœuf and Payan 2001). Thus, water salinity is expected to play a fundamental role shaping species distribution. Indeed, empirical studies often find that community composition changes significantly along salinity gradients (e.g. Martin 1988; Gelwick et al. 2001; Jaureguizar et al. 2004; Fuller et al. 2007; Alcaraz et al. 2008). However, these predictions do not necessarily extend to poeciliid fishes, which in general are considered to be euryhaline (Rosen and Bailey 1993), and several species, including *P. reticulata*, show tolerance to gradual changes in salinity (Gibson and Hirst 1955; Zimmerer 1983; Shikano and Fujio 1997). Thus, the abrupt limit to the distribution of this species at the brackish-freshwater interface was unexpected. Two other lines of evidence support the importance of salinity in creating the observed pattern of distribution of *P. reticulata*. First, if the distribution limit of this species were caused by gradual changes in unmeasured factors, we would expect parallel changes in life-history traits. Instead, we observed no variation in life-history traits between upstream and downstream populations, suggesting a rather abrupt, step change in environmental conditions at the distribution limit (i.e. a physiological threshold; Caughley et al. 1988). Alternatively, it could be that the environment does in fact gradually change, but *P. reticulata* is unable to adapt to the local optimum along the gradient because gene flow swamps the ability for local adaptation (García-Ramos and Kirkpatrick 1997). While we cannot rule out this hypothesis, fish were found in better condition in downstream populations compared to upstream populations, thus swamping due to gene flow seems unlikely. A second line of evidence in support of a threshold response comes from the repeated sampling of localities at the interface between brackish and freshwater in the Caroni and the Guayamare Rivers (Table 3.1). We only found *P. reticulata* at these localities during the wet season, when water was found to be fresh (i.e. 0 ppt). Thus, *P. reticulata* appears to track seasonal changes in salinity and behaviorally avoid brackish water. Behavioral avoidance is expected to evolve if the ecological conditions found outside of the distribution limits predictably result in reduced fitness (Holt 2003). For example, behavioral avoidance of low-salinity water in echinoderm larvae is adaptive because mortality rapidly increases as salinity falls below sea water levels (Sameoto and Metaxas 2008). Similarly, behavioral avoidance of brackish water could explain the abrupt distribution limit of *P. reticulata*, providing a proximate explanation to the observed distribution pattern of this species.

There are several factors that could affect *P. reticulata* fitness in brackish water. First, salinity imposes a physiological stress, which can result in reduced survival probability (e.g., Martin 1988; Bringolf et al. 2005), and/or reduced reproduction success (i.e. by affecting fecundity, number of offspring, growth rate, and/or age at maturity; e.g., Trexler et al. 1990; Vasagam et al. 2005). Second, salinity could affect the competitive interaction of *P. reticulata* with other species (Dunson and Travis 1991). For example, the outcome of the competition between species can vary across environmental gradients, thus species exclusion can be restricted to certain localities along a gradient (Fausch et al. 1994; Davis et al. 1998; Taniguchi and Nakano 2000; Case et al. 2005). Thus, condition-specific competition between *P. reticulata* and *P. picta* may produce the observed distributional pattern. This would be consistent with predictions from niche theory, which suggests abrupt changes in species dominance along abiotic gradients that impose physiological stress (Chase and Leibold 2003). Finally, salinity may be correlated with changes in the predator community (e.g. Winemiller and Leslie 1992), or directly affect prey susceptibility to predators (Hulatbduwa et al. 2007; Squires et al. 2008). Theoretical models suggest that one outcome of the increase in predator-induced mortality along environmental gradients is the formation of abrupt limits to the distribution of prey species (Holt and Barfield 2009). Thus, the interaction between salinity and predation risk might explain the distribution pattern observed in *P. reticulata*.

In contrast to *P. reticulata*, we found several lines of evidence suggesting that salinity plays only a minor role on driving the distribution of *P. picta* (Fig. 3.1, Table 3.2). First, *P. picta* occupies a wide range of salinities, as it is commonly found at brackish and freshwater localities. Second, we found no signs of physiological stress in life-history traits or condition index when comparing populations from brackish and freshwater localities (Table 3.4). Finally, the repeated sampling of localities at the brackish-freshwater interface in the Caroni and the Guayamare Rivers also suggest that salinity plays a minor role in driving the distribution of *P. picta*, as we always found this species at these localities independent of the salinity of the water (Table 3.1). Nonetheless, *P. picta* appears to be limited to elevations below 10 m above sea level. We do not think that elevation *per se* directly causes this species' distribution limit; rather we view this as an indirect metric that captures the joint effects of several unmeasured factors that correlate with elevation (e.g. changes in productivity, competition, and predation). Based on the observed increase in offspring size from *P. reticulata*-absent localities to *P. reticulata*-present localities, we suggest a likely role of competition and productivity in this trait (Price and Kirkpatrick 2009).

Larger offspring size is usually associated with increased competition pressure (Reznick 1982; Bashey 2008; Jørgensen et al. 2011), and competition intensity between *P. reticulata* and *P. picta* could be intensify in upstream localities due to the reduced primary productivity caused by increased canopy cover, as has been experimentally observed in Trinidad streams (e.g. Reznick et al. 2001; Zandonà et al. 2011).

A potential role of predation in shaping the distribution limits of *P. picta* is less clear. Reznick et al. (1992) described this species as a guppy adapted to extremely high levels of predation. Indeed, *P. picta* has faster life histories than what is usually observed in guppy populations that experience increased predation pressures (Reznick et al. 1992; this study). However, *P. picta* did not have a faster life history than sympatric *P. reticulata*. In fact, *P. picta* females produced fewer, larger offspring than *P. reticulata* females, and *P. picta* males matured at a larger size than *P. reticulata* males at sites where they co-occur. Thus, we propose that *P. picta* might use a completely different strategy than *P. reticulata* in order to cope with very high levels of predation risk (Chase 1999; Abrams 2000). For example, *P. reticulata* shows tolerance to predation, increasing reproductive output as mortality increases. *Poecilia picta* might instead show an increased ability to escape predators. In other words, *P. picta* might show behaviors that result in lower mortality rates than *P. reticulata* for a given level of mortality risk (Lima and Dill 1990). Preliminary results from our laboratory suggest that *P. picta* might have higher probability of escaping pike cichlids' (*Crenicichla frenata*) strikes than *P. reticulata* (personal observations).

In summary, we describe a pattern of contiguous distribution along an environmental gradient in the rivers of Trinidad for two closely related and morphologically similar species of poeciliid fish, *P. picta* and *P. reticulata*. *Poecilia picta* occupies the estuarine end of the gradient, spanning a wide range of salinities (Fig. 3.3). However, it is also found in the lowland, freshwater sections of rivers, where it co-occurs with *P. reticulata*. On the other end of the gradient, the distribution of *P. reticulata* begins in freshwater localities and extends further upstream than where *P. picta* is found. Our results suggest that different mechanisms might be responsible for the distribution limits of the two focal species. This asymmetric pattern, where different factors are responsible for the replacement of species along a gradient is observed in other species as well. For example, Taniguchi and Nakano (2000) showed that the altitudinal distribution pattern of two charr species of the island of Hokkaido, Japan, is the result of the interaction between thermal tolerance and competitive abilities. The upstream limit to the distribution of the White-spotted Charr (*Salvelinus leucimaenis*) is caused by the low tolerance of this species to low temperatures. However, in the warmer, downstream sections of the streams, it is competitively dominant over the Dolly Varden (*S. malma*), displacing this species to the upstream, coldest section of the streams. By doing population translocations, Angert and Schemske (2005) showed that one species of monkey-flower (*Mimulus lewisii*) that is commonly found at high elevations in the Sierra Nevada Mountains, California, is unable to survive in low elevation conditions. By contrast, a second species of monkey-flower (*M. cardinalis*) that is usually restricted to low elevations is able to survive when transplanted to high elevations, but it fails to complete its life cycle before senescence. Similarly, reciprocal removal experiments reveal that the Virginia's Warbler (*Vermivora virginiae*) is limited to nesting in a warmer and drier microclimate by the presence of the competitively dominant Orange-crowned Warbler (*V. celata*), which occupies less stressful cooler and wetter habitats (Martin and Martin 2001). In turn, this pattern at the local scale provides an explanation to the pattern at the regional scale where Virginia's Warbler is limited to more arid areas than Orange-crowned Warbler. Similarly, our results suggest that while salinity limits *P. reticulata*, the limits to the distribution of *P. picta* could be driven by interspecific competition. In addition, trade-offs between competitive abilities and predator escape performance between *P. reticulata* and *P. picta* need to be explored as such trade-offs have the potential to explain the coexistence of these two species at the lowland, freshwater sites. Consistent with previous work, our survey of *P. picta* and *P. reticulata* suggest that these two species show a parapatric distribution at regional scales as the result of different processes affecting each species at local scales.

TABLES AND FIGURES

Table 3.1. Temporal variation on salinity and of *P. picta* and *P. reticulata* occurrence at the interface between fresh and brackish water in the Caroni and the Guayamare Rivers.

		Guayamare River W10.59609, W61.42141			Caroni River N10.61813, W61.42884		
Date (mm/yyyy)	Season	Salinity (ppt)	*P. picta*	*P. reticulata*	Salinity (ppt)	*P. picta*	*P. reticulata*
05/2008	Dry	**2**	**Present**	**Absent**	**5**	**Present**	**Absent**
10/2008	Wet	0	Present	Present	0	Present	Present
01/2009	Wet	**10**	**Present**	**Absent**	**1.5**	**Present**	**Absent**
03/2009	Dry	**5**	**Present**	**Absent**	0	Present	Present
07/2009	Wet	0	Present	Present	0	Present	Present
04/2010	Dry	**40.6**	**Present**	**Absent**	**2.7**	**Present**	**Absent**
08/2010	Wet	0	Present	Present	0	Present	Present
02/2011	Dry	0	Present	Present	**2**	**Present**	**Absent**

Table 3.2. Confidence set of candidate models for predicting the presence or absence of Poecilia reticulata and P. picta in lowland rivers of Trinidad. Explanatory variables include elevation (E), salinity (S), dissolved oxygen (DO), and pH.

Response Variable	Model	*K*	AIC_C	ΔAIC_C	w_i
P. reticulata presence	S	4	17.3	0	0.41
	E + S	5	18.9	1.6	0.18
	S + DO	5	19.7	2.4	0.13
	S + pH	5	19.7	2.4	0.12
	E + S + pH	6	21.5	4.2	0.05
	E + S + DO	6	21.5	4.2	0.05
P. picta presence	E	4	17.7	0.0	0.35
	E + S	5	19.5	1.7	0.15
	E + pH	5	19.7	2.0	0.13
	E + DO	5	20.1	2.4	0.11
	E * DO	6	21.0	3.3	0.07
	E + S + pH	6	21.5	3.7	0.05
	E + S + DO	6	21.9	4.1	0.04

Table 3.3. Model-averaged parameter estimates, their unconditional standard errors (SE), and 95% confidence intervals (95% CI) for a composite model for predicting the presence or absence of Poecilia reticulata and P. picta in lowland rivers of Trinidad. Explanatory variables include elevation (E), salinity (S), dissolved oxygen (DO), and pH.

Response variable	Parameter	Estimate	SE	95% CI		Cumulative
				Lower	Upper	w_i
P. reticulata presence	E	0.043	0.117	-0.186	0.273	0.299
	S	-25.310	11.718	-48.276	-2.343	0.999
	pH	0.024	1.212	-2.351	2.399	0.225
	DO	0.012	0.288	-0.554	0.577	0.225
P. picta presence	E	-0.496	0.211	-0.909	-0.082	0.999
	S	0.736	4.507	-8.099	9.570	0.277
	pH	-0.413	1.707	-3.759	2.933	0.275
	DO	0.005	0.207	-0.401	0.411	0.268

Table 3.4. Statistical results (*F*-values) for ANCOVA on fecundity, reproductive allocation, offspring mass (mg), and relative condition for wild caught females, and relative condition and size at maturity for wild caught males.

	df	Fecundity	Reproductive allocation	Offspring size	Female Relative condition	Male Relative condition	Male size
Poecilia picta							
Environment	1	1.4459n.s	0.3303n.s.	6.9427**	1.1689n.s.	0.9658n.s.	20.2088***
Drainage	2	0.2690n.s	4.2127*	2.5854n.s	5.0518**	1.5958n.s.	18.4793***
Interaction (ExD)	2	0.0266n.s.	5.1522**	2.5968n.s.	8.9220***	0.4300n.s.	2.5640†
Size	1	161.7221***	—	—	—	—	—
Stage of development	1	—	11.5670***	19.1479***	—	—	—
Residual sums of squares (df)		1818.1369 (135)	0.2823 (135)	2.6893 (135)	1.5019 (202)	1.6416 (181)	293.1722 (187)
Total sums of squares (df)		4221.3873 (141)	0.3596 (141)	3.3836 (141)	1.7211 (207)	1.6934 (186)	374.9372 (192)
R^2		0.57	0.21	0.21	0.13	0.03	0.22
Poecilia reticulata							
Environment[1]	1	0.2129n.s.	0.4925n.s.	2.9341†	2.5386n.s.	9.0961**	1.5586n.s.
Drainage	3	1.4272n.s.	2.9081*	7.9291***	10.6228***	18.1584***	33.5252***
Interaction (ExD)	3	0.8722n.s.	1.3387n.s.	1.7569n.s.	2.4851†	16.0236***	1.0706n.s.
Size	1	151.4042***	—	—	—	—	—
Stage of development	1	—	4.1345*	14.4071***	—	—	—
Residual sums of squares (df)		1745.5382 (141)	0.3535 (133)	0.1.9129 (133)	1.8831 (220)	2.4982 (227)	209.8810 (235)
Total sums of squares (df)		4062.5933 (149)	0.4021 (141)	2.7711 (141)	2.3422 (227)	3.4691 (234)	314.7129 (242)
R^2		0.57	0.12	0.31	0.20	0.28	0.33

† 0.1> *P* >0.05; * 0.05> *P* >0.01; ** 0.01> *P* >0.001; ****P* <0.001

[1] Environment refers to differences between up- and downstream locales for the two study species.

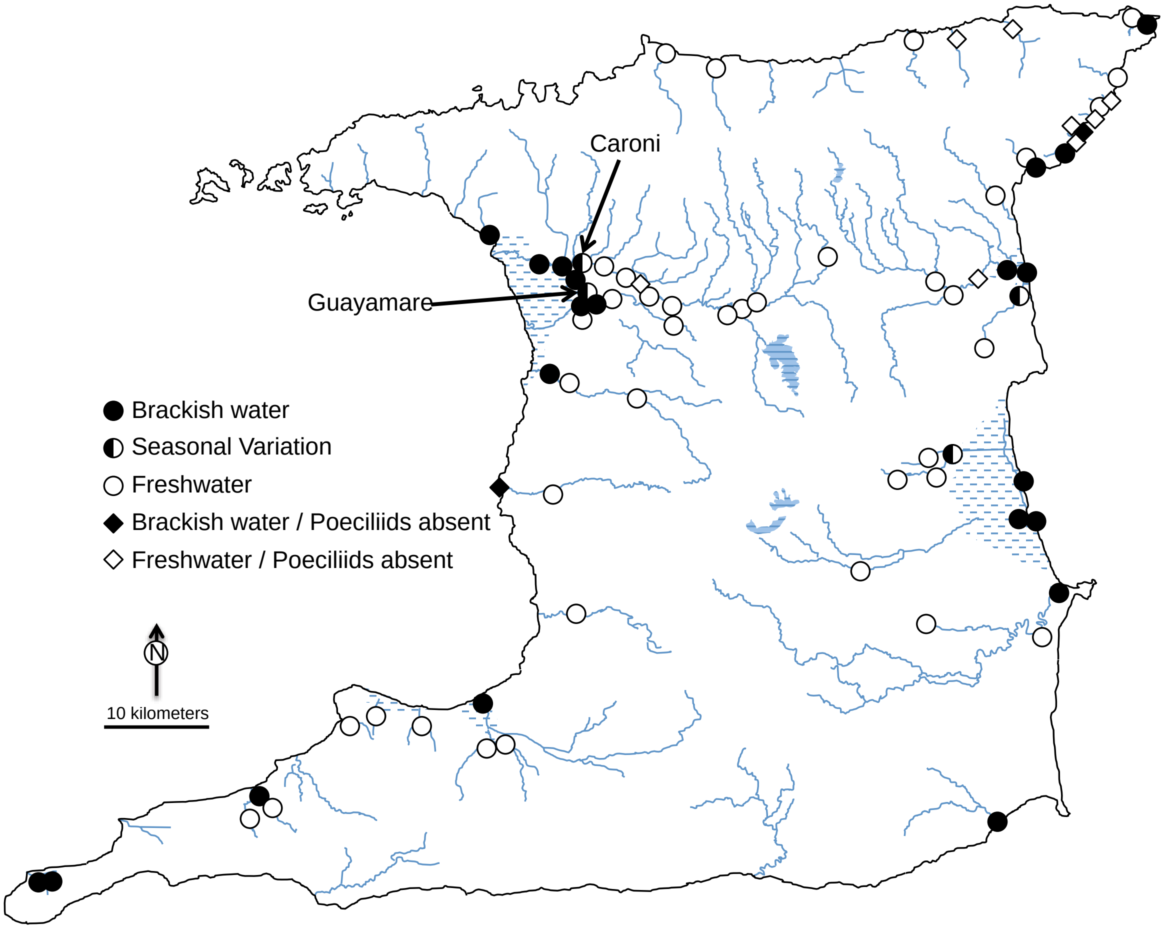

Figure 3.1. Map of Trinidad indicating the position of the sampled locations. Black dots indicate localities where water was brackish when it was surveyed, and white dots indicate localities where water was fresh. Localities where seasonal variation was observed are marked by a divided dot. The localities at the Caroni River and the Guayamare River indicated in the map were sampled on eight different occasions (four occasions on the wet season and four on the dry season).

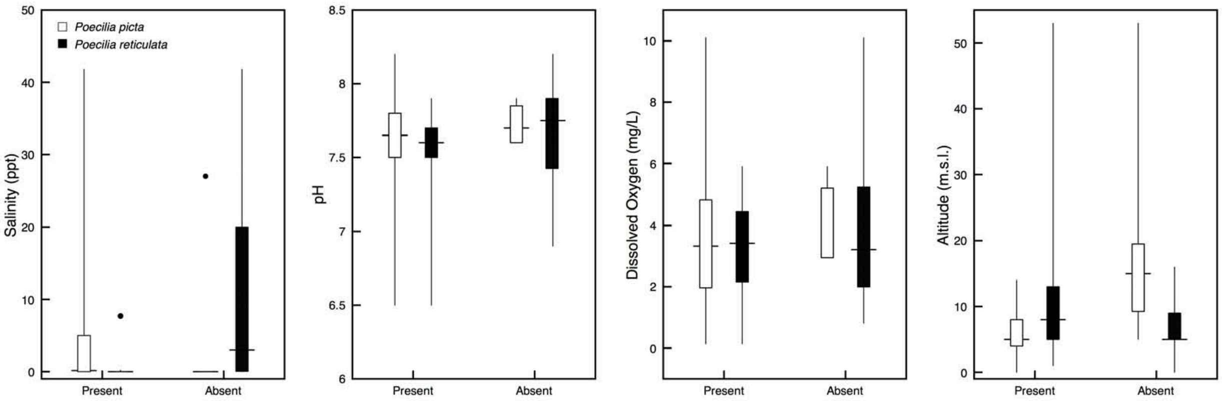

Figure 3.2. Median, interquartile range, total range of salinity, pH, dissolved oxygen, and elevation at which *P. picta* and *P. reticulata* are present or absent in lowland rivers of Trinidad. Two outliers are shown for salinity.

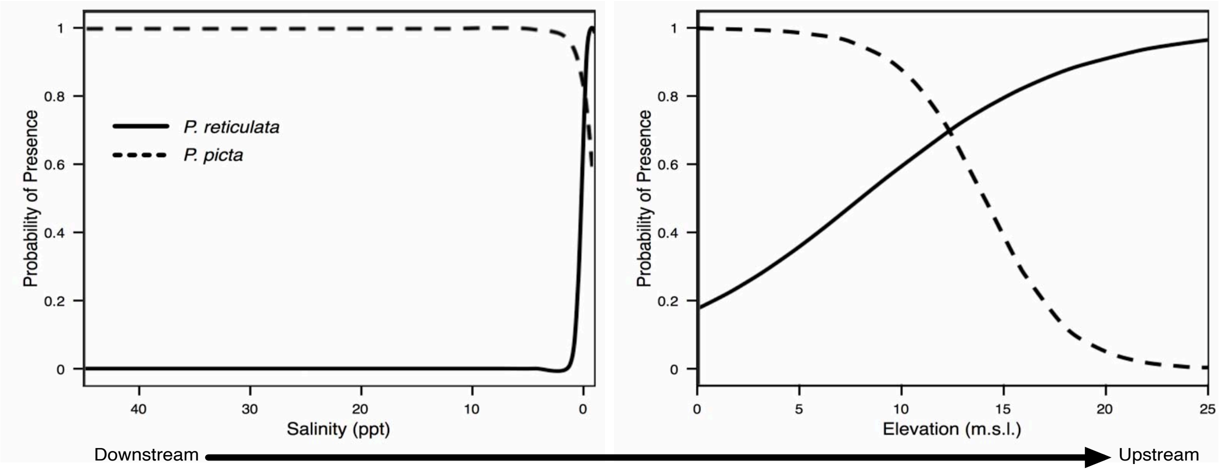

Figure 3.3. Probability of presence curves for P. reticulata (solid lines) and P. picta (dashed lines) against salinity (left panel) and elevation (right panel). Notice that the axis of salinity is reversed.

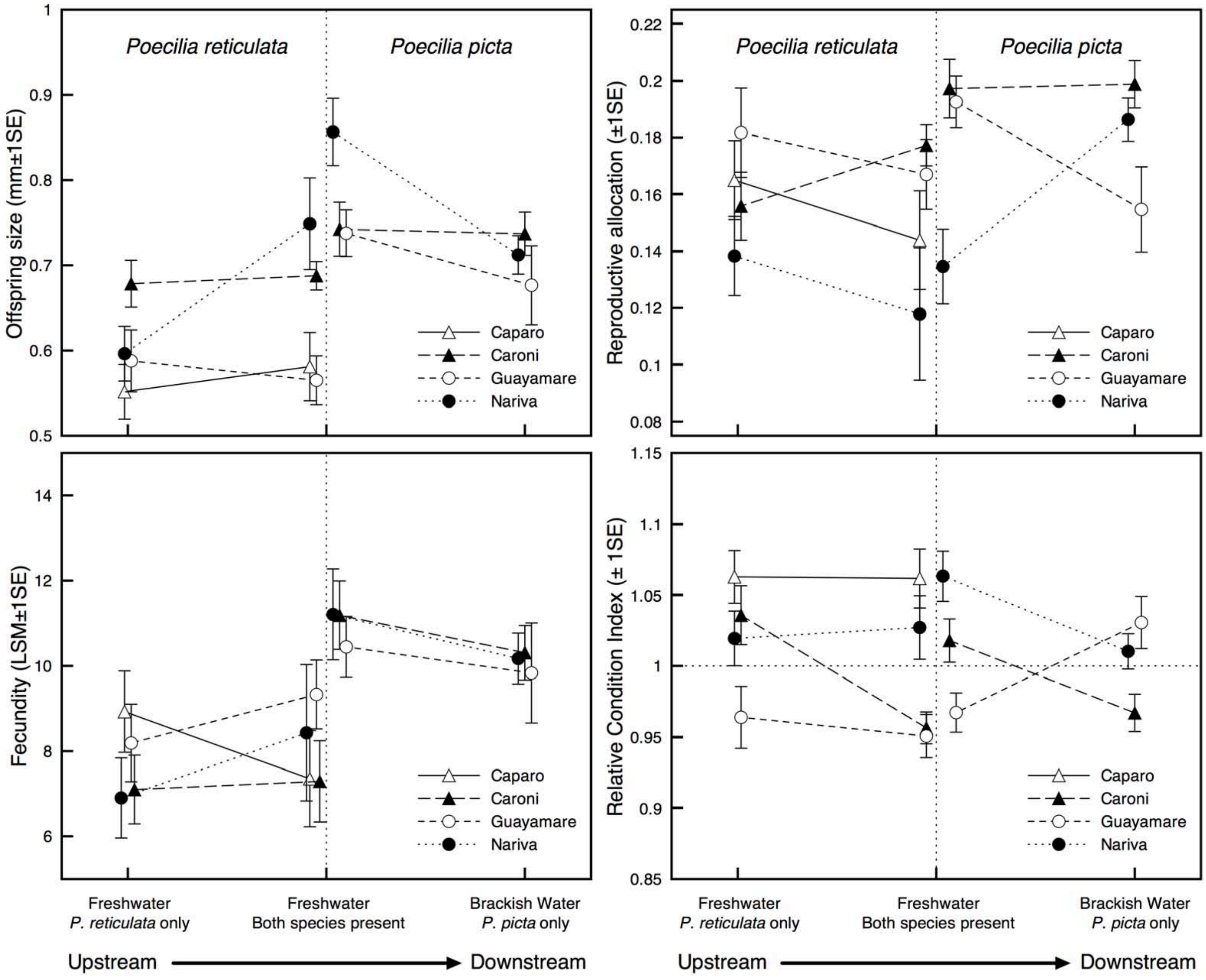

Figure 3.4. *Poecilia reticulata* and *P. picta* females' phenotypic variation along longitudinal gradients along lowland rivers in Trinidad. Localities on the left represent *P. reticulata* only freshwater sites, localities on the right are *P. picta* only, brackish water sites; and the localities in the middle are freshwater sites where both species co-occur.

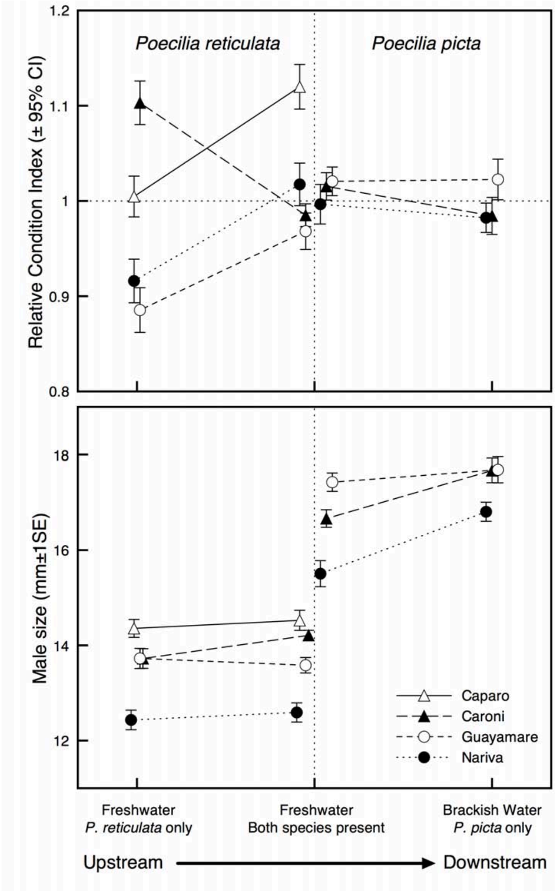

Figure 3.5. *Poecilia reticulata* and *P. picta* males' relative condition and size at maturity along lowland rivers in Trinidad. Localities on the left represent *P. reticulata* only freshwater sites, localities on the right are *P. picta* only, brackish water sites; and the localities in the middle are freshwater sites where both species co-occur.

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CHAPTER 4:

HOW ASYMMETRIC COMPETITION AND SALINITY TOLERANCE EXPLAIN THE PARAPATRIC DISTRIBUTION OF TWO POECILIID SPECIES ACROSS A RIVER GRADIENT

SUMMARY

Understanding how ecological factors influence the turnover of species along environmental gradients is one of the main goals of ecology and biogeography. Traditionally, discussions about the factors responsible for species turnover were centered on the relative roles of biotic versus abiotic factors. However, there is a growing recognition that both biotic and abiotic factors likely interact to shape species distribution limits. Here, we combined a series of laboratory and field experiments to explore how salinity and interspecific competition interact to determine the parapatric distribution of *Poecilia reticulata* and *P. picta* along the brackish-freshwater ecocline in the lowland rivers of Trinidad. Our results suggest that brackish water is an osmotically challenging environment for both studied species. However, the effect of salinity in individual growth rate was more marked in *P. reticulata* than *P. picta*. Furthermore, *P. reticulata* in brackish water had reduced survival compared to freshwater, whereas *P. picta* had similar survival across both brackish and freshwater. It appears that these stressful conditions have selected for behavioral avoidance in *P. reticulata*, as this species exhibited a strong preference for freshwater and avoidance of brackish water. By contrast, we found asymmetric competition as *P. reticulata* is competitively dominant over *P. picta* independently of the salinity level at which competition was tested. Thus, we suggest that asymmetric competition limits the competitively subordinate, but more salinity tolerant, *P. picta* to the harshest end of the salinity gradient, and behavioral avoidance of stressful salinity conditions limits the dominant competitor *P. reticulata* to the mildest end of the gradient.

INTRODUCTION

Understanding how ecological factors influence the turnover of species along environmental gradients is one of the main goals of ecology and biogeography (Clements 1916; Gleason 1926; MacArthur 1972; Holt 2003). However, the relevant factors limiting distributions are only known for a handful of species, and for most species little is known about the ecological and evolutionary processes that shape geographic ranges (Sexton et al. 2009). Arguably, the slow pace of accumulation for conclusive empirical evidence for the processes that limit the distribution of species is due to the complexity of biological systems (Hilborn and Stearns 1982). Historically, discussions about the factors responsible of species turnover along gradients were centered on the relative roles of biotic versus abiotic, which frequently were treated as mutually exclusive (e.g. Clements 1916 versus Gleason 1926 views). Early classic studies focused exclusively on distribution limits caused by abiotic factors such as temperature or precipitation (Griggs 1914; Dahl 1951). Later, with the development of ecological theory and the emphasis on species interactions (Hutchinson 1957; MacArthur 1972), interspecific competition gained importance as a mechanism to explain species distributions (Terborgh 1971; Bowers and Brown 1982). However, there is a growing recognition that both biotic and abiotic factors likely interact to shape species distribution limits (Dunson and Travis 1991; Davis et al. 1998; Taniguchi and Nakano 2000; Martin and Martin 2001). For example, experimental studies using microcosms had shown that in fruit flies (*Drosophila* spp.) the optimum temperature for population growth and the range of temperatures at which populations are viable depends on the presence or absence of congeneric species and the identity of such species (Davis et al. 1998).

The study of parapatric distributions provides the unique opportunity to explore how abiotic and biotic factors interact in the formation of distribution limits (Bull 1991). A parapatric distribution refers to the distribution of two, often closely related, ecologically similar species that have separate, but contiguous distributions with or without a zone of overlap. Hybridization in the zone of contact can result in the formation of stable parapatric distribution in otherwise homogeneous environments (Barton 1979), but more often, parapatric distributed species replace each other along environmental gradients (Heller 1971; Terborgh 1971; Chappell 1978; Fausch et al. 1994; Taniguchi and Nakano 2000; Martin and Martin 2001). The understanding of the mechanisms maintaining ecological parapatry has wide ranging implications for ecological and evolutionary studies (Bull 1991), because of the insights into the processes driving community composition (e.g. Connell 1975) and the process of speciation (e.g. Doebeli and Dieckmann 2003; Fuller et al. 2007).

Estuarine environments provide an excellent system for the study of species turnover along environmental gradients. Traditionally, estuaries were considered just the ecotone between freshwater and marine ecosystems; however, more and more evidence is accumulating that suggest estuaries are complex ecoclines where several factors continuously vary (Attrill and Rundle 2002). The principal abiotic factor changing along the gradient is salinity, which gradually increases as distance from the ocean decreases. Species diversity predictably changes along a salinity gradient, usually decreasing from freshwaters to oligohaline waters due to the loss of freshwater species, but then rapidly increases due to the addition of marine species (Winemiller and Leslie 1992; Attrill and Rundle 2002; Whitfield et al. 2012). Thus, not only does salinity vary along the gradient, species also experience changes in community composition. Thus, parapatric distributions along estuaries can be the result of the interaction of several factors changing along this gradient.

Here we evaluate potential mechanisms responsible for the distribution along a salinity gradient of two closely related livebearer fishes from the island of Trinidad, West Indies. *Poecilia picta* and *P. reticulata* show an overlapping parapatric distribution along the interface between brackish-freshwater in the lowland rivers of Trinidad. Although both species are considered euryhaline (Gibson and Hirst 1955; Rosen and Bailey 1963; Zimmerer 1983; Shikano and Fujio 1997), only *P. picta* is found in brackish environments of Trinidad (Torres-Dowdall 2012). This species is also found in the lowland, freshwater sections of the rivers, where it co-occurs with *P. reticulata*. On the other end of the gradient, *P. reticulata* extends its distribution further upstream than *P. picta*, but it is only found at freshwater localities, dropping abruptly at the brackish-freshwater interface (Torres-Dowdall 2012). Previous work has suggested that several mechanisms could be responsible for the formation of this pattern, emphasizing the roles of behavioral avoidance, osmotic stress due to salinity changes, and condition-specific competition (Torres-Dowdall 2012).

By combining a series of laboratory and field experiments, our main goal is to test the relative roles of different mechanisms that could give rise to the formation of the parapatric distribution pattern. Specifically, we conducted four experiments to test the likelihood of salinity, behavior, and competition contributing to the formation of the observed distribution pattern in the studied species. First, using a Y-maze design, we tested the hypothesis that the abrupt drop of *P. reticulata* at the brackish-freshwater interface is due to behavioral avoidance of brackish water. Second, we used a laboratory experiment to test the effect of osmotic stress by comparing individual growth rate of fish reared in brackish vs. freshwater and at different food levels. Third, we used a similar design to test for intra- and interspecific competition. Finally, we used a field enclosure experiment to test for the roles of osmotic stress and condition-specific competition as drivers of the distribution pattern.

MATERIALS AND METHODS

Experiment 1: behavioral avoidance of brackish water in Poecilia reticulata.

We measured the preference of wild-caught *P. reticulata* for brackish or freshwater using a Y-maze design (Barnett 1977). This experiment gave fish the option to swim either towards brackish or freshwater collected from the river from which the experimental fish were collected. The most important difference between these water sources was the difference in salinity (0–0.23 ppt versus 17–23 ppt). Variation on other physicochemical variables was not as pronounced as variation in salinity (Freshwater: T=28.2°C, DO=42%, pH=7.75; Brackish water: T=29.6°C, DO=51%, pH=7.19); however, other unmeasured factors might co-vary with salinity (see below). Each trial consisted of placing an individual in the base arm of the Y-maze filled with freshwater for a 10-minute acclimation period. After this period, water was simultaneously released into the two response arms, one with freshwater and the other with brackish water (side selected randomly). A drain in the base arm maintained a linear flow from the response to the base arm. A fish was considered to choose a site when it left the based arm and stayed in the selected arm for at least two minutes. Data were analyzed using a Pearson's X^2 test.

Experiment 2: effect of salinity on individual growth rate

We conducted a laboratory experiment to test the effect of salinity and food level on individual growth rate. We collected 20-25 gravid females of *Poecilia picta* and *P. reticulata* from sympatric populations in the Caroni River and transported them to the laboratory at Colorado State University where they were individually housed in 10-liter tanks and kept on a 12:12 light cycle and at 25 ± 1ºC. We propagated fish for two generations in the lab in order to minimize maternal effects. G2 family lines were generated by randomly crossing lab-born fish within each generation. To minimize the influence of maternal effects on our design, G1 litters were split into the two salinity levels used in this study (0 ppt and 20 ppt), thus experimental G2 fish were born at the salinity in which they were tested. Within 24 hours post parturition, three G2 per litter were weighed and each assigned to one of three food levels. The middle food levels equals that reported previously by Reznick (1982). For the two other food levels we provided twice as much or half as much as the intermediate food level. Thus, six fish represented each family line, each assigned to one of six possible treatments resulting from the combination of two salinity levels (0 ppt and 20 ppt) and three food levels. We estimated growth rate by weighing fish at birth and then again 28 days later when they were four weeks old. We measured specific growth rate as $SGR=(LN\ (Mass_{week\text{-}4}*Mass_{birth}^{-1})*days^{-1}*100)$. We analyzed the date using a mixed model ANOVA with Species, Salinity and Food levels as fixed effects; and Family, and its interactions with Salinity and Food levels as random effects. Non-significant higher order terms were eliminated from the final model. Analyses were done in JMP 9, tests were two-tailed, and results are presented as mean ± s.e.

Experiment 3: the interactive effects of salinity and competition on individual growth rate

We followed a similar protocol for our competition experiment in the laboratory as the one described above for our second experiment, with two exceptions. First, all fish were reared at the highest food level. We decided to use this food level because differences between species and salinities were the lowest (see results, Fig. 4.1a), thus by minimizing the direct effect of salinity we increased our power to see the effect of competition and its interaction with salinity. The second difference is that fish were reared in one of three competition treatments (Underwood 1986): (a) none (i.e. fish were alone in the tank), (b) intraspecific competition (i.e. two individuals of the same species in the same tank), and (c) interspecific competition (i.e. one fish of each species in the same tank). As before, all fish were weighed at birth and then again 28 days later when they were four weeks old. We measured specific growth rate as described above. Analyses were conducted as described above for Experiment 2, except that Competition replaced the effect of Food levels.

Experiment 4: field enclosure translocation.

To determine if *P. picta* and *P. reticulata* were maladapted to environmental conditions outside their distribution along rivers of Trinidad, we set replicated enclosures at a *P. picta*-only site (Madame Espagnol River, salinity 15–20 ppt) and at a *P. reticulata*-only site (Guayamare River, salinity < 0.5 ppt), and compared weekly proportion of surviving fish. At each site, we had two treatments per species: one treatment simulated an established population subject only to intraspecific interactions (i.e. 100% conspecifics), and the other simulated an invasion of a habitat occupied by the other species and the ensuing interspecific interactions (i.e. 25% conspecific, 75% heterospecific). These two treatments were replicated six times per species per site (2 species x 2 environments x 2 competition treatments x 6 replicates = 48 enclosures).

We set a total of eight fish per 6.5 l cylindrical enclosure (diameter = 18 cm, height = 25 cm), approximately the natural density at these sites (personal observation). We collected *P. reticulata* from a freshwater site, and *P. picta* from a brackish site, both along the Guayamare River. Fish were brought to the laboratory, where they were measured, weighted, and individually marked using elastomer implants (Northwest Marine Technologies, Inc.). Each fish was randomly assigned to one treatment and locale, and gradually acclimatized to the target salinity. Enclosures were checked weekly, surviving fish were measured and weighed, and dead fish were replaced with previously marked fish held in either a brackish or a freshwater tank.

Probability of survival was analyzed using the Cormack-Jolly-Seber (CJS) model in Program MARK (White and Burnham 1999), setting probability of recapture to 1. The advantage of MARK is that it allows comparison of survival among species, treatments and sites controlling for weekly differences and when the fish were set into the enclosure (i.e. surviving fish or replacement). Model selection methods based on Akaike's Information Criterion for small sample sizes (AIC_C) were used to evaluate a set of candidate models that included the additive and interactive effects on survival of Species, Competition, and Locale (i.e. fresh- versus brackish water sites); including the possibility of weekly variation in survival. We determined the relative strength of each model by comparing the AIC_C values between each model and the best model (ΔAIC_C). Then, we calculated and used Akaike weights (w_i) to rank the candidate models, and report a confidence set of models by including those with at least 10% w_i of the top model (Burnham and Anderson 2002).

RESULTS

Experiment 1: behavioral avoidance of brackish water in **Poecilia reticulata**

We found significant evidence that wild-caught *P. reticulata* actively avoids brackish water. Fish placed inside the Y-maze experiment exhibited a strong preference for freshwater and an avoidance of brackish water ($\text{Probability}_{\text{(choosing freshwater)}}=0.76$, standard deviation= 0.07, Pearson's $\chi^2=8.77$, $P=0.03$), independently of the sex of the fish tested ($\chi^2=0.02$, $P=0.9$).

Experiment 2: effect of salinity on individual growth rate

Both *P. reticulata* and *P. picta* had reduced growth rate in brackish water compared to freshwater (Salinity effect: $F_{1,\,59.60}=20.17$, $P<0.0001$; Fig. 4.1). However, the effect of salinity was more marked in *P. reticulata* than *P. picta*, resulting in a significant Species x Salinity interaction (Species effect: $F_{1,\,48.31}=1.94$, $P=0.17$; Species x Salinity: $F_{1,\,59.96}=4.12$, $P=0.047$; Fig.

4.1a). As expected, growth rate decreased as food level decreased, but, independent of the species, this change in growth rate depended on the salinity at which fish were reared (Food level effect: $F_{2,\ 69.72}=427.85$, $P<0.0001$; Food level x Salinity: $F_{2,\ 66.23}=16.11$, $P<0.0001$; all other interactions $P>0.05$). At the highest food level, salinity had no effect on growth rate, but its effect increased as food level decreased (Fig. 4.1). Thus, growth rate in both species was the lowest in brackish water at the lowest food level. Different families responded differently to the salinity effect, explaining almost 50% of the total variance observed (v.c.=0.36±0.11SE; all other estimates of variance component overlapped zero).

Experiment 3: the interactive effects of salinity and competition on growth rate

Poecilia reticulata appears to be competitively dominant to *P. picta*, as the effect of interspecific competition on growth rate was asymmetric (Species effect: $F_{1,\ 59.36}=25.68$, $P<0.0001$; Competition effect: $F_{2,\ 72.55}=158.50$, $P<0.0001$; Species x Competition: $F_{2,\ 72.55}=71.51$, $P<0.0001$; Fig. 4.2). Whereas intraspecific competition had a stronger negative effect in the growth rate of *P. reticulata* (Fig. 4.2a), interspecific competition reduced growth rate in a higher degree in *P. picta* (Fig. 4.2b). Salinity interacted with competition by increasing its intensity (Salinity effect: $F_{1,\ 55.99}=23.25$, $P<0.0001$; Competition x Salinity: $F_{2,\ 70.07}=5.42$, $P=0.007$; all other interactions $P>0.05$). Both, inter- and intraspecific competition reduced growth rate in both species, but its effect was stronger in brackish water than in freshwater. This effect was consistent in both species as the three-way interaction was not significant. Around 20% of the variation in our data was explained by differences among families or by the different responses of families to salinity and competition, but the 95% confidence intervals for all the terms overlapped zero.

Experiment 4: Field enclosure translocation

When only kept with conspecifics, *P. reticulata* in brackish water had reduced survival compared to freshwater (Fig. 4.3a), whereas *P. picta* had similar survival across both brackish and freshwater (Fig. 4.3b, Table 4.1 and 4.2). Interspecific competition had a similar effect on the survival of *P. picta* and *P. reticulata* (Table 4.2). At the brackish site, competition between species resulted in survival dropping relative to enclosures that contained only conspecifics. However, in freshwater survival did not differ between enclosures with only conspecifics and those with heterospecifics (Fig. 4.3). We also observed weekly variation in survival, but this variation was not consistent between species or treatments (Table 4.1, Fig. S4.1).

DISCUSION

All species in nature have a limited geographic distribution. However, despite a long history of study, we know the relevant factors that limit distribution for only a handful of species (Sexton et al. 2009). Moreover, a general message from those species for which we have a better understanding of the factors limiting their distribution is that simple patterns usually have complex explanations, with biotic and abiotic factors interacting (e.g. Randall 1982; Fausch et al. 1994; Taniguchi and Nakano 2000; Geber and Eckhart 2005). Therefore, studies using a combination of approaches to determine distribution limits are more likely to be informative. Here we used such an approach to study the distribution limits at the drainage scale of two contiguously distributed poeciliid species on the island of Trinidad. We show that abiotic and biotic factors interact to affect the distribution of these species, with salinity being a more important limiting factor for *P. reticulata* and competition playing a larger role in limiting *P. picta*. Below, we first discuss the factors limiting *P. reticulata*'s distribution, emphasizing the role of salinity; second, we discuss the factors limiting *P. picta*'s distribution, emphasizing the role of competition; and finally, we discuss our results in the context of other studies of parapatric distributions along environmental gradients.

***Downstream distribution limits of* Poecilia reticulata**

Our Y-maize experiment results show that *P. reticulata* fish have the capacity to detect and avoid brackish water. This behavior could provide a mechanism explaining the abrupt limit to the distribution of *P. reticulata* at the brackish-freshwater interface. The evolution of behavioral avoidance of osmotically stressful conditions appears to be a frequent response in mobile aquatic species inhabiting haloclines, and it has been observed to play an important role in the distribution limits of several species, including crustaceans (e.g. Jury et al. 1994; Roberts et al. 2008), gastropods (e.g. Roberts et al. 2008), echinoderms (e.g. Sameoto and Metaxas 2008), and fish (e.g. Tosi et al. 1988; Scott et al. 2008). Yet, behavioral avoidance provides only a proximate explanation to the distribution of *P. reticulata*, and in theory salinity should be negatively correlated with fitness for this behavior to evolve (Holt 2003). For example, behavioral avoidance of low-salinity waters in echinoderm larvae is adaptive because mortality rapidly increases as salinity falls below sea water levels (Sameoto and Metaxas 2008).

Here, we found several lines of evidence showing that brackish populations of *P. reticulata* have reduced fitness compared to freshwater populations. First, our growth rate experiment shows that coping with increased salinity is physiologically costly for *P. reticulata*, because fish grew at a slower rate in brackish water than they did in freshwater (Fig. 4.1a). Second, in our field enclosure experiment we found that *P. reticulata* survival decreased in the brackish water locale compared to the freshwater locale (Fig. 4.3a). Thus, even though *P. reticulata* can be acclimatized to brackish water (Gibson and Hirst 1955; Zimmerer 1983;

Shikano and Fujio 1997), it is osmotically challenged in brackish water, with consequences for growth and survival, and potentially for other fitness-related traits.

In addition, for *P. reticulata*, our laboratory competition experiment showed that the intensity of both intra- and interspecific competition, as measured by decreased growth rate, increases in brackish water in comparison to freshwater (Fig. 4.2a). Additionally, the lowest probability of survival in *P. reticulata* was observed in the field transplant treatment that simulated its invasion into brackish water environments where *P. picta* was numerically dominant (i.e. 25% conspecific-75% heterospecific). On the other hand, the presence of *P. picta* did not affect *P. reticulata*'s survival in freshwater, suggesting that competition between these two species is influenced by the specific salinity conditions (Fig. 4.3a). The role of condition-specific competition in shaping species distributions has received substantial empirical support (Dunson and Travis 1991; Davis et al. 1998; Taniguchi and Nakano 2000). For example, Alcaraz et al. (2008) show that a competitive advantage in brackish water favors a native fish (*Aphanius fasciatus*) and limits the distribution of an invasive livebearer fish (*Gambusia holbrooki*) to freshwater sites in Spain. Such results mimic the results observed here, as it appears that condition-specific competition with *P. picta* might contribute to the restriction of *P. reticulata*'s distribution to the freshwater sections of Trinidadian rivers.

***Upstream distribution limits of* Poecilia picta**

We predicted that the distribution of *P. picta* would not be limited by salinity, as it is commonly found occupying a high range of salinities, from freshwater to salinities that exceed marine water (Torres-Dowdall 2012), and expected better performance of this estuarine species in brackish water compared to freshwater. Surprisingly, we found slower growth rates in brackish water, especially at the lower food levels, suggesting that living in brackish water could be physiologically costly for *P. picta* (Fig. 4.1b). However, in our field enclosure experiments we did not see this cost. *Poecilia picta* survival in brackish was as high, or higher, than survival in freshwater (Fig. 4.3b). One explanation that could reconcile the results from the lab and field is that brackish water sites in nature have an abundance of food that ameliorates any physiological costs, or it may be that salinity has different effects on growth versus survival. The response to salinity in other estuarine poeciliids also shows diverse responses depending on the population analyzed (Stearns and Sage 1980), the trait analyzed (Martin et al. 2009; Martin and Leberg 2011), or temporal variation (Trexler and Travis 1990a; Trexler et al. 1992). Moreover, other factors covaring with salinity have been shown to complicate the interpretation of the effects of salinity in phenotypic variation (Trexler and Travis 1990b).

Our results suggest that competition with *P. reticulata* provides a better explanation for the formation of *P. picta*'s distribution limits. Interspecific competition has long been considered a fundamental factor in the determination of community composition and distribution limits at different geographical scales (MacArthur 1972; Tilman 1982; Chase and Leibold 2003; Case et al. 2005). Our laboratory competition experiment shows that *P. picta* is subordinate to *P. reticulata*, independently of the salinity at which competition was tested (Fig. 4.2b). This conclusion is reinforced by our enclosure experiment where we observed an overall reduction on *P. picta* survival in the presence of *P. reticulata* in both brackish and freshwater environments (Fig. 4.3b). *Poecilia picta* being subordinate to *P. reticulata* would be in agreement with patterns of variation in life history traits observed in the wild. Embryo size, a trait associated to competition pressure (Bashey 2008), increases in populations sympatric with *P. reticulata* compared to allopatric populations usually found in brackish water (Torres-Dowdall 2012).

Furthermore, as one moves upstream, productivity decreases as canopy cover increases (Reznick et al. 2001; Zandonà et al. 2011), which would result in increased competition.

Theoretical models considering the effects of competition and gene flow suggest the formation of evolutionary stable parapatric distributions along gradients of productivity, with the species occupying the less productive end of the productivity gradient also showing a wider distribution (Case and Taper 2000). This prediction matches the observed distribution of our studied species, with *P. picta* occupying only the lower, more productive sections of Trinidadian rivers, and *P. reticulata* occurring widely in the less productive sections (Torres-Dowdall 2012). These models also suggest that in the absence of *P. reticulata* we should see an upstream expansion of *P. picta*. Although we currently lack these data, this hypothesis could be tested on the northeastern rivers of Trinidad, where *P. reticulata* is rare (Magurran and Phillip 2001). Note that although the opposite pattern of *P. reticulata* occupying brackish water in the absence of *P. picta* is also predicted from theory, it is not expected in nature based on our data showing *P. reticulata*'s behavioral avoidance of brackish water.

Environmental gradients, asymmetric competition, and the distribution of species

In this study, we found asymmetric competition between the studied species, with *P. reticulata* being competitive dominant over *P. picta,* both in brackish and freshwater (Fig. 4.2). Nonetheless, *P. reticulata* is restricted only to the freshwater sections of the rivers, showing behavioral avoidance of brackish water. Our results suggest that there are physiological costs to inhabiting brackish waters, as individual growth rate and survival probability decreased in this environment, and the negative impacts of intra- and interspecific competition increased (Fig. 4.1-3). By contrast, although brackish water also appears to be stressful for *P. picta,* this species seems to be more tolerant to it (i.e. Fig. 4.1). In addition, interspecific competition had a stronger effect in growth rate and survival probability of *P. picta* than intraspecific competition does (Fig. 4.2b and 4.3b). Therefore, *P. picta* seems to be limited to the most stressful end of the gradient by the competitive dominant *P. reticulata*.

This distributional pattern, with biotic interactions limiting the competitive subordinate, and often more tolerant species to the harshest end of the gradient (e.g. *P. picta*), and abiotic conditions limiting the better competitor to the mildest end of the gradient (e.g. *P. reticulata*), is not unique of our study (Connell 1975; Bull 1991; Bull and Possingham 1995). This same pattern has been observed in a vast array of species, including invertebrates (e.g. Connell 1961; Hemphill and Cooper 1983), fish (e.g. Taniguchi and Nakano 2000; McHugh and Budy 2005), amphibians (e.g. Hairston 1949, 1980; Grover 2000), reptiles (e.g. Dunham 1980), birds (e.g. Ballance et al. 1997; Martin and Martin 2001; Zeng and Lu 2009; Jankowski et al. 2010), and mammals (e.g. Heller 1971; Chappell 1978). Moreover, this pattern, which is usually found at local scales, is analogous to that suggested to occur at larger geographical scales (Dobzhansky 1950; MacArthur 1972). Dobzhansky (1950) suggested that species inhabiting high latitudes are those capable of coping with harsh abiotic conditions, and those inhabiting low latitudes can tolerate the harsh biotic conditions. Later, MacArthur (1972) expanded this idea by proposing that in general, the low latitude edge of the geographic range of species was determined by interspecific competition, but the high latitude edge was caused by the species physiological limitation to deal with stressful climatic conditions. A few studies have provided support for this hypothesis (e.g. Root 1988; Loehle 1998; Gross and Price 2000; but see Price et al. 2011), but it has been rarely tested at these larger geographical scales (Case et al. 2005).

The determination of the mechanisms at local scales can help understanding the distribution patterns at larger spatial scales. For example, Fausch et al. (1994) analyzed the distribution of two charr species from Japan at different scales. They find that the white-spotted charr (*Salvelinus leucomaenis*) is restricted to downstream sections of streams across its range, expanding into upstream reaches only in the warmer southern parts of its range. On the other hand, the Dolly Varden charr (*S. malma*) was more common upstream of the white-spotted charr, and expanded downstream in the northern, colder parts of the range. Later, Taniguchi and Nakano (2000) showed that the white-spotted charr is competitively dominant but more sensitive to low temperatures, whereas Dolly Varden is subordinate but capable of persisting in the coldest upstream reaches of streams. Similarly, reciprocal removal experiments revealed that the Virginia's Warbler (*Vermivora virginiae*) is limited to nesting in a warmer and drier microclimate by the presence of the competitively dominant Orange-crowned Warbler (*V. celata*), which occupies less stressful cooler and wetter habitats (Martin and Martin 2001). In turn, this pattern at the local scale provides an explanation for the pattern at the regional scale, where Virginia's Warbler is limited to more arid areas than Orange-crowned Warbler. Thus, by experimentally determining the factors responsible for parapatric distributions at local scales, we can make predictions about potential mechanisms driving parapatric distributions at geographical scales.

TABLES AND FIGURES

Table 4.1. Akaike's Information Criterion ranking of models describing the effects of species (spp), competition, environmental salinity (salt), and time on weekly survival on *Poecilia reticulata* and *P. picta* in the field enclosure translocation experiment. Shown are the number of parameters (k), the difference in Akaike's Information Criterion (ΔAICc) between each model and the top-ranked model, and the Akaike weights (w_i) of each model in the confidence set ($w_i > 0.1^* w_{best}$). "Main effects" stand for the additive effects of species, competition, salinity and time. "2-way interactions" stand for all the possible interactions between two of the main effects, and "3-way interactions" for all the possible interactions between three of the main effects.

Model	k	ΔAICc	AICc w_i
Main effects + spp*salt + spp*time + salt*competition + competition*time + salt*time + salt*competition*time + spp*salt*time	18	0.0	0.448
Main effects + 2-way interactions + salt*competition*time + spp*salt*time	19	2.1	0.154
Full model	23	2.2	0.150
Main effects + 2-way interactions + salt*competition*time + spp*salt*time + spp*competition*salt	20	2.2	0.149
Main effects + 2-way interactions + 3-way interactions	22	3.1	0.097

Table 4.2. Model parameter values (Estimate), their standard errors (s.e.) and 95% confidence intervals (95% CI) for the fixed effects of species (spp), competition, environmental salinity (salt), and time on weekly survival on *Poecilia reticulata* and *P. picta* in the field enclosure translocation experiment. Effects on bold are those for which the 95% CI does not overlap 0.

			95% CI	
parameter	estimate	s.e.	lower	upper
intercept	-1.995	1.032	-4.017	0.028
spp	1.506	0.834	-0.128	3.140
competition	1.754	0.976	-0.160	3.668
salt	**5.338**	**1.582**	**2.237**	**8.439**
$\text{time}_{\text{(week 1-2)}}$	-0.293	1.227	-2.699	2.112
$\text{time}_{\text{(week 2-3)}}$	1.605	1.131	-0.611	3.821
spp*salt	**-5.601**	**1.380**	**-8.307**	**-2.896**
$\text{spp*time}_{\text{(week 1-2)}}$	1.082	1.016	-0.910	3.074
$\text{spp*time}_{\text{(week 2-3)}}$	-0.878	0.919	-2.679	0.923
competition*salt	**-3.953**	**1.512**	**-6.916**	**-0.989**
$\text{competition*time}_{\text{(week 1-2)}}$	0.692	1.175	-1.612	2.996
$\text{competition*time}_{\text{(week 2-3)}}$	-1.754	1.086	-3.883	0.374
$\text{salt*time}_{\text{(week 1-2)}}$	-2.651	1.779	-6.137	0.836
$\mathbf{salt*time}_{\mathbf{(week\ 2\text{-}3)}}$	**-4.789**	**1.717**	**-8.154**	**-1.424**
$\mathbf{spp*salt*time}_{\mathbf{(week\ 1\text{-}2)}}$	**3.632**	**1.544**	**0.607**	**6.658**
$\mathbf{spp*salt*time}_{\mathbf{(week\ 2\text{-}3)}}$	**4.680**	**1.489**	**1.762**	**7.598**
$\text{competition*salt*time}_{\text{(week 1-2)}}$	0.840	1.717	-2.525	4.206
$\mathbf{competition*salt*time}_{\mathbf{(week\ 2\text{-}3)}}$	**4.640**	**1.657**	**1.392**	**7.889**

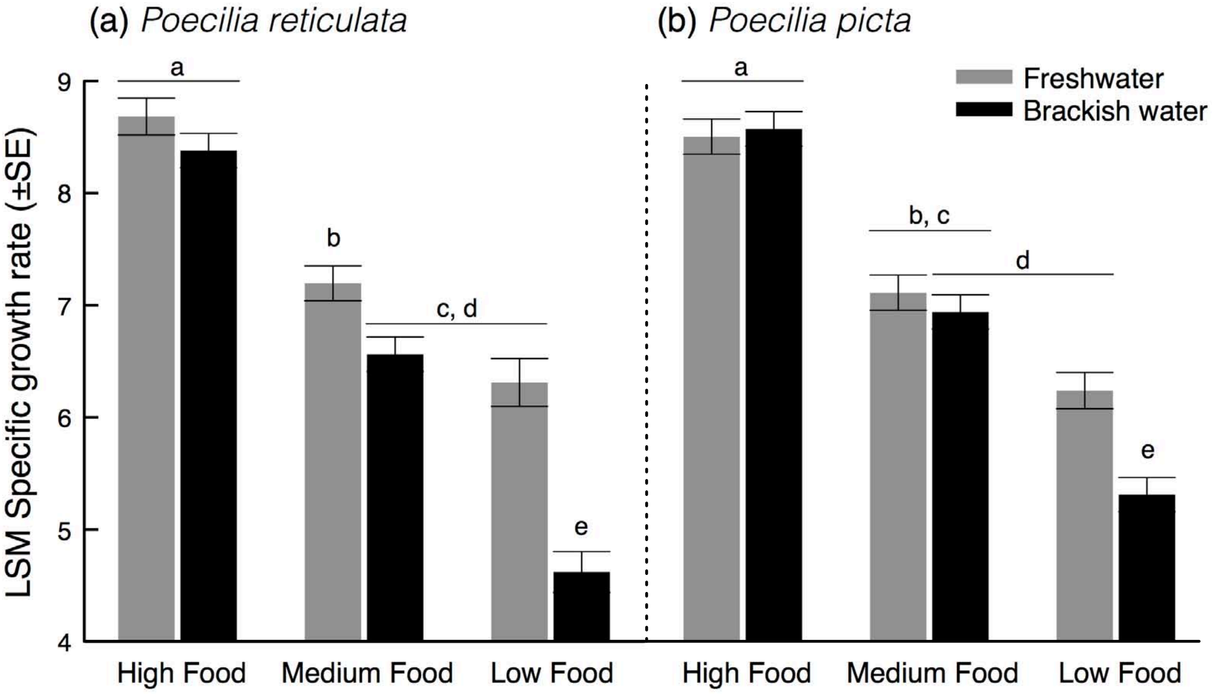

Figure 4.1. Salinity has a negative effect on the growth rate of *P. picta* and *P. reticulata*. Growth rate is higher in freshwater than brackish water for both species, but salinity has a slightly stronger negative effect on *P. reticulata* **(a)** than it does in *P. picta* **(b)**. In both species, the negative effects of salinity are only evident at the lower food levels, but no difference in growth rate was observed between fish reared in brackish and freshwater at the highest food level. Values with different letters are significantly different across species, salinity and food levels (least-squres mean differences with Tukey HSD adjustment).

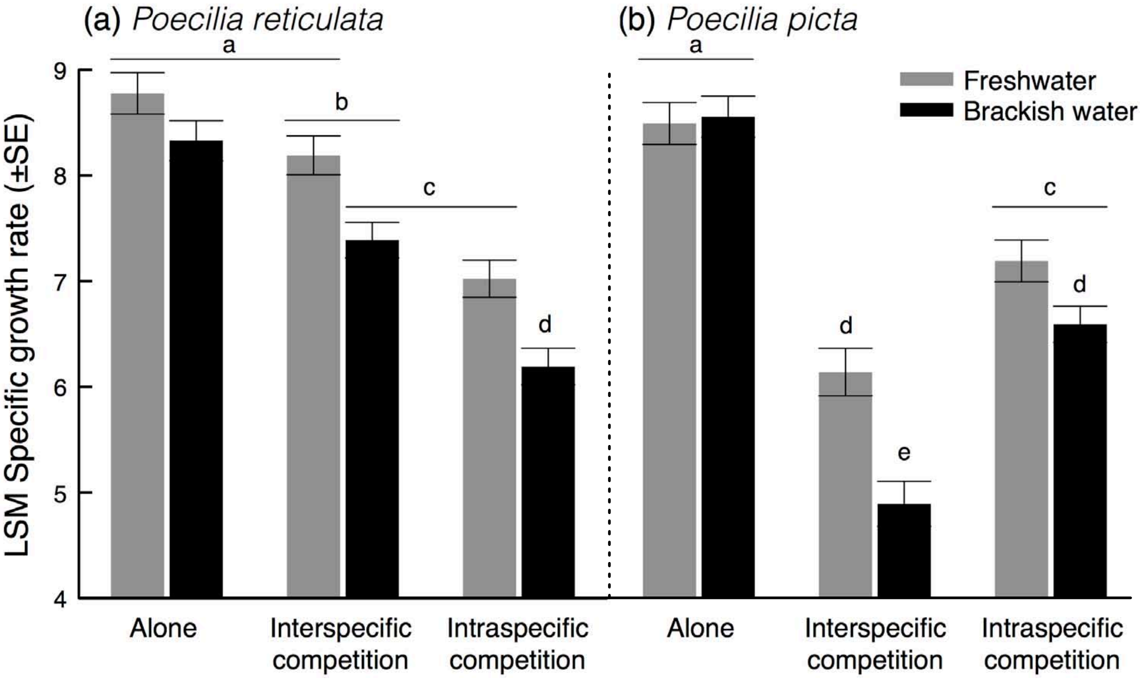

Figure 4.2. The effects of competition in the growth rate of *P. picta* and *P. reticulata* depended on the species and on the salinity level at which fish were reared. Competition is highly asymmetric between the studied species. **(a)** *Poecilia reticulata* growth rate is more affected by the presence of conspecific (i.e. intraspecific competition) than it is by the presence of *P. picta* (i.e. interspecific competition). **(b)** On the other hand, *P. picta* grows at a higher rate in the presence of conspecifics than it does in the presence of *P. reticulata*. In both species, the intensity of both inter- and intraspecific competition became stronger in brackish water. Values with different letters are significantly different across species, salinity and food levels (least-squres mean differences with Tukey HSD adjustment).

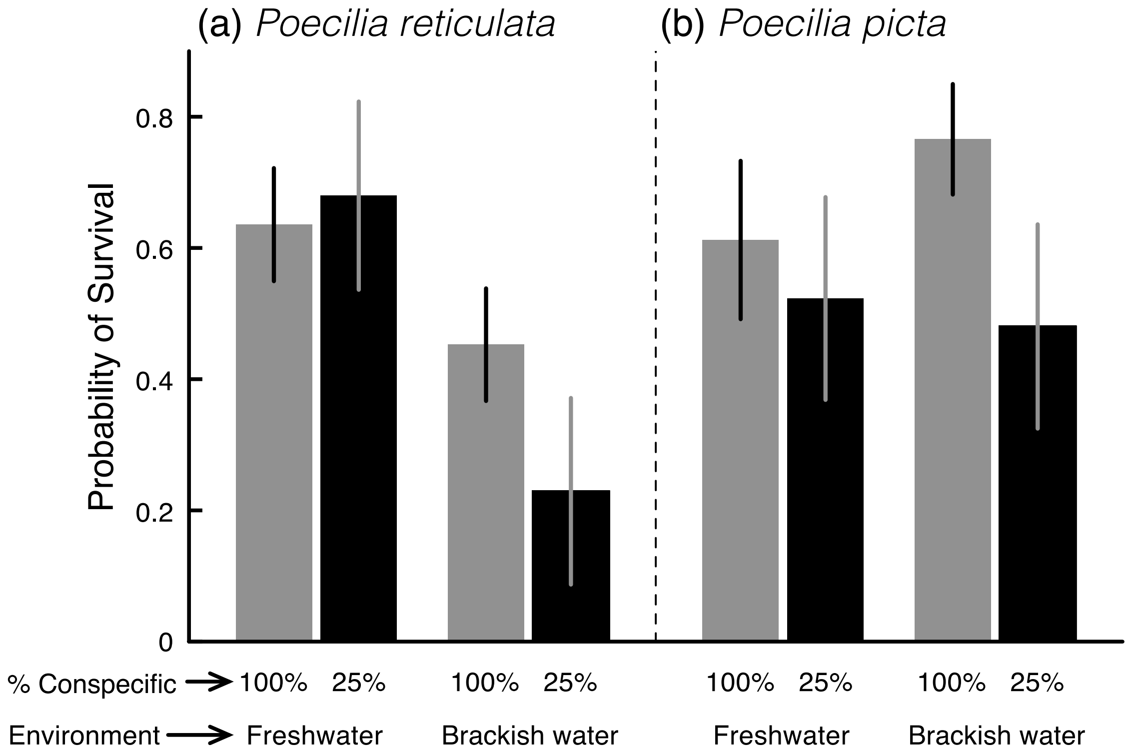

Figure 4.3. Model-averaged mean weekly survival and 90% confidence intervals for *P. reticulata* (left panel) and *P. picta* (right panel) in field enclosures. Survival of *P. reticulata* was dependent on the environment as those allocated to the freshwater locale had a higher probability of survival than those kept at the brackish water locale. There is a negative effect of competition On the other hand, salinity did not have a strong effect on the survival of *P. picta* (right panel). *P. picta* survival decreased in the presence of *P. reticulata*.

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APPENDIX I.I

EFFECTS OF REARING CONDITIONS (10 L GLASS TANKS VS. 1.5 L FLOW THROUGH TANKS) ON GUPPY LIFE HISTORY TRAITS

We established laboratory stocks from wild-caught adult females collected from Guanapo High (high-predation population) and Taylor (low-predation population) to determine the effect of rearing conditions on guppy life-history traits. Female guppies from both populations were individually housed in either 10-liter glass tanks or in 1.5-liter tanks as part of a recirculating system with a 12:12 light cycle at Colorado State University. There were no differences in life-history traits between fish reared in 10-liter glass tanks *versus* 1.5-liters tanks in recirculating systems (all P>0.05, Table S1.1). However, there was a significant interaction between population and rearing environment for offspring number (P=0.048, Table S1.1). Differences in offspring number among high- and low-predation fish was reduced, although maintained, in 1.5-liters flow through tanks compared to 10-liter glass tanks.

Table S1.1. Statistical results (*F*-values) for the contrasts test the effects of rearing condition in fish from the Guanapo High and the Taylor localities on age and size at first parturition, interbrood interval and offspring number of G2 laboratory-reared females.

	df	Age at first parturition	Size at first parturition	Interbrood interval	Offspring Number
Locality	1	16.72***	13.08 ***	4.01*	17.03***
Rearing System	1	0.45^{NS}	0.56^{NS}	3.01^{NS}	2.66^{NS}
L x RS	1	0.03^{NS}	2.48^{NS}	0.16^{NS}	3.92†
Female Size	1	—	—	—	25.16***
L x FS	5	—	—	—	0.2^{NS}
FS x RS		—	—	—	1.75^{NS}
L x RS x FS		—	—	—	0.11^{NS}
Residual sums of squares (df)		5965.78 (85)	0.24 (84)	268.15 (81)	1417.68 (119)
Total sums of squares (df)		7274.13 (88)	0.29 (87)	300.38 (84)	3036.93 (126)
R^2		0.18	0.16	0.11	0.53

†0.1>*P*>0.05; * 0.05> *P* >0.01; ** 0.01> *P* >0.001; *P* <0.001

APPENDIX I.II

CORRELATION BETWEEN PREDATION RISK AND OTHER ENVIRONMENTAL VARIABLES

We sampled fish from six localities along the Guanapo River, which is part of the larger Caroni Drainage in Trinidad and Tobago (Fig. 1.1). At each sampling locale, we determined the predator community by consulting published surveys and by direct observation of fish present at each local. We then arranged the samples from those with the lowest predation risk to those with the highest, resulting in a gradient of predation risk (1_Taylor, 2_Caigual, 3_Guanapo low, 4_Guanapo middle, 5_Guanapo high, and 6_Caroni, Fig. S1.1). At each locale we also characterized the abiotic environment by establishing six stations, one every 50 meters, along the stream at each of these six localities. At each station we measured stream channel width and took three measures of stream depth (at one fourth, one half, and three fourths of the stream width). We recorded temperature, dissolved oxygen, and pH at each station. We took four canopy openness readings at each station using a spherical densiometer (one reading in the direction of each cardinal point). We then performed a Principal Component Analysis to reduce the number of variables. The first principal component axis explained almost 70% of the variation, while the second explained an additional 25% of the variation among sites in the abiotic environment (Table S1.2). The first principal component is correlated with predator community (Fig. S1.1), showing the most abrupt change when going from Guanapo high to Caroni. Temperature, depth, width and canopy all loaded positively on the first principal component and dissolved oxygen loaded negatively on this axis (Table S1.2). The second principal component is not correlated with the risk of predation, and pH is the variable that loaded most heavily on PC2 (Fig S1.1, Table S1.2). When life-history traits were plotted against principal component 1, a similar pattern to that seen for predation risk was observed (Fig. S1.2 left panel). Age at maturity, offspring size, and interbrood interval decreased, while fecundity and reproductive allocation increased as the river became wider and deeper, the canopy became more open and water temperature increased while the percent of dissolved oxygen decreased. None of the life-history traits appeared to be correlated with PC2 (Fig. S1.2 right panel). Exceptions to this general pattern were offspring size and female size at maturity in females reared in the laboratory. Also, reproductive allocation in wild-caught females appeared to be weakly correlated with PC1, but more strongly correlated with PC2 (Fig. S1.2).

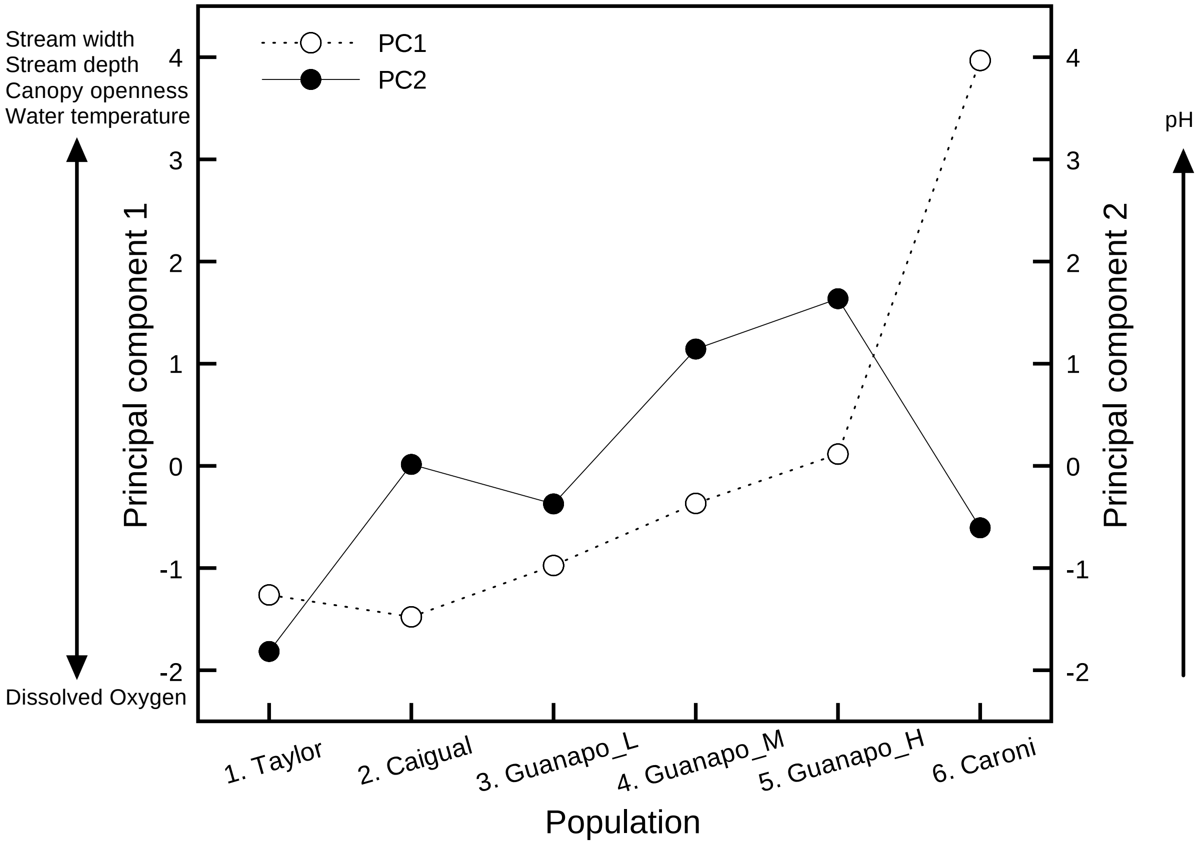


Figure S1.1. Population scores for the first two axes of variation from a Principal Component Analysis on environmental variables including stream width and depth, canopy cover, temperature, and pH (loading of these variables in each axes are depicted on the figure and in Table S1.2).

Table S1.2. Loading matrix, associated eigenvalues and proportion of variation explained by each principal component form a Principal Component Analysis on environmental variables including stream width and depth, canopy cover, temperature, and pH.

	Principal Component				
	1	2	3	4	5
Water temperature	0.93	0.27	-0.18	0.18	0.01
% dissolved oxygen	-0.83	0.53	-0.06	0.15	0.01
pH	0.04	0.96	0.26	-0.04	-0.01
Stream width	0.99	-0.04	0.09	0.08	-0.05
Stream depth	0.95	-0.21	0.23	0.02	0.05
Canopy openness	0.83	0.48	-0.24	-0.16	0.01
Associated Eigenvalue	4.12	1.55	0.22	0.09	0.005
% variation	68.74	25.96	3.73	1.48	0.08
χ^2	36.88	19.45	–	–	–
P-value	0.01	0.15	–	–	–

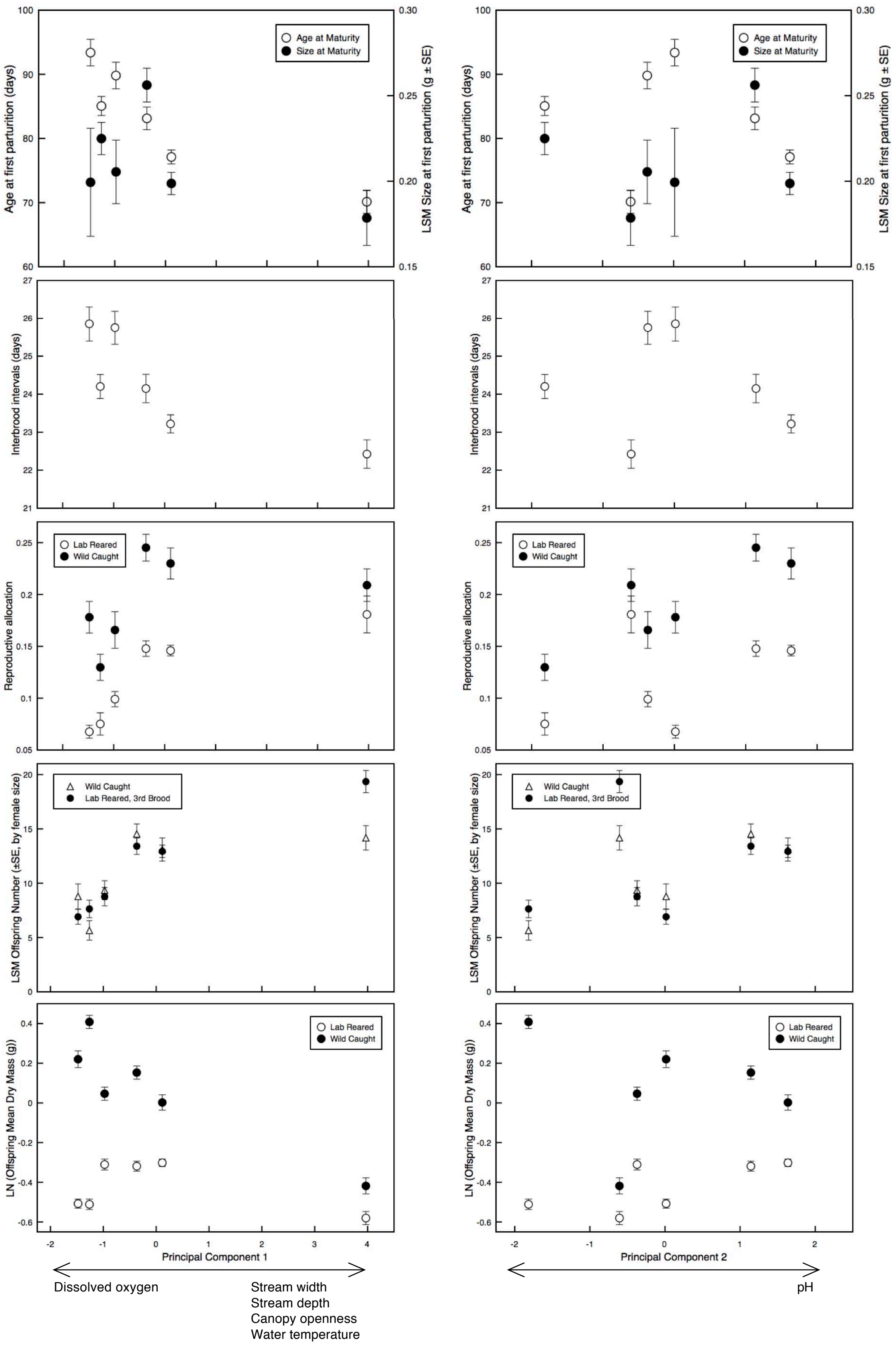
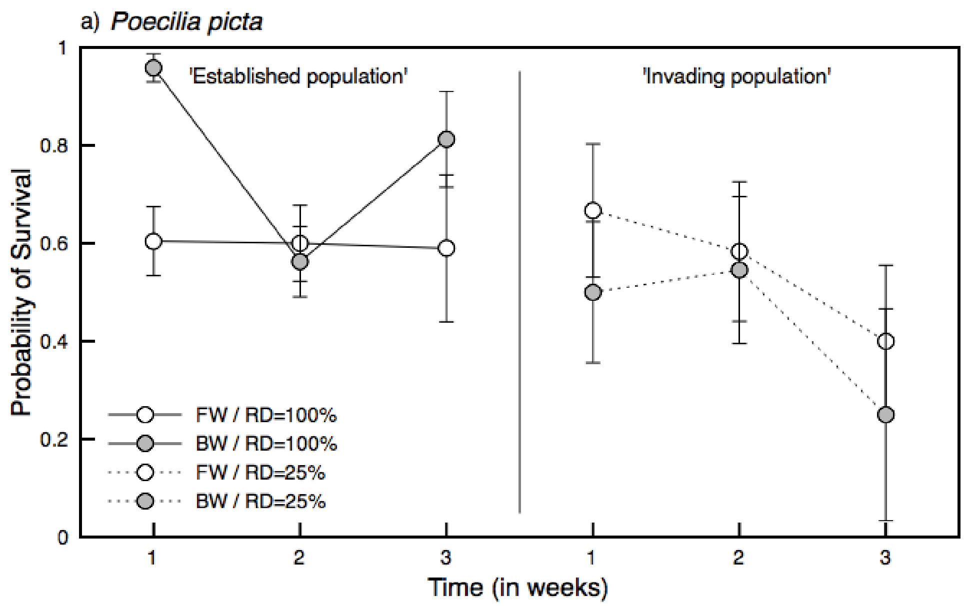

Age at first parturition (days)
LSM Size at first parturition (g ± SE)
Age at Maturity
Size at Maturity
Interbrood intervals (days)
Reproductive allocation
Lab Reared
Wild Caught
LSM Offspring Number (±SE, by female size)
Wild Caught
Lab Reared, 3rd Brood
LN (Offspring Mean Dry Mass (g))
Lab Reared
Wild Caught
Principal Component 1
Principal Component 2
Dissolved oxygen
Stream width
Stream depth
Canopy openness
Water temperature
pH

Figure S1.2. Least square mean (± SE) for guppy females' life-history traits (age and size at maturity, interbrood interval, reproductive allocation, offspring number and offspring size) produced from the third brood of G2 laboratory-reared females and wild caught females plotted against the first two principal component axes from a Principal Component Analysis on environmental variables including stream width and depth, canopy cover, temperature, and pH (loading of these variables in each axes are depicted on the figure and in Table S1.2).

APPENDIX IV.I


a) *Poecilia picta*

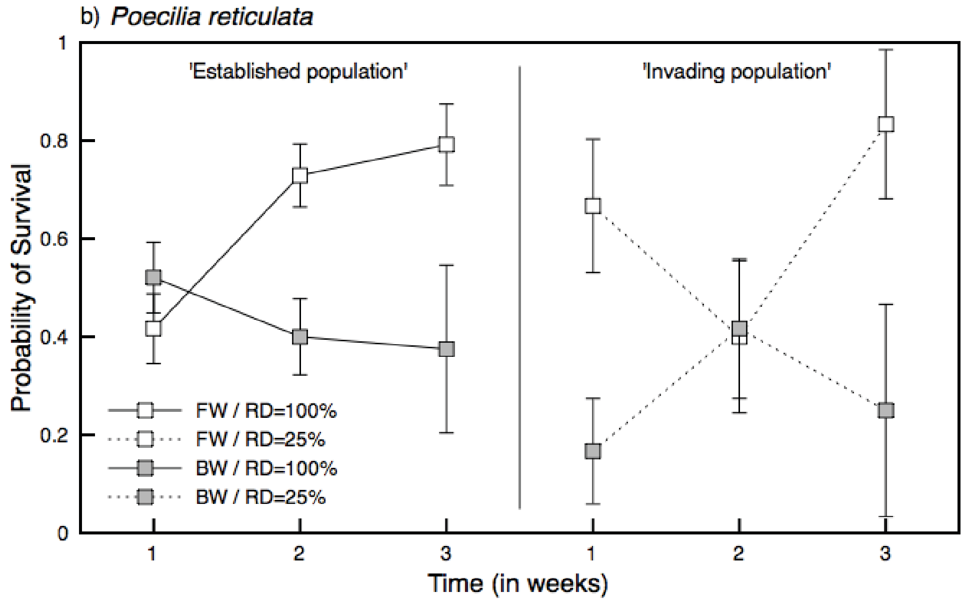

b) *Poecilia reticulata*

Figure S4.1. Detailed temporal variation in survival in field enclosures. Time was a significant variable in the model as estimations of survival varied across time; however, there is no consistent pattern of variation among species or treatments.