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NATURAL SELECTION AND ITS INTERACTIONS WITH MIGRATION AND POPULATION DEMOGRAPHY: EXPERIMENTS IN WILD GUPPY POPULATIONS

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A THESIS

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NATURAL SELECTION AND ITS INTERACTIONS WITH MIGRATION AND POPULATION DEMOGRAPHY: EXPERIMENTS IN WILD GUPPY POPULATIONS

By Dylan Jones Weese

Thesis Advisor: Dr. Michael T. Kinnison

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
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In this dissertation I quantify spatial and temporal variation in the pattern and strength of natural selection in wild populations of Trinidadian guppies (*Poecilia reticulata*), and assess the demographic costs of ongoing contemporary evolution in the form of selection against migrants. First, I describe the results of ten mark-recapture experiments to test hypotheses concerning the role of natural selection in geographic patterns of trait variation. Previous work has reported that guppies inhabiting high- and low-predation sites differ in both body shape and color. These patterns of phenotypic variation have been theorized to reflect differences in the balance between functional trade-offs among various aspects of performance. For example, natural selection is hypothesized to disfavor bright male color (owing to predation) and sexual selection is hypothesized to favor bright color (owing to female choice). My results support some of the predictions generated from considering these functional trade-offs. However, for many color and shape traits, my results do not support the prediction that viability

selection is weaker in low-predation experiments. Instead, some of the most intense bouts of selection occurred in low-predation experiments. My results illustrate considerable spatiotemporal variation in selection among experiments. It seems more complex selective interactions, possibly including the indirect effects of predators on variation in mating behavior, as well as other environmental factors, might be required to more fully explain patterns of color and shape variation in this system.

Second, I quantify the demographic costs of ongoing contemporary evolution. Fine-scale genetic diversity and contemporary evolution can theoretically influence ecological dynamics in the wild. Such eco-evolutionary effects may be particularly relevant to the persistence of species facing acute or chronic environmental change. One way that ongoing evolution may influence the dynamics of threatened populations is through the role that selection plays in mediating the "rescue effect", the ability of migrants to contribute to the recovery of populations facing local disturbance and decline. I combined field experiments with natural catastrophic events to show that ongoing evolution is a major determinant of migrant contributions to population recovery in Trinidadian guppies.

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CHAPTER 1: INTRODUCTION

1.1 Natural Selection

The process of evolution by natural selection is widely acknowledged to be the primary mechanism generating ecological and phenotypic diversity (Rose and Lauder 1996; Schluter 2000; Coyne and Orr 2004), and was the major contribution of Charles Darwin to the field of biology (Darwin and Wallace 1858; Darwin 1959). Simply, natural selection can be defined has a process whereby a set of three conditions will necessarily lead to phenotypic evolution. If: 1) a population has variation among individuals in some trait, 2) there is a consistent relationship between that trait and fitness, and 3) there is a consistent association, for that trait, between expression of that trait in parents and offspring (variation in the trait is heritable); then evolution by natural selection is anticipated. Quantitatively rigorous methods for studying natural selection in nature are, however, in their relative infancy. John Endler (1986), in his classic work on the subject comprehensively examined different methodological approaches to describe and quantify selection in the wild. The most commonly implemented of these methods include: 1) testing whether or not observed patterns of trait variation are consistent with biophysical first principles; 2) testing whether or not observed patterns of trait variation are consistently correlated with particular ecological or environmental features in phylogenetically distinct taxa (the comparative method); 3) Experimental manipulation of natural populations, followed by an assessment of whether or not resulting trait changes are in the predicted direction, and 4) the direct observation of natural selection in the wild by establishing a relationship between fitness and trait variation in contemporary populations.

This last approach (direct estimation of natural selection) has benefited from the development of analytical techniques that have merged the fields of evolutionary ecology and quantitative genetics. Lande and Arnold (1983) describe a method for quantifying indirect and direct selection through the use of multivariate linear regressions to estimate selection coefficients that describe the relationship between traits and fitness. Meta-analyses of these selection coefficients (Kingsolver et al. 2001; Hoekstra et al. 2001) estimated for diverse taxa suggest that natural selection in the wild is relatively common and frequently quite strong. The possibility that strong directional selection in the wild is relatively common belies the expectation that adaptation causes populations to approach adaptive optima, potentially making contemporary selection less apparent. This paradox might be explained by 1) the possibility that the direction and magnitude of selection is highly variable in the wild (Siepielski et al. 2009), and 2) the possibility that many traits are subject to functional trade-offs whereby selection optimizing one aspect of performance necessarily compromises another aspect of performance (Arnold 1992; Walker 2007). Both of these non-exclusive possibilities are usefully addressed by studies that provide spatiotemporal replication of selection estimates, and that evaluate specific predictions regarding the fitness consequences of functional trade-offs between different aspects of performance (Ghalambor et al. 2004).

1.2 Reciprocal Interactions of Ecology and Evolution

Ultimately, the above approach to directly study natural selection in the wild is an exercise in understanding how fundamentally ecological processes, specifically nuances of population demography (survival and reproduction), shape the traits of wild populations. But what of potential reciprocal interactions? How does the evolution of populations in turn influence their ecological dynamics, including population demography? One might suspect that such a fundamental question would have received focused attention in evolutionary theory or ecology, but that has not been the case. Ever since Darwin, research on the biological relevance of natural selection has targeted its importance to the speciation process (Schluter 2000, 2001; Rundle and Nosil 2005). Ecologists have somewhat implicitly followed suite in their attentions, focusing largely on the processes that determine the distribution, abundance and interactions of diversity at or above the species level. However, the last few years have seen an explosion of theoretical and applied interest in the potential relevance of natural selection to ecological processes and conservation biology.

This is not to say there is no precedence for such reciprocal effects. Classic works in the disciplines of both evolutionary biology (Fisher 1930; Lande 1982) and population ecology (Chitty 1952; Charlesworth 1971) have on occasion acknowledged the potential for reciprocal influences between natural selection and population dynamics; but for some reason empirical work on these eco-evolutionary interactions (sensu Kinnison and Hairston 2007) has lagged. This discrepancy could reflect the relative difficulty of performing suitable experiments (which require the simultaneous estimation or manipulation of ecological and evolutionary parameters). However, a more parsimonious explanation may simply be that the fields of evolutionary biology and ecology have developed largely in isolation over the last half century, owing perhaps to a widely held perception that ecological and evolutionary timescales rarely overlap (Slobodkin 1961); that perception has been empirically challenged by a number of recent meta-analyses (for example Hendry and Kinnison 1999; Hairston et al. 2005; Kinnison and Hairston 2007).

Oddly enough, most ecologists would acknowledge that genetic factors, such as loss of genetic variation and inbreeding depression, may cause eventual population declines (Frankham 2005). However, recent lab and field studies suggest a much broader link between genetic variation, adaptation, and ecological dynamics. In what is becoming a classic paper, Yoshida et al. (2003) manipulated genetic variation of algae in a simple predator-prey system with rotifers (predators) and showed that the opportunity for evolution changed population cycles from those expected in a purely ecological model. Quantitative approaches applied in field studies have also provided support that demographic fluctuation are partly attributable to concurrent natural selection on life history traits in Soay sheep (Pelletier et al. 2007), aspects of beak morphology in Galapagos finches (Hairston et al. 2005), and allelic variation in a glycolytic enzyme in fritillary butterflies (Hanski and Saccheri (2006). Such eco-evolutionary effects (see Figure 1.1) have even been shown to have important community- (Palkovacs and Post 2008; Post et al. 2008; Palkovacs and Post 2009) and ecosystem-level (Palkovacs et al. 2009; Harmon et al. 2009; Bassar et al. 2010) consequences. Parallel work from the field of community genetics has similarly demonstrated a wide range of emergent community and ecosystem effects linked to the "extended phenotypes" of "foundation" species of plants (Whitham et al. 2006; Barbour et al. 2009).

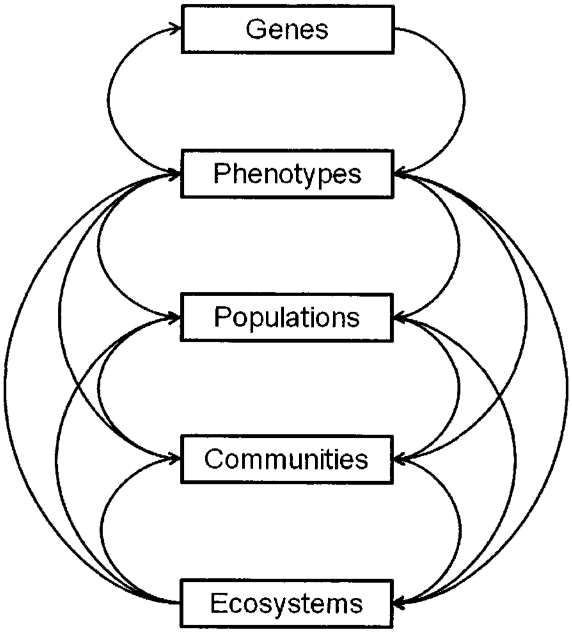

Figure 1.1. Conceptual Model of Eco-Evolutionary Feedbacks across multiple levels of ecological organization (recreated from Bailey et al. 2009). Arrows on the left side of the boxes represent the concept that ecology affects evolutionary processes through direct and indirect ecological effects. Arrows on the right side of the boxes represent the concept that evolution feeds back on ecology through direct and indirect emergent effects of the phenotype.

1.3 Selection and Eco-Evolutionary Effects in Metapopulations

Although not traditionally referred to as such, eco-evolutionary dynamics can be appreciated in the intersection of evolutionary and ecological contributions to metapopulation theory (Hanski 1999). Theoretical work on evolutionary source-sink dynamics within environmentally heterogenous metapopulations incorporate interactions between divergent natural selection, immigration, gene flow and population demography (for example, Holt and Gomulkieicz 1997; Kawecki and Holt 2002; Kimbrell and Holt 2007). Unlike purely ecological models where resident and migrant individuals are adaptively equivalent, these models show that migration and evolution can variously facilitate or impede the persistence and conversion of sink populations. The interactions between divergent natural selection, adaptive divergence, and gene flow can be theoretically shown to both mediate and be mediated by population demography (Garant et al. 2007a; Kinnison and Hairston 2007). For example, the influence of population demography on evolution can be seen in the way that population growth reduces the effectiveness of a given amount of gene flow and so contributes to isolation favoring further adaptive divergence (Gomulkiewicz et al. 1999; Tufto 2001; Kawecki and Holt 2002; Hendry 2004). Conversely, the influences of gene flow-selection interactions on population demography give rise to predictions for limits on population colonization (Holt and Gomulkiewicz 1997), the size of species ranges (Kirkpatrick and Barton 1997; Garcia-Ramos and Rodriguez 2002), the ability of populations to persist in the face of environmental disturbance (Burger and Lynch 1995; Boulding and Hay 2001), and demic dynamics of metapopulations (Saccheri and Hanski 2006).

Despite the fundamental importance of natural selection and its interactions with dispersal and population demography, what little empirical evidence we have for these interactions comes from relatively few descriptive studies (for example Hendry et al. 2002; Hanski and Saccheri 2006; Duckworth and Badyaev 2007). Even fewer such study systems have performed manipulative experiments (Reichert 2001; Moore and Hendry 2009; Nosil 2009) to assess evolutionary consequences, and these have generally lacked sufficient data on selection, gene flow and demography to truly assess reciprocal eco-evolutionary interactions.

1.4 Dissertation Objectives

Generally, the goal of my dissertation is to assess the reciprocal effects of demography on population trait distributions (evolution) and of population trait distributions on demography. Any broader understanding of the ecological relevance of ongoing evolutionary processes requires studies that reveal the spatial and temporal grain over which selection and evolution may shape such dynamics. Towards this goal, I have conducted a replicated series of mark-recapture experiments in natural populations of Trinidadian guppies (*Poecilia reticulata*) to test hypotheses concerning how spatiotemporal variation in selection influences geographic patterns of trait variation. Chapter two tests hypotheses related to spatiotemporal variation in selection on male guppy coloration, and chapter three considers variation in selection on male and female body shape. Perhaps the most salient outcome of these two chapters is that traditional explanations for population divergence in guppies, as gleaned from comparative and laboratory studies, are often not supported by the actual study of selection in the wild.

If the emerging field of eco-evolutionary dynamics is to be broadly integrated into the traditional fields of ecology, evolution and conservation biology, then experimental approaches are also needed to empirically demonstrate the ecological consequences of ongoing evolution. The "rescue effect" predicts that migrants may serve to sustain populations that would otherwise go extinct. However, selection against migrants complicates predictions for such a demographic effect. In chapter four, I use a combination of mark-recapture and population genetic techniques, to experimentally evaluate the demographic contribution of migrants to recovery of adaptively divergent populations subjected to natural catastrophic disturbance (flooding). Here, I discovered that selection against migrants was very strong (compared to local guppies) and drastically reduced the possibility of a demographic "rescue". This research provides a novel perspective on the complexity of eco-evolutionary interactions and their relevance to the conservation of disturbed populations.

CHAPTER 2: SPATIOTEMPORAL VARIATION IN LINEAR NATURAL SELECTION ON BODY COLOR IN WILD GUPPIES (*POECILIA RETICULATA*)

2.1 Introduction

The role of natural selection in generating diversity is frequently inferred from associations between phenotypic variation and environmental features or habitat types (Endler 1986; Schluter 2000). Such evidence for natural selection is indirect because selection itself is not actually quantified (Lande and Arnold 1983, Endler 1986, Kingsolver et al. 2001). Direct estimates of selection in the wild can therefore provide additional insight into adaptive hypotheses by suggesting whether contemporary patterns of selection are consistent with those predicted to produce observed patterns of phenotypic variation (Lande and Arnold 1983; Endler 1986). The best opportunity to witness such selection might often be cases where trade-offs exist between different components of fitness. This follows from the recognition that although selection might be expected to shift trait values toward adaptive optima, potentially making contemporary selection less apparent, such trade-offs will often prevent phenotypes from being optimized with respect to any one component of selection (for example, survival or mating success) (Schluter et al. 1991). Here, I consider natural (i.e., viability) selection on secondary sexual traits, which are generally considered subject to a selective trade-off between natural and sexual selection (Fisher 1930; Endler 1980; Svensson and Gosden 2007). In so doing, I assess the contribution of viability selection to contemporary phenotypic variation in nature.

In addition to the balance between fitness trade-offs, phenotypic evolution will be sensitive to spatiotemporal variation in selection. This variation is likely common in nature, presumably because of fluctuating environmental conditions (reviewed in Siepielski et al. 2009). This spatiotemporal variation in the intensity or direction of selection is commonly proposed as a primary mechanism responsible for the maintenance of trait variation both within and between populations (Barton and Turelli 1989; Merilä et al. 2001; Brooks 2002). Although spatiotemporal variation in natural or sexual selection has been directly documented in some systems (Siepielski et al. 2009), such variation is more commonly indirectly surmised. Importantly, although it is relatively straightforward to test for the statistical significance of any estimate of selection at a given time and place (*H_0: no selection is apparent*), such a test is not in itself a statistical evaluation of whether patterns of trait variation are likely the result of variable selection. Rather, the generality of adaptive hypotheses must be statistically assessed by contrasting multiple spatiotemporal estimates of selection (*H_0: selection is spatiotemporally consistent*).

My objectives were to quantify spatiotemporal variation in patterns of natural selection in a classic study system of secondary sexual trait evolution – color patterns of Trinidadian guppies (*Poecilia reticulata*). Using survival data from ten separate mark-recapture experiments, I estimated linear natural selection coefficients (Lande and Arnold 1983) associated with male coloration (male guppies have colored spots that vary in size and number). My estimates of natural (viability) selection were then used to evaluate support for current hypotheses for the origin and maintenance of color diversity within and among habitat types.

2.1.1 Evolution of Guppy Color

Typically, Trinidadian guppy habitats are characterized as either high predation or low predation (Endler 1995). High-predation habitats are usually found in the lower reaches of streams and contain a variety of large, predatory fishes. These predator communities differ somewhat between the south and north slopes of Trinidad's Northern mountain range. The south slope contains a 'mainland' community of predators (a sub-set of the icthyofauna of South America), whereas the north slope contains a marine-derived 'Caribbean' icthyofauna (Endler 1983). Low-predation habitats, in contrast, are usually found upstream of barrier waterfalls that have prevented colonization by the above predatory fishes (Endler 1978; Magurran 2005). These low-predation habitats do contain some guppy predators, although these predators are considered less "dangerous." They include a species of killifish (*Rivulus hartii*) on both slopes and several species of predatory prawns (*Macrobrachium* spp) on the north slope (Endler 1978, 1983; Millar et al. 2006; Gordon et al. 2009; Mckellar et al. 2009). Both *Rivulus* and *Macrobrachium* are also found in high-predation habitats, the latter only on the north slope. Regardless of slope-specific differences in predator communities, the broad contrast between high- and low-predation habitats has been suggested to drive parallel patterns of adaptive divergence in numerous traits, including male color, in many streams (Endler 1978, 1983, 1995; Magurran 2005).

The color patterns of male guppies are influenced by both sexual and natural selection (Endler 1978, 1983). Sexual selection (female mate choice) often (although not always) favors more colorful males (Houde 1987; Endler and Houde 1995; Brooks and Endler 2001). On the other hand, natural selection imposed by predators is expected to favor less conspicuous color patterns (Endler 1978, 1980, 1983; Godin and McDonough 2003; Millar et al. 2006). Broadly consistent with this prediction, males in high-predation guppy populations on both slopes are often (but not always) less colorful than their low-predation counterparts (Endler 1978, 1980, 1983; Millar et al. 2006; Alexander et al. 2006; Karim et al. 2007; Schwartz and Hendry 2007; Kemp et al. 2008). The role of predators in color pattern evolution has been further supported by an introduction of guppies from a high-predation site to a low-predation site, and by multi-generation greenhouse experiments (Endler 1980). In both cases, colored spots were smaller and less numerous in guppies that coexisted with large fish predators compared to those that inhabited control, low-predation treatments, or natural low-predation streams (Endler 1980).

Despite these broadly deterministic patterns, a large amount of local color diversity exists both within and among guppy populations, even within a given predation regime (Endler 1978; Brooks 2002; Millar et al. 2006; Olendorf et al. 2006; Karim et al. 2007). As a result, guppies are commonly regarded as a model system in which to study the factors maintaining variation in adaptive traits. Numerous mechanisms have been advanced as potential explanations (reviewed in Brooks 2002), including frequency-dependent natural selection (Olendorf et al. 2007), frequency-dependent sexual selection (Hughes et al. 1999), local variation in female color preferences (Endler and Houde 1995; Schwartz and Hendry 2007), spatial variation in selection coupled with gene flow (Brooks 2002; Crispo et al. 2006), and temporal variation in selection (Brooks 2002; Gamble et al. 2003). My study will address the possible contribution of spatiotemporal variation in natural (viability) selection to the patterns of trait variation.

Despite extensive work on the geographic distribution and evolution of male guppy color, no study has yet directly tested the basic expectation that more conspicuous and colorful guppies face a survival deficit in natural habitats. That is, no studies have actually calculated selection coefficients for color in natural populations of guppies. I suggest that such estimates would be valuable in extending and refining this now classic example of evolution in the wild, and would contribute to a growing body of work emphasizing the biological implications of spatiotemporal variation in natural and sexual selection. Based on previous work in the guppy system, I tested the following hypotheses.

1. Mortality rates are higher in high-predation environments than in low-predation environments (see also Reznick et al. 1996; Gordon et al. 2009).

2. Natural selection generally disfavors more colorful guppies (Endler 1978, 1980), and estimated linear selection coefficients are therefore predominantly negative.

3. The strength of natural selection (selection coefficients) against color is greater in high-predation habitats than in low-predation habitats (Endler 1978, 1980). This prediction is distilled from the general notion, derived from geographic patterns, field introductions, and laboratory evolution, that selection against color is more intense (sensu Endler 1978) in high-predation habitats. I interpret this notion as predicting that the slope describing the relationship between color and survival should be more strongly negative where guppies coexist with visual-hunting fish predators.

4. Linear selection coefficients and mean trait values should be correlated among populations: populations with less of a given color should experience strong selection against that type of color.

2.2 Methods

2.2.1 Study Sites

My study sites were located within three rivers (Marianne, Damier, and Aripo) that flow from Trinidad's Northern Mountain range (Table 2.1). The Marianne and Damier rivers drain the north slope, whereas the Aripo River drains the south slope. Additional environmental information about the Marianne River sites (M1, M10, M15, M16, M17), can be found in Crispo et al. (2006) and Millar et al. (2006). The Aripo River sites (AH, AL) are described in Schwartz and Hendry (2007) and the Damier River sites (DH, DL) are those described in Karim et al. (2007) and Gordon et al. (2009). I conducted the majority of my field work during the dry season (March-June) (Table 2.2) – because flow rates and stream morphology are less variable at this time (Reznick et al. 1996). The sites chosen for my mark-recapture experiments were all characterized by distinct pool-riffle structure. Study sites were typically pools or sets of pools (guppies are rarely found in riffles) selected for features that would minimize emigration (for example, partial barriers to upstream or downstream movement). In one case, separate mark-recapture experiments were conducted in the same site (Aripo high-predation) in two different years (2005 and 2006).

Table 2.1. Locations of Sites used for our selection experiments in Trinidad's Northern range mountains. Grid references are from the Trinidad National Grid System 1: 25, 000 map series (Lands and Surveys Division, Port of Spain, Trinidad).

Site	Predation risk	Slope	Drainage	Grid reference
M16	Low	North	Marianne	PS 856 882
M1	Low	North	Marianne	PS 846 892
M10	Low	North	Marianne	PS 868 914
DL	Low	North	Damier	PS 823 936
AL	High	South	Aripo	PS 933 818
M15	High	North	Marianne	PS 852 912
AH	High	South	Aripo	PS 940 780
M17	High	North	Marianne	PS 855 899
DH	High	North	Damier	PS 824 937

Table 2.2: Summary of Male Mark-Recapture Information for the ten experiments. Capture efficiency is the proportion of guppies captured at the first recapture episode (Recap 1), divided by the number known to be alive based on the second recapture episode (Recap 2). Daily mortality rate (Mort rate) is the estimated percentage of the original number released fish that died per day. Killing power (daily exponential mortality rate) is Log10(N released) minus Log10(N at final recap) then divided by the duration of the experiment (T). Information for Recap 2 and Capture efficiency are not applicable (n/a) for experiments with only a single recapture event.

			Recap 1		Recap 2	
Experiment	Release date	*N* released	*N*	T (days)	*N*	T (days)
Low predation						
M16	3/26/2005	65	61	19	45	52
M1	6/29/2004	132	71	11	36	67
M10	5/19/2005	211	147	14	118	30
DL	3/27/2004	87	63	12	n/a	n/a
AL	5/5/2005	95	34	25	n/a	n/a
High predation						
M15	3/28/2004	248	93	13	n/a	n/a
AH05	5/10/2005	100	23	10	n/a	n/a
AH06	4/3/2006	210	79	15	31	44
M17	6/26/2004	111	41	13	21	66
DH	3/28/2004	62	39	12	n/a	n/a

Table 2.2 continued.

Experiment	Capture efficiency	Mort rate	Killing power
Low predation			
M16	97.97	0.006	0.003
M1	90.87	0.011	0.008
M10	85.90	0.015	0.008
DL	n/a	0.023	0.012
AL	n/a	0.026	0.006
High predation			
M15	n/a	0.048	0.011
AH05	n/a	0.077	0.014
AH06	82.78	0.019	0.019
M17	89.76	0.012	0.011
DH	n/a	0.031	0.012

Guppy populations from the Damier River were the result of a 1996 experimental introduction of guppies that originated from the high-predation section of the nearby Yarra River (Karim et al. 2007; Gordon et al. 2009). The Damier selection experiments thus provide a particularly direct test of the hypothesis that colonization of different predation habitats leads to differential selection, since trait values in these populations may not have achieved optimum values. All other sites contained indigenous populations.

2.2.2 Mark-Recapture Techniques

I employed standard mark-recapture techniques for guppies (Rodd and Reznick 1991; Reznick et al. 1996; Bryant and Reznick 2004; Olendorf et al. 2006; Van Oosterhout et al. 2007; Gordon et al. 2009). For each experiment, virtually all of the adult guppies in each pool were captured and transported to the field station in Trinidad. These guppies were kept in aerated tanks that had been treated to prevent fungal infection (Fungus Eliminator – Jungle Inc.), reduce stress from handling (Stresscoat – Aquarium Pharmaceuticals), and neutralize toxic chemicals in the water (Amquel – Kordon). All guppies were anaesthetized with tricanine methanesulfonate (MS-222), placed on a standard metric grid under full spectrum fluorescent lights (which mimic the daylight spectrum), photographed with a digital camera (Sony MVC-500), and then individually marked with sub-cutaneous injections of elastomer dye (Northwest Marine Technology). Using a combination of six different colors and (up to) six different anatomical locations, two sub-cutaneous injections provided 540 individually identifiable marking codes per sex per experiment. Mortality rate due to tagging was very low (<1%) in the period between tagging and release. Within a few days of tagging, guppies were released back into their site of origin. On several occasions, subsets of marked guppies were retained in the lab, verifying that no appreciable delayed mortality resulted from marking.

Recapture episodes occurred at approximately two week intervals after the original release date, with some variation (10-14 days) due to field logistics and anticipated mortality rates (for example, the Aripo low-predation experiment was sampled after 25 days). These are standard and appropriate lengths of time for studies of mortality in adult male guppies because approximately 50% of adult male guppies perish over two weeks in high-predation experiments (Reznick 1996; Gordon et al. 2009). Recapture episodes involved intensively sampling each site, where I attempted to catch all guppies. I used butterfly nets, in conjunction with bait stations (wire boxes holding dog food), to capture guppies from particular pools before identifying their marks in the lab or field. Very few (<0.1 %) guppies lost one of their original marks between marking and recapture. In most of these cases, color patterns recorded in photos (in addition to the single remaining mark) allowed determination of fish identity.

Typically, recapture episodes lasted several days, depending on the number of guppies and the size and complexity of a particular stream site. I stopped fishing when no more guppies were observed, and then returned on one or two subsequent days to capture any remaining guppies. I also sampled upstream and downstream pools within 300-400 m of the release sites (unless an absolute barrier to guppy movement was present), which prevented potential emigrants from leaving the focal sites. This distance corresponds roughly with the maximum observed movement for male guppies as described by Croft et al. (2003). The vast majority of marked guppies were captured within the focal study areas.

Five experiments (M16, M1, M10, AH06, and M17) included a second recapture episode that targeted individuals surviving the first episode (which had been re-released following the first recapture episode) (Table 2.2). For these experiments, I was able to estimate approximate capture efficiency as the number of guppies known to be alive during the first episode (including guppies missed during the first episode but subsequently captured during the second episode) divided by the number of guppies that were captured during the first episode. To compare mortality patterns between regimes, I calculated (for each experiment) the daily mortality rate as the percentage of the original number of guppies released that had died divided by the number of days in each experiment (Begon et al. 1996). To represent the concept that the probability of death can be considered as a rate applied over time, and to account for differences in experimental sample size and duration, I also calculated (for each experiment) the killing power (daily exponential mortality rate) as Log10(number of fish released) minus Log10(number of fish present at the final recapture episode) then divided by the number of days in each experiment (Begon et al. 1996). I used t-tests with the different selection experiments as the unit of replication to evaluate regime differences in both daily mortality rates and killing power.

2.2.3 Photo Analysis

Using the photographs, each color spot on each guppy was assigned to one of seven color categories (Black, Silver, Orange, Yellow, Green, Blue, Violet). I generally followed the methods of Millar et al. (2006), except that I did not differentiate between fuzzy black and black, or between bronze-green and green. I then measured body area, and the area of each individual color spot with the program ImageJ (Scioncorp.com). No measurements were taken from any fins (including the caudal fin), because such structures are difficult to position in a standard fashion and due to concerns that extra handling time could contribute to mortality. A substantial amount of color can be located on the caudal fin (less so on the dorsal fin); this limitation should be addressed in future work, but does not obviate any inferences with specific respect to selection on body color. I likewise did not take spectroradiometer measurements from each color spot because the required procedure (Kemp et al. 2008) was again considered too stressful for fish being used in a mark-recapture experiment in the wild.

For each individual, the areas of all spots of the same color were summed to obtain the total area of a particular category of color spot. The colors yellow, silver, and violet were all very rare and so they were not considered individually. They were, however, included in analyses that pooled colors into biologically relevant categories (Endler 1978): structural color (includes violet, silver, and blue spots) and carotenoid color (includes yellow and orange spots). Total color was computed as the entire color spot area on an individual guppy (all color spots pooled).

2.2.4 Measurement of Natural Selection

For experiments with a single recapture episode, captured guppies were assigned an absolute fitness of one, and guppies that were never recaptured were assigned an absolute fitness of zero. In experiments with two recapture episodes, guppies that survived the entire duration of the experiment were assigned an absolute fitness of one, and guppies that survived to the first recapture episode (but not the second) were assigned an absolute fitness proportional to the interval of time between the first and second recapture episode (Brodie and Janzen 1996). Assigning absolute fitness in this manner is suitable for adult male guppies because they attempt to copulate with females continuously following maturity (Magurran 2005), and thus the number of days a male guppy is alive is likely a good surrogate for potential reproductive success. As is customary for direct estimates of natural selection (Lande and Arnold 1983; Brodie et al. 1995), every individual's absolute fitness was converted to relative fitness by dividing by the population mean (independently for each experiment). Trait values were also standardized to a mean of zero and a standard deviation of unity (Lande and Arnold 1983; Brodie et al. 1995), which facilitates the comparison of selection coefficients (Kingsolver et al. 2001).

For experiments with two recapture episodes, simple linear regressions (fitness predicted by a single trait) were used to calculate selection differentials (regression coefficients). Multiple regressions (fitness predicted by all color traits and body area) were used to calculate selection gradients (partial regression coefficients associated with particular traits) (Lande and Arnold 1983; Brodie et al. 1995). Selection differentials indicate total selection (both direct and indirect) acting on a trait, whereas selection gradients estimate selection on a trait while removing the effects of selection on all other measured traits. I did not include total color in the full multiple regression model as this would result in complete collinearity (Mitchell-Olds and Shaw 1987), since total color was determined by adding up all of the individual color elements. I did, however, calculate selection gradients associated with total coloration in a multiple regression that included only body area and total color. In experiments with two recapture events, I assessed temporal variation in selection by calculating selection differentials and gradients separately for each recapture episode. To estimate selection over the second episode, I only considered variation in fitness among the subset of the population that survived the first recapture episode. Therefore, for these analyses I implemented the analytical approaches appropriate for studies with a single recapture episode (described below). Episode-specific estimates of selection necessarily have reduced sample size and experimental duration; therefore, I consider the selection coefficients that estimate selection over the duration of the experiment to be the more accurate representation of selection.

For experiments with only a single recapture episode, relative fitness can only have two possible values and selection differentials were calculated by standardizing trait values and then subtracting the mean trait value of survivors from the population mean value. Statistical significance was tested by performing a logistic regression between the standardized trait value and fitness. For these experiments, selection gradients were calculated using multiple logistic regressions. The relevant coefficients resulting from the logistic regressions were converted to their linear equivalents following the methods of Janzen and Stern (1998). For all experiments, linear selection coefficients represent the number of standard deviations that selection will change the mean value of a trait within a generation (Lande and Arnold 1983; Kingsolver et al. 2001).

Given the well-documented challenges associated with detecting significant selection in the wild (Mitchell-Olds and Shaw 1987; Kingsolver et al. 2001; Hersch and Phillips 2004), I set my alpha level at 0.10, but considered *P*-values between 0.05 and 0.10 as less conclusive support for a hypothesis than *P*-values less than 0.05. Furthermore, given the many recent criticisms of Bonferroni corrections (for example, Nakagawa 2004), I did not perform such corrections when considering the results of individual selection experiments. Instead, I addressed the issue of multiple comparisons by also implementing statistical models that simultaneously considered data from multiple experiments (see below).

To visualize the pattern of natural selection associated with particular color traits in different selection experiments, I generated cubic spline diagrams for each trait in every experiment (Schluter 1988). I do not here present a formal analysis of non-linear selection because my specific objective was to test hypotheses concerning linear selection and mean trait values (see Introduction).

I was also interested in comparing the broader pattern and strength of natural selection within and between predation regimes, combining data from all experiments. For these analyses, differences in temporal interval were approximately standardized by only considering the first recapture episode from each experiment, except in the low-predation experiment M16 where the longer interval was used (because only 4 guppies died by the end of the first episode). Also, since I was interested in documenting natural (unmanipulated) selection in the wild, I excluded the Damier River experiments (which were recently introduced) from these analyses. To compare the pattern of selection between predation regimes, I approximately followed the ANCOVA methods described by Caruso (2000). Using the combined data from the 8 experiments, I generated an ANCOVA model that included, as independent variables, standardized traits including body area (due to collinearity I excluded total coloration from this analysis), predation regime, experiment nested within predation regime, and an interaction term between each trait and predation regime. The dependent variable, relative fitness, was calculated separately for each experiment; therefore the coefficients resulting from these ANCOVA selection models should not be considered formal selection gradients. Statistical significance of the trait-by-predation-regime interaction terms would indicate statistical support for a difference in the pattern and strength of natural selection between predation regimes. I next considered the predation regimes separately and used a similar ANCOVA selection model with independent variables that included traits (excluding total color), experiment, and interaction terms. Models without the interaction terms (none were significant) were then run in order to estimate regime-wide selection coefficients for each trait. Finally, I generated a model with all experiments pooled and no regime effect, independent variables were experiment and traits (excluding total color). This model estimates universal selection coefficients for each color trait and for body area (there were no significant interactions). In order to generate comparable selection coefficients for total coloration identical models were constructed with only body area and total coloration as traits.

2.2.5 Relating Selection to Divergence

I was interested in qualitatively comparing the estimates of selection to differences in trait values between high- and low-predation regimes. To test for, and characterize, trait differences between predation regimes I used an ANOVA to test for an effect of predation regime and experiment (nested within regime) on body area (body size). For each color individually, I used a similar ANOVA model but here included body area as a covariate (i.e., ANCOVA), thus controlling for possible allometric effects of body size. I initially consider models where the amount of a particular color depended on predation regime, experiment nested within predation regime, body area, and an interaction term between predation regime and body area. No significant interactions between body area and predation regime were found, indicating that I could assume parallel slopes between predation regimes. I then considered a reduced ANCOVA model (separately for each color), wherein a particular color depended on predation regime, experiment nested within predation regime, and body area. From these models, I estimated the least squares adjusted mean values for each color trait associated with each predation regime. Additionally, a discriminant functional analysis (DFA) was employed to identify the axis of color variation that maximized discrimination between high- and low-predation individuals.

To formally explore the possibility that variation in color trait values among sites is correlated with variation in the strength of linear selection acting on color traits, I calculated the relative areas of the different colors for each experiment (mean area of a color divided by mean body area), and then arcsine square-root transformed these values. Separately for each color trait (black, green, carotenoid color, structural color, and total color), I then used general linear models to determine if selection gradients measured during the experiments were related to these transformed mean trait values. I evaluated two models in this regard, each conducted separately for each color pattern element: 1) to assess the relationship between selection gradients and population-level mean trait values, the first model considered only selection gradients as the predictor variable; and 2) to assess whether such relationships differ among predation regimes the second model also considered predation regime and the interaction between predation regime and selection gradient as the predictor variables.

2.3 Results

2.3.1 Sampling Efficiency and Mortality Rates

My estimated recapture efficiencies were high (range = 83-98%, mean = 90%) (Table 2.2). The guppies I did not capture were thus assumed to have perished, particularly because I also sampled from pools below and above the study sites. Consistent with my predictions, daily mortality rates were higher, on average, in high-predation experiments (mean = 3.8%, range = 1.2 – 7.7%) compared to low-predation experiments (mean = 1.6%, range = 0.6 – 2.6 %), although statistical support was modest ($P_{1,9} = 0.057$). Similarly, daily killing power was significantly higher on average in the high-predation experiments (mean = 0.013, range = 0.011 – 0.019), compared to low-predation experiments (mean = 0.0074, range = 0.003 – 0.012) ($P_{1,9} = 0.012$; Table 2.2). Note, however, that some estimates of mortality rate and killing power in low-predation experiments were higher than some estimates in high-predation experiments (Table 2.2). Overall, mortality rates for the high- and low-predation experiments were in the same ranges as those reported for mature males in previous work: Rodd and Reznick 1991 (LP = 3.8%), Reznick et al. 1996 (HP average = 3.8%, LP average = 2.0%) (estimated from Figure 2C), Olendorf et al. 2006 (HP = 1.6 – 2.2%, LP = 1.3 – 2.5%), Van Oosterhout et al. 2007 (LP = 1.2%), and Gordon et al. 2009 (HP = 0-5.0%, LP = 0.5 – 2.0%).

2.3.2 Estimates of Natural Selection

I first consider the results of individual selection experiments. Results for selection differentials (Table 2.3) and gradients were similar (Table 2.4), and so I here focus on selection gradients (which estimate direct selection – see Methods).

Table 2.3. Linear Selection Differentials (*S*) for Color Traits in the ten selection experiments.

	Size		Black		Green	
Site	*S*	*P*	*S*	*P*	*S*	*P*
Low predation						
M16	-0.08	0.11	-0.02	0.72	-0.06	0.22
M1	-0.05	0.60	-0.16	0.07	0.06	0.47
M10	-0.08	0.07	-0.09	0.04	-0.12	0.01
DL	0	0.94	-0.03	0.66	-0.08	0.22
AL	-0.23	0.02	-0.2	0.15	-0.01	0.92
High predation						
M15	-0.08	0.33	-0.2	0.02	-0.11	0.17
AH05	-0.05	0.75	0.33	0.07	0.06	0.73
AH06	-0.01	0.90	-0.12	0.20	0.06	0.53
M17	0.04	0.81	-0.09	0.54	-0.12	0.43
DH	0.07	0.48	0.08	0.45	0.14	0.16

Table 2.3 continued

	Carotenoid		Structural		Total	
Site	*S*	*P*	*S*	*P*	*S*	*P*
Low predation						
M16	0	0.98	-0.04	0.40	-0.07	0.19
M1	-0.11	0.20	-0.15	0.10	-0.21	0.02
M10	-0.16	0.00	-0.14	0.002	-0.16	0.001
DL	0.03	0.65	-0.03	0.65	-0.04	0.54
AL	-0.25	0.07	-0.24	0.08	-0.34	0.01
High predation						
M15	-0.18	0.03	-0.04	0.66	-0.24	0.004
AH05	-0.08	0.65	-0.08	0.65	0.09	0.61
AH06	0.04	0.67	-0.14	0.15	-0.07	0.49
M17	-0.09	0.53	0.05	0.75	-0.09	0.54
DH	-0.11	0.27	0.02	0.86	0.05	0.61

Table 2.4. Linear Selection Gradients (β) for Color Traits in the ten selection experiments.

	Size		Black		Green	
Experiment	β	*P*	β	*P*	β	*P*
Low predation						
M16	-0.05	0.54	-0.02	0.72	-0.05	0.47
M1	0.15	0.22	-0.17	0.07	-0.07	0.51
M10	-0.01	0.89	-0.01	0.89	-0.02	0.70
AL	-0.08	0.75	0.01	0.94	-0.08	0.58
DL	0.01	0.92	-0.03	0.64	-0.08	0.20
High predation						
M15	-0.01	0.93	-0.16	0.08	-0.07	0.41
M17	0.10	0.61	-0.10	0.55	-0.16	0.33
AH05	-0.11	0.71	0.32	0.10	-0.03	0.89
AH06	0.06	0.67	-0.14	0.19	0.01	0.90
DH	0.02	0.86	0.09	0.39	0.15	0.21

Table 2.4 continued.

	Carot		Struct		Total	
Experiment	β	*P*	β	*P*	β	*P*
Low predation						
M16	0.02	0.66	-0.02	0.81	-0.01	0.84
M1	-0.12	0.18	-0.23	0.06	-0.30	0.01
M10	-0.11	0.06	-0.06	0.39	-0.16	0.001
AL	-0.26	0.09	-0.32	0.10	-0.31	0.11
DL	0.03	0.71	-0.04	0.55	-0.05	0.49
High predation						
M15	-0.13	0.14	0.00	0.99	-0.24	0.01
M17	-0.11	0.52	0.07	0.71	-0.15	0.39
AH05	-0.06	0.74	-0.13	0.54	0.19	0.41
AH06	0.08	0.45	-0.13	0.26	-0.11	0.40
DH	-0.12	0.22	-0.02	0.87	0.02	0.87

Consistent with expectations, the majority of estimated selection gradients (for individual experiments considered separately) for color were negative (Table 2.4, Figures 2.1, 2.2). Of the seven gradients that were significant ($P < 0.10$), six were negative. The only significant case of positive selection on color was for black coloration in one selection experiment (2005) at the Aripo high-predation site (Table 2.4, Figure 2.1). The absolute values of significant selection gradients measured in this study range from 0.11-0.32, which exceed 40-78% (respectively) of the gradients from the literature (Kingsolver et al. 2001). I did not detect significant selection gradients associated with fish body size (body area).

Significant selection gradients were most commonly associated with black (i.e., the aforementioned estimate from Aripo high-predation), carotenoid colors, structural colors, and total color (Table 2.4, Figure 2.1). Green appeared to be the most selectively benign color: no selection gradients associated with green were significant. (Table 2.4, Figure 2.1). Power to detect significant selection coefficients can be influenced by sample size (Kingsolver et al. 2001; Hersch and Phillips 2004). However, I sometimes failed to detect statistically significant selection in experiments with relatively large sample sizes (e.g., M17 = 111, AH06 = 210) and, in other cases, detected selection with comparatively modest sample sizes (e.g., AH05 = 100, AL = 95). These results suggest that sample size did not strongly influence the patterns of selection that I detected.

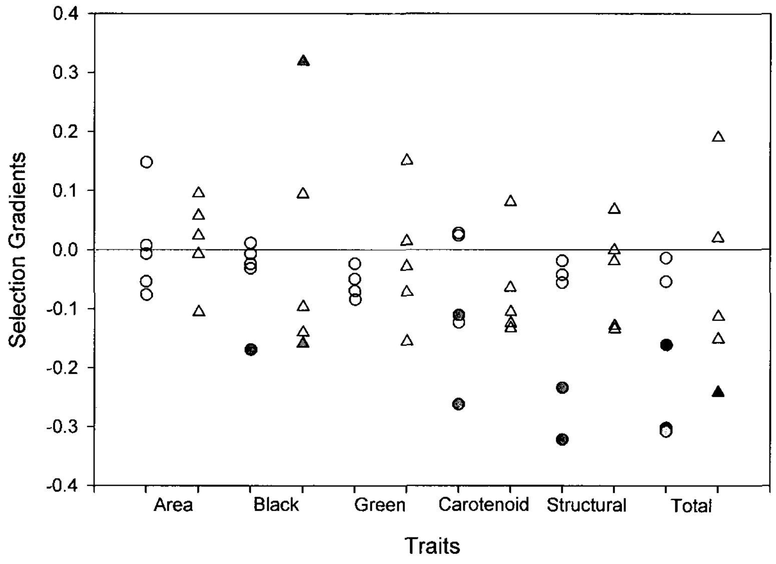

Figure 2.1: Selection Gradients for Color Traits for all experiment/trait combination considered in this study. Circles represent gradients associated with low-predation experiments. Triangles represent gradients associated with high-predation experiments. Statistical support is represented by the shading in the symbol interiors (grey fill $P < 0.1$; black fill $P < 0.05$).

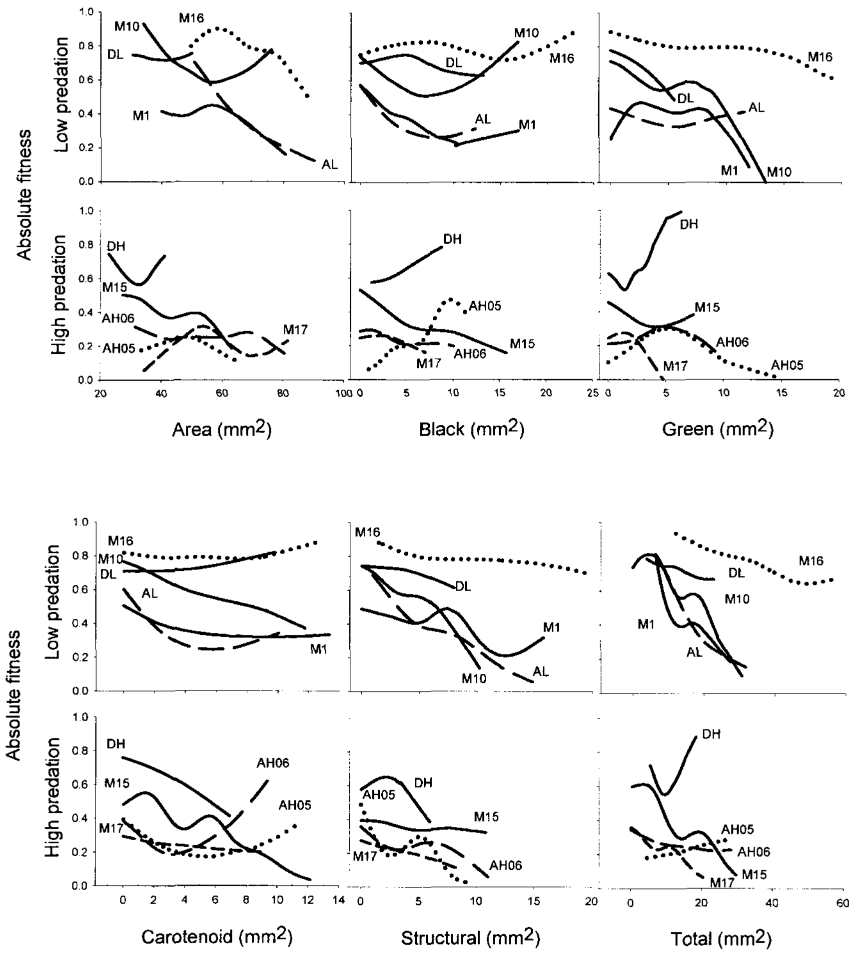

Figure 2.2. Cubic Splines for Color Traits depicting the relationship between various color traits considered in this study and absolute fitness. To facilitate interpretation, experiments with lines that are very close together (on the figure) have been assigned different dash patterns.

Differences in the prevalence and strength of selection between high- and low-predation experiments were not as overt as I had expected (Figures 2.1, 2.2). Indeed, more bouts of significant selection *against* color components or total color were encountered in low-predation experiments than in high-predation experiments (Table 2.4, Figure 2.1). The ANCOVA analyses, combining all selection experiments, revealed no support for differences in selection associated with any color traits between high-predation and low predation experiments (interaction terms: Table 2.5) , suggesting that the magnitude and direction of selection was similar in both regimes. Therefore, I focus on interpreting the results of the ANCOVA models that estimated universal selection gradients (experiments from both regimes pooled). In these models, selection against structural and total color was strong and well-supported statistically, but coefficients associated with body area, green color, black color, and carotenoid color were not.

Fluctuating selection was occasionally suggested by comparisons of selection gradients (Table 2.6) between the early and late episodes for the five experiments with two sequential recapture events (M1, M10, M16, M17, AH06). For example, in the high-predation experiment at AH06 the sign of selection gradients for four out of five color traits was reversed in the second recapture episode. In this experiment, selection gradients for green, carotenoid, structural and total colors were *negative* in the first episode (only structural and total were significant), and *positive* in the second episode (all significant except total color). Note that for this experiment, the selection gradients that estimated selection over the entire duration of the experiment (both recapture episodes) were insignificant for all traits (Table 2.4).

Table 2.5. Results of the ANCOVA Selection Models for Color Traits that 1) tested for significant differences in selection between the two putative regimes (Trait x Regime *P*), 2) estimated selection coefficients separately within low-predation (LP β) and high-predation regimes (HP β), and 3) estimated universal selection coefficients (Universal β) with experiments from both regimes pooled. These analyses do not include the Damier River experimental introductions.

Trait	Trait x Regime *P*	LP β	*P*	HP β	*P*	Universal β	*P*
Area	0.91	-0.024	0.70	-0.01	0.92	-0.027	0.65
Black	0.82	-0.031	0.41	-0.013	0.86	-0.23	0.57
Green	0.65	-0.027	0.50	-0.068	0.40	-0.033	0.45
Carotenoid	0.17	-0.048	0.18	-0.152	0.02	-0.094	0.128
Structural	0.33	-0.097	0.028	-0.20	0.037	-0.12	0.011
Total	0.28	-0.13	0.0078	-0.24	0.011	-0.17	0.001

Table 2.6: Selection Gradients for Color Traits Calculated Separately for Early (β1) and late (β2) Recapture Episodes (*$P < 0.1$, **$P < 0.05$) (only experiments with two recapture episodes).

	Area		Black		Green	
Experiment	β1	β2	β1	β2	β1	β2
M1	0.15	0.00	-0.05	-0.23	-0.06	-0.02
M10	-0.03	0.04	0.03	-0.07	0.02	-0.09
M17	-0.04	0.23	-0.09	-0.02	-0.07	-0.08
M16	0.12	-0.13	0.06	-0.07	-0.04	-0.08
AH06	0.02	0.12	-0.02	**-0.33***	-0.08	**0.31****

Table 2.6 continued.

	Carotenoid		Structural		Total	
Experiment	β1	β2	β1	β2	β1	β2
M1	-0.05	-0.15	**-0.17***	-0.13	-0.15	**-0.33****
M10	-0.07	-0.07	-0.07	0.03	**-0.09****	**-0.16****
M17	-0.18	0.13	0.06	-0.04	-0.19	0.03
M16	0.05	0.02	-0.04	-0.02	0.04	-0.07
AH06	-0.03	**0.26***	**-0.24****	**0.28***	-0.17	0.16

2.3.3 Relating Selection to Divergence

Consistent with previous work, male guppies from low-predation experiments were larger, on average, than were those from high-predation experiments (Table 2.7). Also, the DFA identified body size as an important variable discriminating between predation regimes (Table 2.7, Figure 2.3). I also found significant differences in coloration between males from high- and low-predation experiments. Consistent with previous work, low-predation guppies were more colorful for their size than were high-predation guppies (Table 2.7, Figure 2.3), particularly in structural colors. Note, again, however, that for each color trait average values overlapped somewhat between high- and low-predation experiments: e.g., some high-predation experiments had more structural color than some low-predation experiments. Moreover, not every color category followed the predictions; high-predation guppies actually had a greater total area of carotenoid color spots for their size than did low-predation guppies (Table 2.7, Figure 2.3). The multivariate DFA supported this trend, with low-predation guppies toward the structural color end of the discriminant function and high-predation guppies toward the carotenoid color end (Table 2.7, Figure 2.3). Low predation populations had more black and green, but these colors did not load as strongly on the DFA (Table 2.7, figure 2.3).

Table 2.7: Population Mean Values for Color Traits (± SEM) for all ten sites. "Regime means" represent the LS adjusted mean values for each type of predation regime, F-ratio and p-values correspond to the predation effect in the model (described in text). "DFA loading" is the canonical loading of each trait on the DFA axis that maximized differences between the high-predation (negative loading) and low-predation (positive loading) populations.

	Body area	Black	Green	Carotenoid	Structural	Total
Low predation						
M16	68.29 ± 1.43	9.16 ± 0.72	7.58 ± 0.54	4.74 ± 0.33	7.67 ± 0.5	29.16 ± 1
M1	55.15 ± 0.72	4.17 ± 0.21	3.33 ± 0.21	2.67 ± 0.18	6.36 ± 0.32	16.53 ± 0.42
M10	51.44 ± 0.51	2.5 ± 0.18	1.76 ± 0.15	3.13 ± 0.2	2.1 ± 0.14	9.48 ± 0.52
DL	38.76 ± 0.45	4.85 ± 0.28	1.51 ± 0.15	3.68 ± 0.24	2.64 ± 0.19	12.67 ± 0.45
AL	67.83 ± 0.79	4.37 ± 0.27	5.04 ± 0.26	2.98 ± 0.19	7.01 ± 0.3	19.4 ± 0.54
High predation						
M15	46.12 ± 0.43	4.24 ± 0.18	1.96 ± 0.09	4.45 ± 0.13	2.04 ± 0.1	12.69 ± 0.32
AH05	48.35 ± 0.6	5.05 ± 0.24	3.84 ± 0.28	3.54 ± 0.2	3.58 ± 0.2	16.01 ± 0.43
AH06	49.87 ± 0.52	3.15 ± 0.16	3.31 ± 0.16	3.19 ± 0.13	3.3 ± 0.15	12.95 ± 0.31
M17	51.88 ± 0.87	3.01 ± 0.16	0.73 ± 0.11	3.64 ± 0.19	2.11 ± 0.19	9.49 ± 0.42
DH	33.67 ± 0.58	3.52 ± 0.21	2.11 ± 0.15	3.46 ± 0.17	1.35 ± 0.13	10.44 ± 0.34
Regime means						
LP	56.58 ± 0.34	4.72 ± 0.13	3.47 ± 0.11	3.15 ± 0.1	4.5 ± 0.11	15.85 ± 0.22
HP	45.98 ± 0.31	4.15 ± 0.12	2.78 ± 0.1	4.03 ± 0.1	3.12 ± 0.1	14.07 ± 0.2
F-ratio	540.05	8.42	19.79	32.84	79.37	30.31
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
DFA loading	0.515	0.102	0.082	-0.257	0.561	n/a

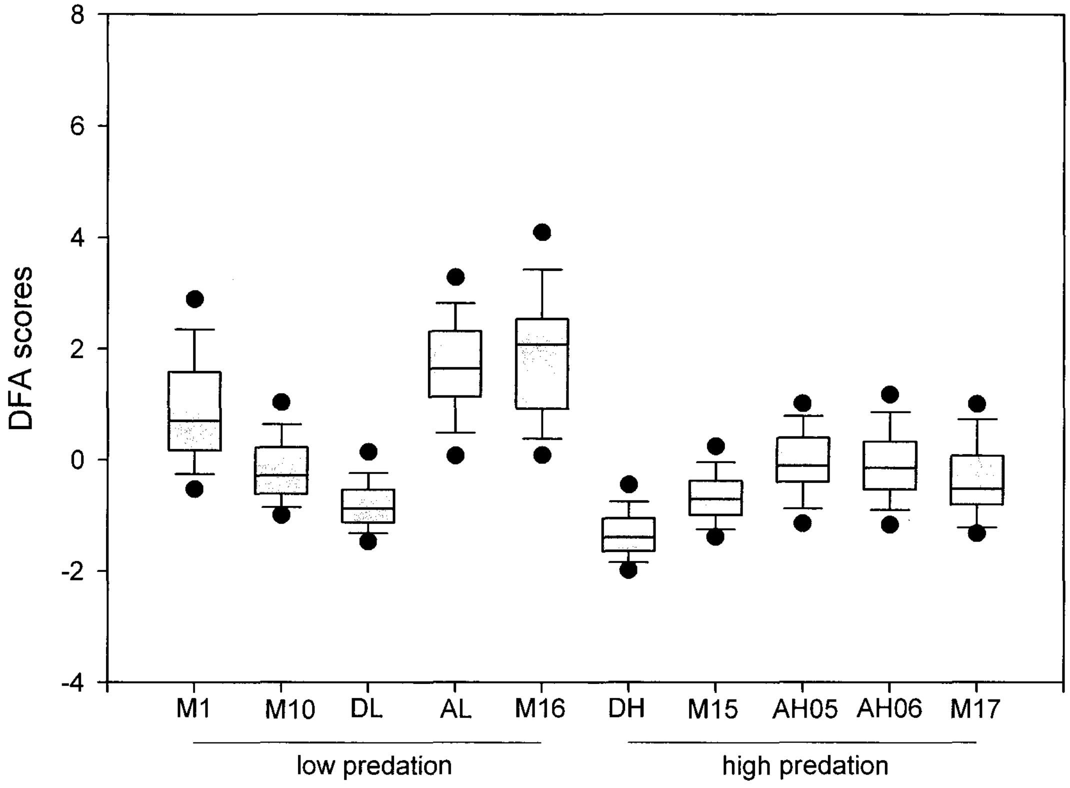

Figure 2.3. Visual Summary of Discriminant Function Analysis (DFA) that differentiated between high- and low-predation sites based on body area and color traits (black, green, carotenoid and structural). Boxplots of DFA scores (based on variation in body area, black, green, carotenoid, and structural colors) for all ten sites. Dots represent the 95th and 5th percentiles. Note that DH and DL were the result of an experimental introduction of guppies that originated from the high-predation section of the nearby Yarra River in 1996, for more detail regarding trait divergence in these populations consult Karim et al. (2007) and Gordon et al. (2009).

Analyses of the relationship between experiment-specific values of male color and experiment-specific estimates of selection were significant for only a single color trait. Here I detected a significant negative relationship between the strength of selection against structural colors and the mean relative area of structural colors ($R^2 = 0.46$, $F_{1,9} = 6.71$, $P = 0.032$, Figure 2.4). Experiments with initially more structural color experienced stronger selection against structural coloration. I found no evidence that the relationship between selection and trait values differed between predation regimes (all interactions were insignificant).

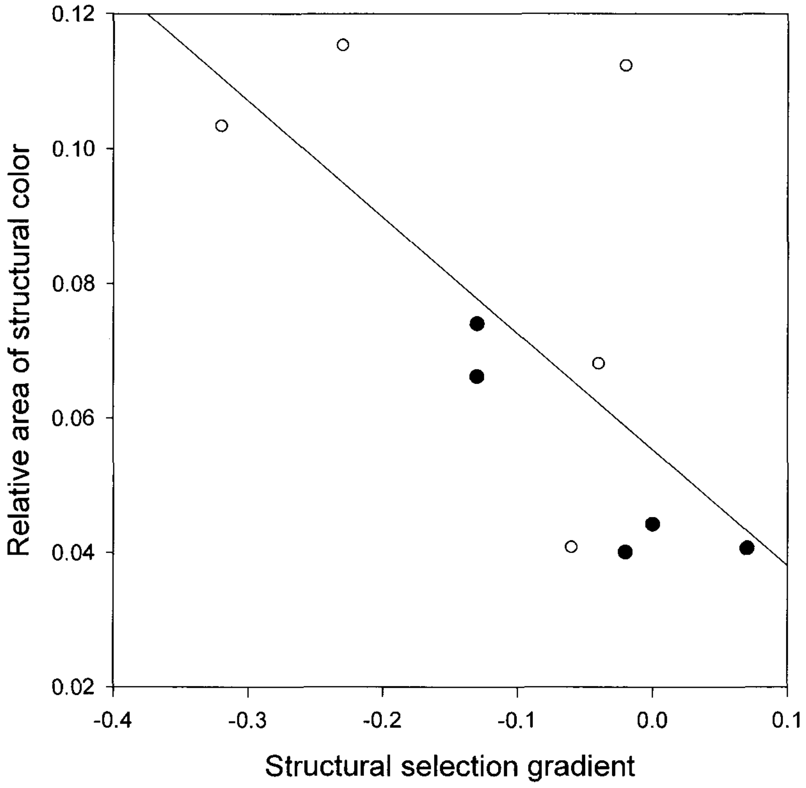

Figure 2.4. Relationship Between the Strength of Selection on Structural Color and the Average Amount of Structural Color among the 10 experiments ($R^2 = 0.46$, $F_{1,9} = 6.71$, $P = 0.032$). Open symbols represent low-predation experiments. Filled symbols represent high-predation experiments. The negative relationship suggests that I measured stronger selection against structural coloration in experiments that had higher initial mean values for structural color.

2.4 Discussion

My objective was to obtain the first formal linear estimates of selection on male guppy coloration from replicated mark-recapture experiments in the wild, and to then use those replicate estimates to test *a priori* hypotheses about the role of natural selection in shaping geographic patterns of male color variation. My results broadly support many existing perspectives about the selective basis for color variation, but also suggest some important nuances.

2.4.1 Prevalence and Strength of Natural Selection

Consistent with my first hypothesis, the presumed effects of predators, and several other guppy mark-recapture studies (Reznick et al. 1996; Gordon et al. 2009), mortality rates were, on average, higher in high-predation experiments than in low-predation experiments. However, mortality rates were also quite variable, especially in high-predation experiments (Table 2.2). It is worth noting that not all of the unrecaptured guppies were necessarily eaten by aquatic predators; other agents of mortality (starvation, parasitic infection, or bird predators) may account for some of the variable mortality in my mark-recap experiments. It would be very useful to consider these factors in future work.

My second hypothesis was that selective tradeoffs between natural and sexual selection in guppies should cause more colorful male guppies to experience greater mortality. Consistent with this prediction, selection coefficients for color pattern elements were mostly negative, directly confirming for the first time that viability selection in the wild generally disfavors male guppies with exaggerated color patterns. Within this general pattern, however, selection varied extensively in space and time. Indeed, in one high-predation experiment in particular (AH06), separate selection gradients for the early and late recapture episodes (of the same selection experiment) revealed opposite directions of selection – a complexity that was obscured by selection estimates that spanned both recapture intervals. Without experimental manipulation of potential causative agents, I can only speculate about specific causes of this spatiotemporal variation in selection. One possibility is frequency-dependent selection by predators results in a rare-type viability advantage (Olendorf et al. 2006). Another possibility is spatiotemporal variation in the abundance and distribution of predators or other environmental factors that influence risk of predation, such as light availability and spectral properties of the water, both of which could vary spatially and temporally (for example, increased turbidity during periods of higher rainfall).

Spatiotemporal variation in selection is not entirely unexpected (Siepielski et al. 2009). For example, general reviews of selection and contemporary evolution in the wild (Hendry and Kinnison 1999; Hoekstra et al. 2001; Kinnison and Hendry 2001) indicate that estimates over longer periods of time (multi-generational) are weaker, probably owing to a tendency for temporal averaging over periods of fluctuating selection across generations. Even within generations or cohorts, however, conflicting selection on a trait during an individual's ontogeny is predicted to result in evolutionary trade-offs that constrain the direction of evolution (Schluter et al. 1991). Indeed, consistent directional selection might be relatively rare outside of specific contexts; for example, immediately following the colonization of a novel habitat (Clegg et al. 2008), in the face of ongoing gene flow (Bolnick and Nosil 2007), or owing to human perturbations (Darimont et al.

2009). That said, my own assessment of selection in introduction sites on the Damier River (nine years after these population were established) did not reveal any significant selection.

The biological relevance of spatiotemporal variation in selection has been stressed in recent work examining the spatial scale of adaptation (Svensson and Sinervo 2004; Garant et al. 2007b), apparent trait stasis (Hendry and Kinnison 1999; Merilä et al. 2001), the maintenance of genetic variation (Roff 1997), and the evolution of phenotypic plasticity (Huber et al. 2004). Perhaps one of the most enduring questions in guppy biology surrounds hypothesized mechanisms that maintain extreme levels of male color polymorphisms. Thus far, support has been provided for the roles of mate choice (Endler and Houde 1995; Hughes et al 1999; Gamble et al. 2003), frequency dependent selection (Olendorf et al. 2006), and negative genetic correlation between attractiveness and survival (Brooks 2000). Here I provide evidence that high spatiotemporal variation in natural selection, coupled with the relatively short lifespan of guppies, should be considered an additional candidate mechanism (but see Hedrick 2000 for a theoretical discussion of the role of spatiotemporal variation in maintaining genetic polymorphism).

The pattern and strength of spatiotemporal variation in selection also have important consequences for a broader set of eco-evolutionary interactions (Yoshida et al. 2003; Hanski and Saccheri 2006; Kinnison and Hairston Jr. 2007; Pelletier et al. 2007). Clearly, any broader understanding of the ecological relevance of ongoing evolutionary processes requires not only a demonstration of the population, community, and ecosystem consequences of intraspecific trait variation (for example, Palkovacs et al. 2009), but also studies that reveal the spatial and temporal grain over which selection and evolution may shape such dynamics (Siepielski et al. 2009). The variation in selection documented in this study suggests that such eco-evolutionary effects might occur on very fine spatiotemporal scales.

2.4.2 Regime Specific Selection

Given the general pattern of selection against color and the considerable variability in selection among mark-recapture experiments, it is noteworthy that I did not find support for the hypothesis that the strength of selection is generally greater in high-predation habitats (hypothesis 3). This can be seen by considering patterns in the experiment-specific selection estimates and in the ANCOVA analyses that combined experiments. Despite evidence of higher mortality rates in high-predation experiments, experiment-specific estimates detected *significant* selection against color in only a single high-predation experiment (M15). In contrast, selection against color was evident for at least three color traits in three out of five low-predation experiments (Fig. 2.1; Tables 2.3 and 2.4). When I combined the multiple experiments into a single ANCOVA analysis comparing predation regimes, I found support for universal selection against structural color and total color, but no significant regime-by-color interaction terms that would suggest differences in selection between the high- and low-predation habitats at large. On the other hand, this finding is consistent with suggestions by some authors that predators in low-predation sites (e.g., *Rivulus harti* and *Macrobrachium* spp.) might impose significant mortality and color selection in their own right (Endler 1978, 1980, 1983; Millar et al. 2006; Kemp et al. 2008; McKellar et al. 2009).

The fact that I did not find evidence of divergent selection between predation regimes warrants further discussion. It is difficult to rule out the possibility that the spatiotemporal replication of selection experiments (which estimated selection during relatively narrow windows of time) was inadequate to detect some rare, but strong, bouts of natural selection that might have disproportionate effects on color traits in high-predation sites. Some previous authors have also suggested that predators are not the only environmental factor shaping geographic variation in male color in Trinidadian guppies. Other environmental variables that may mediate color pattern evolution include canopy openness, primary production, and variation in water transmission properties have been suggested as factors (Kodric-Brown 1989; Grether et al. 1999, 2001a; Millar et al. 2006). Guppy population are known to differ in susceptibility to parasite infection (Van Oosterhout et al. 2003), such variation may also influence color pattern evolution through viability and sexual selection (Houde and Torio 1991; Van Oosterhout et al. 2003).

2.4.3 Relating Selection to Divergence

Although I did not detect significant differences in selection at a regime level, it is important to note that I did nonetheless detect differences in male color between predation regimes. Phenotypic color comparisons from this study were broadly consistent with the major conclusions of previous studies comparing males from high- and low-predation sites (Endler 1978, 1983; Reznick 1982; Reznick and Endler 1982; Magurran 2005; Millar et al. 2006; Kemp et al. 2008). For example, the observation of greater structural coloration (i.e., blues and iridescent colors) in low-predation experiments (compared to high-predation experiments) is similar to earlier surveys and experiments (Endler 1978, 1980). In contrast, I did not find greater carotenoid color in low-predation experiments, but other studies have also found variable results in this regard (Endler 1978, 1980, 1983). Likewise, recent studies that have compared the coloration of high- and low-predation guppies have documented a diverse range of outcomes despite some generally recognized trends (for example, Alexander et al. 2006; Millar et al. 2006; Karim et al. 2007; Schwartz and Hendry 2007; Kemp et al. 2008).

Several explanations have been advanced for why variable results are obtained when comparing high- and low-predation guppy populations (Schwartz and Hendry 2007; Millar et al. 2006; Kemp et al. 2008). I can here address one of these explanations: divergent selection might be temporally variable or even episodic. Under these conditions, different geographic surveys of standing variation might yield different patterns, particularly if strong bouts of selection had recently acted in different populations in different studies. In this framework, focused and temporally-replicated studies of selection in the wild can supplement surveys of geographical variation in trait values (for example, Endler 1978; Millar et al. 2006; Alexander et al. 2006) by drawing attention to the finer dynamics that likely contribute to, but also complicate, geographic patterns.

Irrespective of regime-specific patterns of selection and color, it is nonetheless useful to inquire whether experiment-specific patterns of selection are in any way related to local color variation (hypothesis 4). In general, patterns of contemporary viability selection did not strongly predict broad patterns of trait divergence. If so, I would have expected stronger selection against color to be associated with less male coloration. If anything, the converse appears to be truer – greater amounts of structural color were positively associated with stronger selection against structural colors. (Figure 2.4). This might suggest that the detection of selection on structural color is in part linked to the phenotypic opportunity for selection (i.e., the total amount of color variation present). For most color traits, however, no clear associations were detected, which may suggest that past selection and local adaptation have reduced the phenotypic opportunity for contemporary selection on some traits (for example, Clegg et al. 2008), particularly in high-predation sites.

The discordant relationship between patterns of natural selection and trait variation (greater areas of particular colors in experiments where those colors are disfavored by natural selection) might best be explained by dynamic tradeoffs between natural (i.e., viability) selection and sexual selection. In some sites, stronger sexual selection for male color might pull male traits further from the optimal with respect to natural selection, and thereby generate stronger, and detectable, natural selection. Of course, this hypothesis in turn raises the question of what factors might cause spatiotemporal variation in the strength or pattern of sexual selection?

Research on sexual conflict has revealed that male guppies can obtain fertilizations either by attracting a female through mating displays or through coercive mating behaviors (Houde 1997; Magurran 1998, 2005; Godin 1995). The elevated mortality risk in high-predation sites might favor males that engage in sneaky copulation attempts, instead of complex mating displays (Godin 1995). Moreover, guppies in high-predation sites often show more schooling behavior (Seghers and Magurran 1995), which might further enhance opportunities for males to succeed in coercive matings. Finally, females might be less attracted to, or discriminating against, displaying males if such displays increase predation risks for females (Godin and Briggs 1996). Accordingly, some studies have reported that female preferences for male colors are weaker in high-predation sites (Schwartz and Hendry 2007). Collectively, these possibilities suggest that predator environment could influence net selection on color indirectly through its effects on the strength and pattern of sexual selection.

In short, geographical patterns of color variation may in some cases be more directly explained by environmental (predatory) modulation of sexual selection than by the direct effects of viability selection. However, additional studies that simultaneously estimate natural and sexual selection (for example, Hamon and Foote 2005; Svensson et al. 2006) would be required to formally address this hypothesis in the guppy system. Interestingly, sexual selection gradients have been estimated for low-predation guppies under laboratory conditions by Brooks and Endler (2001). In that study, estimates of sexual selection gradients for areas of black, iridescent and orange colors were comparable in scale but opposite in sign to my estimates of natural selection (0.077, 0.205 and 0.127 respectively: Brooks and Endler 2001). Such comparability of scale, but opposing sign, suggests that natural and sexual selection might interact strongly, leading to diverse and unstable evolutionary outcomes for male color at various sites and times.

2.4.4 Summary

I documented considerable spatiotemporal variation in viability selection both within and among the classically categorized predation regimes experienced by wild guppy populations. My study supports previous inferences in that mortality rates are greater in high-predation sites, and that natural selection broadly disfavors male guppies with more color. However, it does not support the prediction that natural selection against color would generally be stronger in high-predation sites. Indeed, natural selection measured in any given low-predation experiment was often as strong, or stronger, than natural selection measured in any given high-predation experiment. Moreover, patterns of natural selection did not readily predict geographic variation in guppy color. I suggest that one explanation for this discordance with geographical patterns of color divergence may be that the role of visual-hunting fish predators should be deconstructed into direct effects (viability selection by predators against colorful males) and indirect effects resulting from predation's influence on sexual behavior (decreased sexual selection favoring colorful males in habitats with higher extrinsic mortality). Real-time studies of the interaction of natural and sexual selection in the wild, perhaps using natural pedigrees, could ultimately yield more definitive insights into the relative importance of these components of selection on male color in this classic evolutionary system.

CHAPTER 3: SPATIOTEMPORAL VARIATION IN LINEAR NATURAL SELECTION ON BODY SHAPE IN WILD GUPPIES (*POECILIA RETICULATA*)

3.1 Introduction

The study of adaptation requires the consideration of functional trade-offs between different aspects of performance (Schluter et al. 1991; Arnold 1992; Ghalambor et al. 2003; Ghalambor et al. 2004; Walker 2007). In many circumstances, the process of natural selection is unable to optimize trait values in regards to one component of fitness (for example, survival), without compromising another aspect of fitness (for example, reproduction). Ideally, diverse types of studies are implemented to evaluate how interactions between multiple components of fitness influence phenotypic evolution in wild populations (Reznick and Travis 1996). The comparative method has been the most commonly used approach to infer adaptation by testing for significant associations between environmental features and phenotype (Endler 1986; Harvey and Pagel 1991; Schluter 2000); however, such studies typically make assumptions regarding the specific ecological agent of selection since neither individual performance, nor fitness, are measured. Laboratory experiments can be used to test for trade-offs between trait variation and aspects of performance, and can therefore more directly evaluate assumptions regarding the agent of selection suggested by comparative studies (Blake et al. 2005; Blake et al. 2009; Langerhans 2009a, 2009b). However, laboratory studies must assume that relationships between traits and performance discovered in the laboratory are consistent, and relevant to individual fitness, in the wild. Given these limitations, studies that directly measure the relationship between fitness and multiple, correlated traits in wild populations can offer an important complement to comparative and laboratory studies by directly assessing how specific traits directly and indirectly relate to variation in fitness among individuals (Lande and Arnold 1983; Endler 1986; Kingsolver et al. 2001).

This is not to say that studies of selection in nature are not without their own limitations. Selection studies must assume that the relationship between traits and fitness measured over a limited spatiotemporal scale are relevant to broader evolutionary patterns (Siepielski et al. 2009); that correlated, unmeasured traits are not strongly influencing the relationship between fitness and the measured traits (Mitchell-Olds and Shaw 1987); and that fitness gains conferred by a particular trait regarding one component of fitness are not cancelled out by fitness costs associated with another, unmeasured, component of fitness (Schluter et al. 1991). Nonetheless, inconsistent conclusions derived from selection studies and other approaches have the potential to suggest nuances regarding the process of adaptation, and may reveal important knowledge gaps in the trait – performance – fitness pathway (Arnold 1983). Here, I use direct estimates of natural selection in wild populations of Trinidadian guppies to test predictions distilled from comparative and laboratory studies of the functional significance and trade-offs of variation in fish body shape.

3.1.1 Ecomorphology of Pociliidae Body Shape

The performance trade-offs resulting from variation in body shape among fishes are one of the most widely-appreciated and intensively studied form-function relationships in vertebrate biology (for example, Webb 1982, 1984; Webb 1998; Walker 1997; Domenici 2003; reviewed in Blake 2004). In particular, livebearing fishes of the family Poeciliidae have become a model system for studying aspects of fish shape that are thought to influence predator avoidance (Langerhans et al. 2003; Langerhans and Dewitt 2004; Langerhans et al. 2004; Alexander et al. 2006; Hendry et al. 2006; Gomes and Monteiro 2008; Langerhans 2009a; 2009b; Langerhans and Makowicz 2009; Burns et al. 2009). Poeciliids, like many other fish species, evade attempted strikes by predators using an escape response referred to as "fast-start" swimming, which involves a rapid burst of unsteady swimming that propels the fish away from threatening stimuli (Webb 1978, Harper and Blake 1990; Domenici and Blake 1997; Walker et al. 2005; Langerhans 2009a). Biophysical principles are fairly clear in predicting that fast-start performance is maximized by a shape that is dorso-ventrally expanded posteriorly (which increases thrust), and dorso-ventrally compressed anteriorly (which decreases drag) (Walker 1997 and references therein). This "rear-weighted" morphology, while maximizing fast-start swimming, necessarily results in decreased swimming efficiency (Walker 1997; Langerhans et al. 2004). Thus, the general expectation is that poeciliids experiencing greater risk of predation should have body shapes that produce greater fast-start speeds than fish inhabiting habitats with a lower risk of predation which should have body shape maximizing efficiency.

This ecomorphological paradigm (Langerhans et al. 2004) has been tested by several studies that have compared the morphologies of poeciliids from high- and low-predation habitats (Langerhans et al. 2003; Langerhans and Dewitt 2004; Langerhans et al. 2004; Alexander et al. 2006; Hendry et al. 2006; Gomes and Monteiro 2008 Langerhans and Makowicz 2009; Burns et al. 2009), and laboratory experiments that consider the performance (Langerhans et al. 2004; Langerhans 2009a, 2009b) and survival (Langerhans 2009b), consequences of variation in shape among individuals. However, regarding the Trinidadian guppy in particular, differences in body shape between high- and low-predation populations have not been consistent among several studies (Burns et al. 2009). In some cases, thicker caudal peduncles are associated with low-predation sites; while in other cases, thicker caudal peduncles are associated with high-predation sites (see Table 1 in Burns et al. 2009). A possible explanation for this inconsistent pattern is spatiotemporal variation in selection (Burns et al. 2009). The direction and magnitude of selection associated with shape may vary between regimes, among populations within regimes, and temporally within populations (Siepielski et al. 2009).

Furthermore, regarding Trinidadian guppy females specifically, this ecomorphological paradigm is nuanced by a functional trade-off between swimming performance and reproduction. In high-predation sites, elevated levels of extrinsic mortality have resulted in the evolution of earlier maturity, increased fecundity, shorter interbrood intervals, and larger reproductive allocation compared to low-predation populations (Reznick and Endler 1982; Reznick et al. 1990). Ghalambor et al. (2003, 2004) hypothesized that these life-history adaptations in Trinidadian guppies may constrain the adaptive evolution of fast-start swimming performance. Empirical support for the functional trade-off suggested by Ghalambor et al. (2003, 2004) has been provided by laboratory studies that have compared fast-start swimming performance between high- and low-predation guppies over several stages of pregnancy. Consistent with predictions, high-predation guppies had better fast-start swimming performance, compared to low-predation guppies (Ghalambor et al. 2004). However, performance declined with increasingly advanced stages of pregnancy, and this decline was more dramatic for the high-predation guppies, suggesting that that the evolution of swimming performance and reproductive allotment were mutually constrained. However, whether or not decreased fast-start swimming performance in laboratory trials translates into decreased survival in the wild is unknown. I propose to evaluate the fitness costs of this functional trade-off by measuring natural selection associated with abdomen distension (a proxy for pregnancy) in replicated populations of wild Trinidadian guppies.

Despite extensive work on the adaptive significance of variation in fish shape, I am unaware of any study that has directly tested the prediction that aspects of fish body shape thought to maximize fast-start performance are significantly related to fitness in wild populations. I suggest that measurements of natural selection associated with fish shape will provide valuable insights into the functional trade-offs between different aspects of performance (acceleration versus efficiency, and predator evasion versus reproduction). Specifically, I propose to directly measure natural selection associated with body shape in replicated high- and low-predation populations of Trinidadian guppies (see chapter 2 for a more comprehensive description of the Trinidadian guppy system). Based on previous work (see above), I made the following predictions. 1) For both males and females, natural selection should favor individuals with thicker caudal peduncles and relatively smaller heads reflecting improved ability to evade predator strikes resulting from elevated fast-start swimming performance (for example, Langerhans et al. 2004; Langerhans 2009b). 2) For females, increased abdomen distension should be disfavored by natural selection (Ghalambor et al. 2003, 2004). 3) Selection associated with both of these aspects of shape variation should be stronger in high-predation habitats, reflecting the increased predation risk associated with predatory fishes (see chapter two).

3.2 Methods

Recapture data and photographs from the 10 experiments described in chapter two were used to estimate selection associated with variation in shape (refer to chapter two for information regarding specific sample sites, mark-recapture techniques, and photography techniques). Unlike my consideration of selection associated with color (chapter two), I was interested in quantifying selection associated with shape for both males and females, and thus table 3.1 contains sample size and basic mark-recapture data for females for seven of the ten selection experiments described in chapter two. Females were only available for seven experiments, because the females from three of these mark-recapture experiments (AH05, AL, and M16) were used for a separate experiment that is incompatible with the measurement of natural selection. As in chapter two, I used t-tests with the different selection experiments as the unit of replication to evaluate regime differences in both daily mortality rates and killing power.

Table 3.1. Summary of Female Mark-Recapture Information for the 7 experiments used to estimate selection associated with body shape. Capture efficiency is the proportion of guppies captured at the first recapture episode (Recap 1), divided by the number known to be alive based on the second recapture episode (Recap 2). Daily mortality rate (Mort rate) is the estimated percentage of the original number released fish that died per day. Killing power (daily exponential mortality rate) is log10(N released) minus Log10(N at final recap) then divided by the duration of the experiment (T). Information for Recap 2 and Capture efficiency are not applicable (n/a) for experiments with only a single recapture event.

			Recap 1		Recap 2	
Site	Release date	*N* released	*N*	T (days)	*N*	T (days)
Low predation						
M1	6/29/2004	193	154	11	74	67
M10	5/19/2005	280	236	14	187	30
DL	3/27/2004	134	111	12	n/a	n/a
High predation						
M15	3/28/2004	311	176	13	n/a	n/a
AH06	4/3/2006	202	173	15	109	44
M17	6/26/2004	211	117	13	55	66
DH	3/28/2004	105	72	12	n/a	n/a

Table 3.1 continued.

Site	Capture efficiency	Mort rate	Killing power
Low predation			
M1	90.94	0.009	0.006
M10	86.94	0.011	0.006
DL	n/a	0.014	0.012
High predation			
M15	n/a	0.033	0.011
AH06	93.97	0.010	0.006
M17	80.84	0.011	0.009
DH	n/a	0.026	0.012

3.2.1 Shape Analysis

Fish shape was quantified using geometric morphometrics following the techniques described in Hendry et al. (2006) and Burns et al. (2009). This technique implements a landmark-based approach that eliminates the effect of variation in the location, orientation, and scale of the different specimens (Bookstein 1991). To obtain landmark coordinates, a standardized grid was superimposed onto the digital image of each fish along the nose-to-tail axis (e.g. Langerhans et al. 2003; Hendry et al. 2006; Burns et al. 2009). This grid consisted of a line, 100 arbitrary units in length, which was anchored at the middle of the eye and the tip of the caudal peduncle. This line was divided by perpendicular vertical lines placed at 0, 20, 40, 60, 80, 90, and 100 units (Figure 3.1). For each fish, the grid was resized so that the proportions of the grid remained constant while the grid was stretched to accommodate fish of varying sizes. Grid construction, superimposition, positioning, and resizing were performed using Adobe Photoshop. TPSDIG (Rohlf, 2003) was then used to place 18 landmarks on the digital photo of each fish (that now had a superimposed grid). Six of these landmarks can be considered homologous points: landmark 1 (tip of snout), landmark 3 top of eye orbit, landmark 4 (middle of the eye orbit), landmark 5 (bottom of the eye orbit), and landmark 12 (end of caudal peduncle), and landmark 18 (inflection point where the operculum meets the body outline). The remaining 12 landmarks (2, 6-11 and 13-17) were placed where the vertical lines of the grid intersected the outline of the fish (Figure 3.1). Fin insertions were not used as landmarks because I was concerned that the excessive handling required to properly and consistently display the fins would have negatively impacted the health of the fish which were to be used in mark-recapture experiments. The grid method nonetheless captures shape variation that would have certainly been missed by only considering the available homologous landmarks. Burns et al. (2009) performed an analysis using guppy shape data quantified using the grid method, and compared it to results obtained using a "non-grid" method and found the results to be qualitatively similar. Scale was established using markings on the laminated background included in each digital image.

Due to high levels of sexual dimorphism, the following analyses were performed separately for males and females. TPSRelW (Rohlf, 2003) was used to rotate, translate and scale landmark coordinates using generalized least squares superimposition (Bookstein, 1991). TPSRelW was also used to compute partial warps (which describe small-scale localized variation in shape) and uniform components (which describe variation along the x and y-planes). Additionally, TPSRelW computed a series of $2p - 4$ orthogonal relative warps (RWs: p = number of landmarks). RW scores are calculated for each individual and represent the extent of deviation from the consensus configuration (the multidimensional mean for the entire sample) along the particular axis of shape variation described by that RW. Morphological variation described by particular RWs can be visualized using deformation diagrams. As is customary for studies using geometric morphometrics, body size was quantified as centroid size. Although I am not here interested in selection associated with size, centroid size provided a covariate in some multivariate analyses (see below) to measure selection acting directly on shape variables.

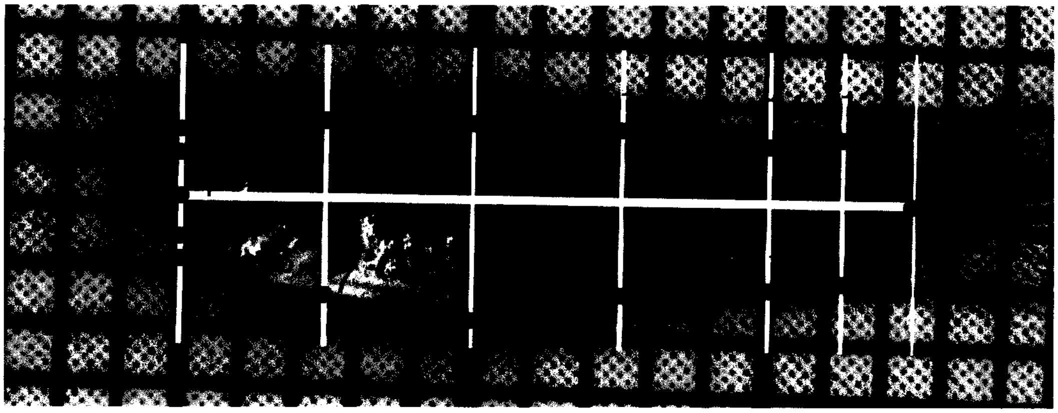

Figure 3.1. Landmarks Used for Geometric Morphometric Analyses.

3.2.2 Measurement of Natural Selection

Generally, techniques used to assign individual fitness were identical to the approaches described in chapter two. An individual's absolute fitness was the length of time it was known to be alive. For experiments with one recapture episode, surviving individuals were assigned an absolute fitness of one and individuals that were not recaptured were assigned an absolute fitness of zero. In experiments with two recapture episodes, guppies that survived the entire duration of the experiment were assigned an absolute fitness of one, and guppies that survived to the first recapture episode (but not the second) were assigned an absolute fitness proportional to the interval of time between the first and second recapture episode (Brodie and Janzen 1996). Every individual's absolute fitness was converted to relative fitness by dividing by the population mean (Lande and Arnold 1983). Relative fitness was calculated separately for males and females for each experiment.

Relatively few studies have attempted to measure natural selection associated with shape in the wild, and no formal method has been developed (Gomez et al. 2006). Therefore, I used multiple approaches to quantify and visualize the relationship between body shape and fitness, but I will focus on interpreting selection gradients (see below) as this approach (calculating selection gradients associated particular RWs) has some precedence (Klingenberg and Leamy 2001; Klingenberg and Monteiro 2005; Gomes et al. 2006; Gomes et al. 2008; Benitez-Vieyra et al. 2009). Selection coefficients (differentials and gradients) associated with the RWs were calculated using techniques similar to those described in chapter 2. To decrease the number of parameters in regression models, I analyzed only the RWs explaining more than one percent of the variation in shape (Klingenberg and Leamy 2001: Gomez et al. 2006; Gomez et al. 2008). For both males and females this criteria resulted in the inclusion of RWs 1 -11 (Table 3.2). All selection analyses were performed separately for males and females within each selection experiment using sex-specific RWs. RWs were standardized within each experiment to a mean of zero and a standard deviation of unity (Lande and Arnold 1983; Brodie et al. 1995). For experiments with two recapture episodes, simple linear regressions (fitness predicted by individual RWs) were used to calculate selection differentials (regression coefficients) and multiple regressions (fitness predicted by all RWs and centroid size) were used to calculate selection gradients (partial regression coefficients associated with particular RWs). As in chapter two, selection gradients were calculated separately for the first and second recapture episodes (for experiments with two recapture episodes) in order to assess temporal variation in the pattern and strength of selection. For selection experiments with only one recapture episode, selection differentials were calculated using logistic regressions, and selection gradients were calculated using multiple logistic regressions. For these experiments, the relevant coefficients resulting from the logistic regression were converted to their linear equivalents following the methods of Janzen and Stern (1998). As in chapter 2, I set my alpha level at 0.1, but considered *P*-values between 0.05 and 0.10 as less conclusive support for a hypothesis than *P*-values less than 0.05. To visualize the pattern of natural selection associated with particular RWs for individual selection experiments, I generated cubic spline diagrams for RWs 1-11 in every experiment (Schluter 1988).

Table 3.2. Phenotypic Variation Explained by Relative Warps (RWs). Only RWs 1-11 were used for selection analyses (see Methods).

	Females		Males	
Relative Warp	% Variance	Cumulative	% Variance	Cumulative
1	30.33	30.33	34.09	34.09
2	24.91	55.24	25.29	59.38
3	10.92	66.16	7.27	66.65
4	8.82	74.99	6.63	73.27
5	6.41	81.40	4.88	78.16
6	5.00	86.40	4.42	82.58
7	2.34	88.75	2.85	85.42
8	2.00	90.75	2.49	87.92
9	1.56	92.31	1.86	89.78
10	1.34	93.65	1.56	91.34
11	1.00	94.65	1.15	92.49
12	0.74	95.39	0.99	93.48
13	0.67	96.06	0.95	94.43
14	0.60	96.66	0.78	95.21
15	0.52	97.18	0.68	95.89
16	0.45	97.63	0.56	96.46
17	0.38	98.01	0.52	96.97
18	0.31	98.33	0.47	97.44
19	0.16	98.49	0.27	97.72
20	0.16	98.64	0.25	97.97
21	0.15	98.79	0.23	98.20
22	0.15	98.94	0.23	98.42
23	0.14	99.08	0.22	98.64
24	0.14	99.22	0.21	98.85
25	0.13	99.34	0.20	99.05
26	0.12	99.47	0.19	99.24
27	0.12	99.58	0.18	99.42
28	0.11	99.69	0.16	99.57
29	0.10	99.79	0.14	99.72
30	0.09	99.88	0.12	99.84
31	0.06	99.94	0.09	99.92
32	0.06	100.00	0.08	100.00

As in chapter two, I was interested in comparing the broader pattern and strength of natural selection within and between predation regimes, combining data from all experiments. To do this, I generally followed the methods describing the ANCOVA selection models used to test for divergent selection associated with color traits between predation regimes (described in chapter two). For these analyses, differences in temporal interval were standardized by only considering the first recapture episode from each experiment, except for the males used in the low-predation experiment M16 in which the longer interval was used because only 4 fish died in the first interval. In contrast to the ANCOVA selection models described in chapter two, I included the experiments from the Damier river, because without the Damier low-predation population (DL) there would only be two low-predation experiments with females. Also, due to the increased number of independent variables (RWs 1 – 11), I did not enter all traits into a single ANCOVA model, and instead generated a series of ANCOVA models that considered each trait separately. First, using the combined data from all experiments, I generated a series of ANCOVA selection models with relative fitness as the dependent variable, independent variables included trait (RWs 1 -11 considered separately by different ANCOVA models), predation regime, experiment nested within predation regime, and an interaction term between trait and predation regime. Statistical significance of the trait x regime interaction terms would indicate that selection on that particular trait was different between regimes. Next, I considered each predation regime separately and generated a similar series of ANCOVAs with independent variables that included trait, experiment, and an interaction term between trait and experiment. Models without the interaction terms were then run to estimate regime-wide selection coefficients for each trait. Finally, I generated a model with all experiments pooled and no regime effect: independent variables were experiment and traits. As in chapter two, the coefficients resulting from these ANCOVA models cannot be considered true selection differentials because traits were standardized and relativized separately in each experiment. In contrast to chapter two, the coefficients resulting from these ANCOVA selection models describe both direct and indirect selection acting on a trait.

I was particularly interested in testing specific predictions regarding the relationship between fitness and aspects of shape variation (see introduction). However, other aspects of shape may also be under selection, and thus I performed an exploratory analysis to determine which aspects of shape variation were most directly linked to fitness – possibly including aspects of shape variation that I did not predict would be strongly related to fitness. To do this, I used TPSRegr (Rohlf 2003) to implement a multivariate general linear model that predicted shape variation (as described by the full series of partial warps and the uniform shape components) as a function of absolute fitness. Significance of the relationship between absolute fitness and multivariate shape was assessed using permutation tests for Wilks' lambda implemented in the same program. This program also permits the visualization of the deformation in shape from the consensus configuration that corresponds most directly to absolute fitness. These analyses were performed separately for males and females within each population.

3.3 Results

3.3.1Sampling Efficiency and Mortality Rates for Females

As was the case for males (see chapter two), my estimated recapture efficiencies for females were high (range = 81%-94%, mean = 88%) (Table 3.1). Females not recaptured were thus assumed to have perished. Consistent with my predictions and the results for males (described in chapter two), daily mortality rates were higher in high-predation experiments (mean = 2.0%, range = 1.0% - 3.3%) compared to low-predation experiments (mean = 1.1%, range = 0.9% - 1.4%), however, the difference in mean values was not significant ($P_{1,6} = 0.12$). Similarly, daily killing power was higher, on average, in high-predation experiments (mean = 0.0093, range = 0.006 – 0.012) compared to low-predation experiments (mean = 0.0079, range = 0.006 – 0.012), but this difference in mean values was also insignificant ($P_{1,6} = 0.27$). The lack of significant differences between the high and low-predation experiments in daily mortality rate and killing power, for females, is likely the result of lower statistical power (three low-predation experiments versus four high-predation experiments) compared to males (five high-predation experiments versus five low-predation experiments) for which differences in daily mortality rate and killing power were significant.

3.3.2 Selection on Female Body Shape

For females, there were nine significant selection differentials (*S*) (Table 3.3), absolute values ranging from 0.07 to 0.19, and four significant selection gradients (β) (Table 3.4), absolute values ranging from 0.09 – 0.19. I will first consider the analyses that assessed the relationship between fitness and select RWs that describe aspects of shape variation that I predicted *a priori* to be strongly related to survival (see introduction) – specifically RWs 2, 3, and 8 (see below). I will then consider *a posteriori*, any strong patterns that emerge from the remaining RWs and the multivariate general linear model, implemented in TPSRegr.

Table 3.3. Linear Selection Differentials (*S*) for Female RWs 1-11 in the 7 selection experiments. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	RelW1		RelW2		RelW3		RelW4	
Experiment	β	*P*	β	*P*	β	*P*	β	*P*
Low Predation								
M1	-0.02	0.74	**0.11**	**0.04**	0.03	0.57	0.04	0.43
M10	0.04	0.22	0.02	0.49	0.03	0.35	0.02	0.57
DL	0.00	0.95	1.74	0.68	-0.03	0.43	0.04	0.30
High Predation								
M15	-0.02	0.73	-0.01	0.77	**-0.11**	**0.03**	-0.05	0.32
M17	-0.05	0.58	0.11	0.21	**-0.19**	**0.03**	-0.04	0.69
AH06	0.06	0.16	0.00	0.96	0.02	0.59	**-0.07**	**0.09**
DH	**0.14**	**0.03**	-0.04	0.57	-0.03	0.62	-0.06	0.40

	RelW5		RelW6		RelW7		RelW8	
Experiment	β	*P*	β	*P*	β	*P*	β	*P*
Low Predation								
M1	-0.01	0.81	-0.06	0.26	-0.04	0.40	-0.02	0.74
M10	0.01	0.72	-0.04	0.17	0.02	0.47	0.02	0.45
DL	-0.05	0.25	0.01	0.72	0.03	0.44	-0.02	0.56
High Predation								
M15	0.01	0.80	-0.05	0.32	-0.01	0.80	**-0.10**	**0.04**
M17	-0.09	0.31	-0.14	0.11	-0.05	0.60	0.05	0.60
AH06	-0.02	0.70	0.00	0.93	-0.02	0.72	**-0.09**	**0.03**
DH	-0.01	0.85	0.07	0.33	-0.01	0.90	**0.12**	**0.07**

Table 3.3 continued.

	RelW9		RelW10		RelW11	
Experiment	β	*P*	β	*P*	β	*P*
Low Predation						
M1	-0.02	0.76	-0.02	0.71	-0.04	0.41
M10	-0.04	0.19	0.04	0.18	-0.03	0.29
DL	0.03	0.46	0.02	0.70	-0.02	0.54
High Predation						
M15	0.06	0.26	-0.03	0.54	-0.02	0.75
M17	0.00	0.99	**-0.17**	**0.05**	0.12	0.19
AH06	-0.04	0.34	-0.03	0.42	-0.07	0.13
DH	-0.09	0.19	0.00	1.00	0.02	0.71

Table 3.4. Linear Selection Gradients (β) for Female RWs 1-11 in the 7 selection experiments. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	RelW1		RelW2		RelW3		RelW4	
Experiment	β	*P*	β	*P*	β	*P*	β	*P*
Low Predation								
M1	-0.02	0.77	0.07	0.32	0.05	0.53	0.00	0.96
M10	-0.02	0.68	-0.03	0.56	0.02	0.73	0.03	0.32
DL	0.00	1.00	-0.04	0.43	-0.08	0.14	0.01	0.79
High Predation								
M15	0.04	0.54	-0.03	0.61	-0.08	0.26	-0.04	0.47
M17	0.00	1.00	0.00	0.98	-0.19	0.19	-0.01	0.96
AH06	0.07	0.27	-0.06	0.36	-0.02	0.73	-0.08	0.15
DH	0.11	0.18	0.01	0.91	0.00	0.96	-0.01	0.92

	RelW5		RelW6		RelW7		RelW8	
Experiment	β	*P*	β	*P*	β	*P*	β	*P*
Low Predation								
M1	-0.05	0.47	-0.10	0.12	-0.04	0.46	-0.06	0.37
M10	-0.05	0.20	-0.05	0.24	0.03	0.28	0.03	0.37
DL	-0.08	0.13	-0.01	0.87	0.03	0.40	-0.02	0.56
High Predation								
M15	0.09	0.17	-0.05	0.50	-0.04	0.48	**-0.15**	**0.01**
M17	-0.11	0.32	-0.08	0.49	-0.14	0.15	0.02	0.84
AH06	0.02	0.77	-0.06	0.36	0.01	0.81	**-0.13**	**0.01**
DH	0.06	0.49	0.01	0.98	0.01	0.90	0.11	0.12

Table 3.4 continued.

	RelW9		RelW10		RelW11	
Experiment	β	*P*	β	*P*	β	*P*
Low Predation						
M1	-0.02	0.70	-0.06	0.30	-0.06	0.36
M10	-0.01	0.75	0.01	0.80	-0.05	0.16
DL	0.07	0.18	-0.01	0.79	-0.01	0.88
High Predation						
M15	0.03	0.59	-0.02	0.67	0.07	0.26
M17	0.06	0.58	**-0.19**	**0.07**	0.08	0.42
AH06	-0.04	0.46	**-0.09**	**0.08**	-0.03	0.61
DH	-0.10	0.19	0.09	0.30	0.06	0.36

For females, RW2 quantifies variation in abdomen distension (figure 3.2), and explains 24% of the shape variation among individuals. Fish with a high score for RW2 have a less distended abdomen. Therefore, I predicted that selection coefficients would be positive. Surprisingly, selection associated with this RW appears to be quite weak. Considering the seven selection experiments separately, only one selection differential associated with female RW2 was significant, which was from the low-predation experiment M1 ($S = 0.11$, $P = 0.04$) (Table 3.3). The direction of the selection differential associated with female RW2 from the M1 experiment was consistent with my predictions; fish with a less distended abdomen had higher fitness. None of the selection gradients associated with female RW2 were significant (Table 3.4). The ANCOVA selection model for RW2 found no evidence of divergent selection between regimes (trait x regime $P = 0.37$), nor was there any evidence of a significant relationship between RW2 and fitness when data from all experiments were pooled (Table 3.5).

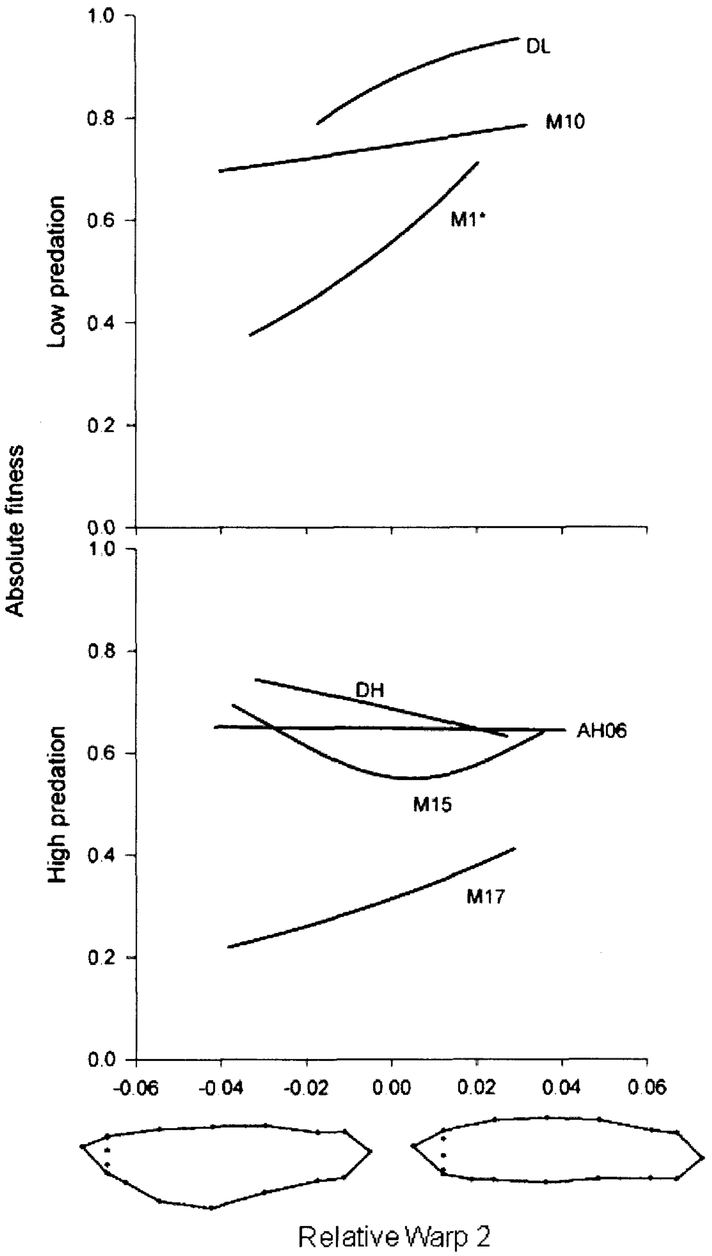

Figure 3.2. Cubic Splines for Female RW2. Cubic splines depict the relationship between phenotypic variation in female RW2 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant ($P < 0.05$) or marginally significant ($0.1 > P > 0.05$).

Table 3.5. Results of the ANCOVA Selection Models for Female RWs 1-11 that (1) tested for significant differences in selection between the two putative regimes (Trait x Regime *P*), (2) estimated selection coefficients separately with low-predation (LP *S*) and high-predation regimes (HP *S*), and (3) estimated universal selection coefficients (Universal *S*) with experiments from both regimes pooled. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	Trait x Regime					Universal	
Trait	*P*	LP *S*	*P*	HP *S*	*P*	*S*	*P*
RelW1	0.70	0.020	0.29	0.007	0.81	0.012	0.48
RelW2	0.37	0.017	0.37	-0.015	0.58	-0.001	0.94
RelW3	**0.02**	**0.033**	**0.08**	**-0.050**	**0.06**	-0.015	0.39
RelW4	**0.05**	0.018	0.34	**-0.050**	**0.06**	-0.021	0.22
RelW5	0.98	-0.009	0.65	-0.008	0.77	-0.008	0.64
RelW6	0.56	-0.003	0.88	-0.024	0.38	-0.015	0.39
RelW7	0.31	0.024	0.21	-0.012	0.66	0.003	0.85
RelW8	0.23	0.008	0.66	-0.034	0.20	-0.016	0.35
RelW9	0.54	-0.015	0.44	0.007	0.79	-0.002	0.91
RelW10	0.18	0.022	0.23	-0.024	0.36	-0.005	0.79
RelW11	0.83	-0.014	0.46	-0.006	0.81	-0.010	0.58

For females, RW3 quantifies variation in relative head size and explains 10.92 percent of the variation among individuals. Fish with a higher value for RW3 have a relatively larger head (Figure 3.3), and thus I predicted that selection coefficients associated with this RW should be negative. Some results from individual selection experiments are consistent with my predictions. Selection differentials were negative and significant for two high-predation experiments M15 ($S = -0.11$, $P = 0.03$) and M17 ($S = -0.19$, $P = 0.03$) (Table 3.3). However, selection gradients associated with RW3 were all insignificant (Table 3.4). The ANCOVA selection models did produce evidence of significant divergent selection between regimes (trait x regime $P = 0.02$) (Table 3.5). Consistent with predictions, selection coefficients were negative in high-predation experiments, although marginally significant ($S = -0.055$, $P = 0.06$); suggesting that guppies with relatively larger heads had reduced fitness. However, in low predation populations, there was a marginally significant positive relationship between RW3 and survival (fish with relatively larger heads had higher survival) ($S = 0.033$, $P = 0.08$).

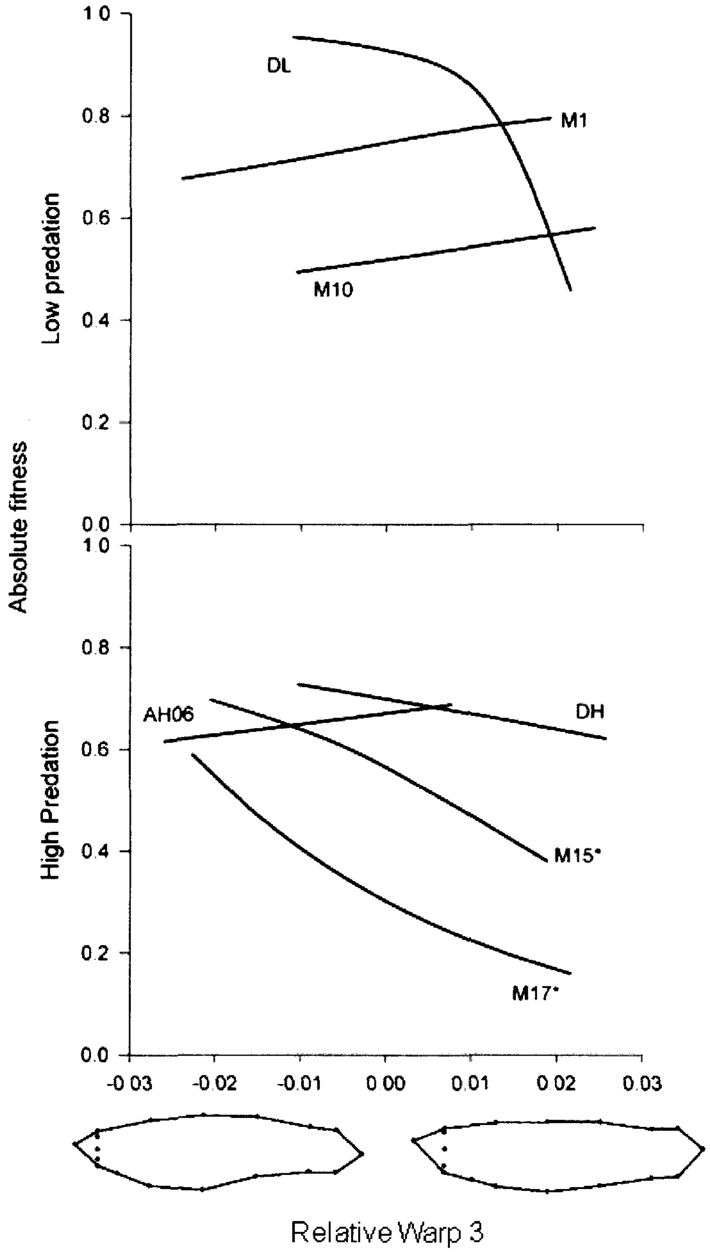

Figure 3.3. Cubic Splines for Females RW3. Cubic splines depict the relationship between phenotypic variation in female RW3 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant ($P < 0.05$) or marginally significant ($0.1 > P > 0.05$).

For females, RW8 quantifies variation in relative caudal peduncle depth. Fish with a higher score for RW8 have relatively deeper caudal peduncles (Figure 3.4), and thus I predicted that selection coefficients associated with RW8 should be positive. Consistent with my predictions, in one high-predation experiment (DH) there was a marginally significant, positive selection differential ($S = 0.12$, $P = 0.07$). However, results from other selection experiments were in contrast to my predictions. In two high-predation experiments, M15 and AH06, selection gradients and differentials were significant and negative (M15: $S = -0.1$, $P = 0.04$; AH06 $S = -0.09$, $P = 0.03$) (M15: $\beta = -0.15$, $P = 0.01$; AH06: $\beta = -0.13$, $P = 0.01$). The ANCOVA selection models did not produce any evidence of divergent selection between regimes (trait x regime $P = 0.23$) (Table 3.5), nor was there any evidence of a significant relationship between RW8 and fitness when data from all experiments was pooled.

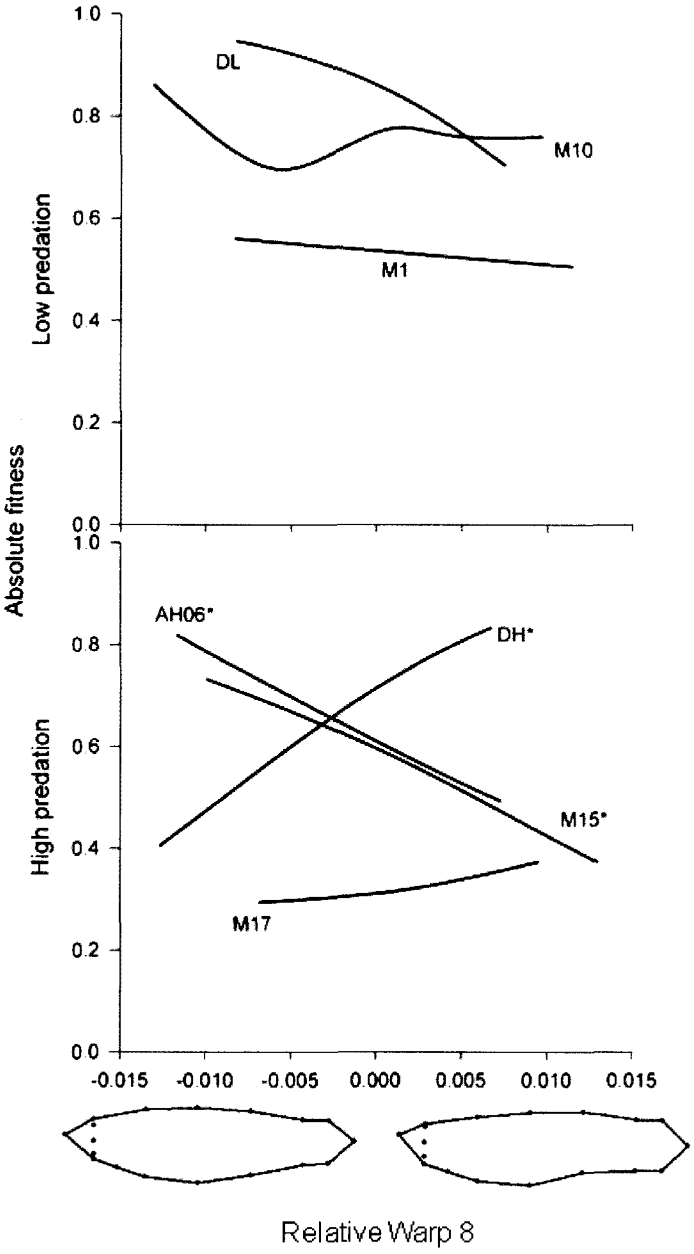

Figure 3.4. Cubic Splines for Female RW8. Cubic splines depict the relationship between phenotypic variation in female RW8 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant ($P < 0.05$) or marginally significant ($0.1 > P > 0.05$).

In addition to evaluating the relationship between fitness and the *a priori* selected RWs described above, I was interested in assessing whether any other aspects of shape variation influenced fitness. Therefore, I *post hoc* inspected the selection coefficients (from the individual selection experiments and the ANCOVA selection models) associated with the remaining RWs, in order to determine if there were any RWs that were consistently related to fitness. For females, two other RWs seemed to have an influence on fitness, RW4 and RW10. RW4 again quantifies variation in abdomen distension and explains 8.8% of the total phenotypic variance, females with a higher score have a more distended abdomen (Figure 3.5). Therefore, a negative selection coefficient would be consistent with my original predictions. The ANCOVA selection model indicates that RW4 is under divergent selection between regimes (trait x regime $P = 0.05$) (Table 3.5), in high predation experiments the selection coefficient was negative and marginally significant ($S = -0.05$, $P = 0.06$), in low-predation populations the selection coefficient was insignificant ($S = 0.018$, $P = 0.34$). A single high-predation experiment (AH06) had a marginally significant, negative selection differential ($S = -0.07$, $P = 0.09$) associated with RW4. Selection coefficients describing the relationship between RW4 and fitness are generally consistent with my original predictions; in high-predation experiments, females with a distended abdomen have lower fitness.

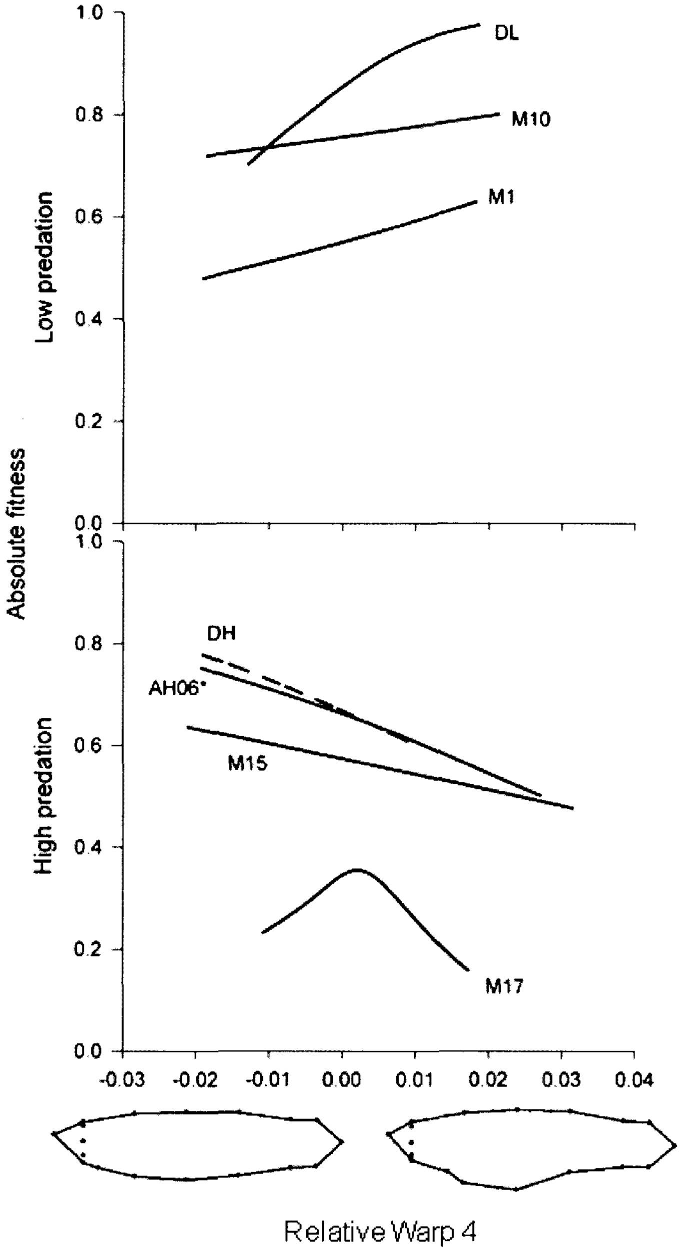

Figure 3.5. Cubic Splines for Female RW4. Cubic splines depict the relationship between phenotypic variation in female RW4 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant ($P < 0.05$) or marginally significant ($0.1 > P > 0.05$).

Female RW10 quantifies variation in caudal peduncle thickness and explains 1.3% of the total phenotypic variation; individuals with a high score have a narrower caudal peduncle (Figure 3.6). Therefore, a negative selection coefficient would be consistent with my original predictions. Despite low variability, two high predation experiments (M17 and AH06) had negative, marginally significant selection gradients associated with RW10 (M17 $\beta = -0.19$, $P = 0.07$; AH06 $\beta = -0.09$, $P = 0.08$) (Table 3.4). Similar to the selection coefficients associated with RW8 (see above), which also quantified variation in caudal peduncle thickness, these results are in contrast to my original predictions - fish with narrower caudal peduncles seem to have higher fitness.

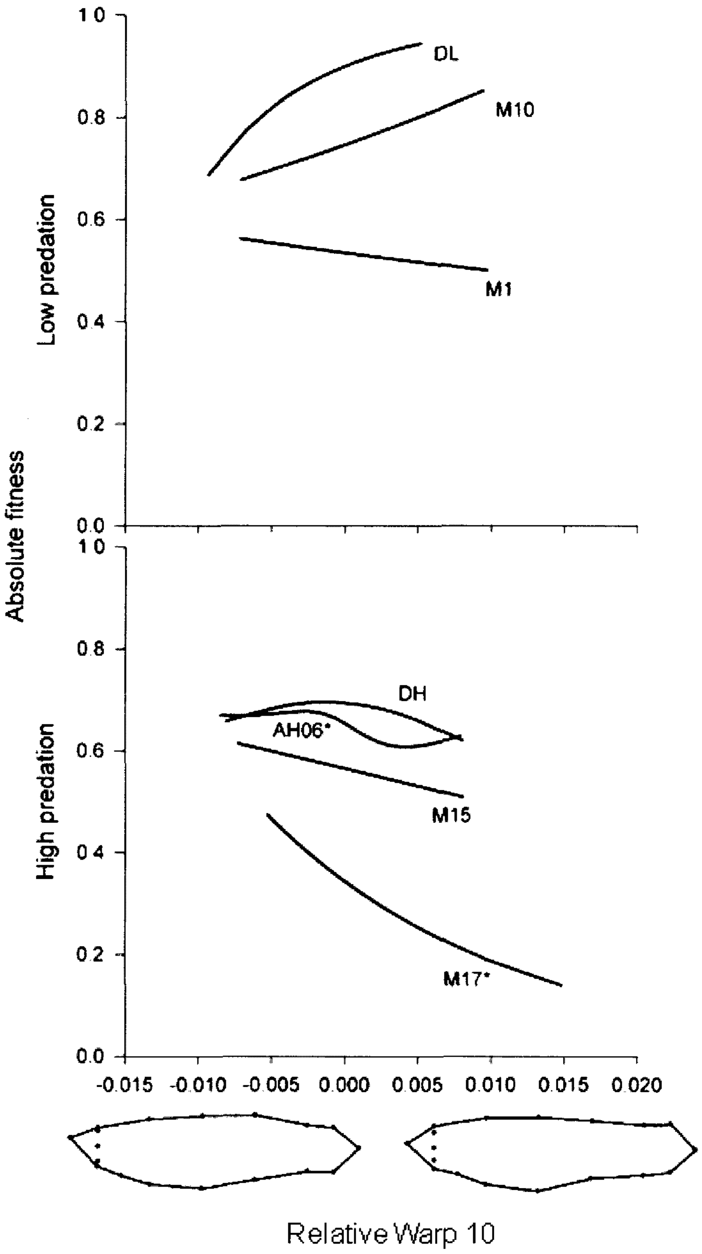

Figure 3.6. Cubic Splines for Female RW10. Cubic splines depict the relationship between phenotypic variation in female RW10 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant ($P < 0.05$) or marginally significant ($0.1 > P > 0.05$).

To assess temporal variation in selection, I compared the selection gradients associated with early and late recapture episodes for the RWs discussed above (Table 3.6). For one particular high-predation experiment (M17) selection associated with body shape was much stronger over the second interval. Selection gradients associated with RW3 were positive and insignificant over the first interval ($\beta = 0.11$, $P = 0.29$), but strongly negative and significant over the second episode ($\beta = -0.4$, $P = 0.01$) – consistent with the prediction that fish with larger heads would have reduced survival (see above). For the same site (M17), selection gradients associated with RW10 were similarly variable, over the first episode selection was insignificant ($\beta = 0.01$, $P = 0.83$), but over the second episode the selection gradient was strongly negative and significant ($\beta = -0.32$, $P = 0.01$) – consistent with the prediction that fish with thicker caudal peduncles would have higher fitness.

I will not specifically interpret data from the remaining RWs (1, 5, 6, 7, 9, and 11), since I did not predict they would be associated with fitness *a priori*, nor did any strong post hoc patterns emerge. However, the selection differentials and gradients quantifying the relationship between the each remaining RW and fitness are provided in tables 3.3 and 3.4, respectively. A general linear model, implemented in TPSRegr, was used to test for a relationship between absolute fitness and the comprehensive, multivariate shape phenotype (shape data represented by all partial warps and uniform components). For females, the permutation tests assessing the significance of the relationship between shape and fitness were all insignificant ($P > 0.1$). These results suggest that for these 7 experiments, shape variation was not strongly associated with survival and this conclusion is supported by the observation that selection coefficients are generally weak and insignificant, especially those associated with RWs explaining large amounts of phenotypic variation (Tables 3.3 and 3.4).

Table 3.6. Selection Gradients for Female RWs 1-11 Calculated Separately for Early (β1) and Late (β2) Recapture Episodes, only experiments with two recapture episodes. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	RelW1				RelW2			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	-0.04	0.29	0.05	0.60	0.02	0.60	0.11	0.31
M10	0.03	0.43	**-0.10**	**0.02**	-0.02	0.67	-0.02	0.66
M17	0.02	0.84	-0.06	0.70	0.07	0.44	-0.14	0.27
AH06	0.04	0.35	0.05	0.49	-0.02	0.63	-0.07	0.43

	RelW3				RelW4			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	0.07	0.13	-0.07	0.55	-0.06	0.20	0.11	0.25
M10	0.01	0.80	0.01	0.85	0.04	0.24	-0.01	0.84
M17	0.11	0.29	**-0.40**	**0.01**	-0.01	0.88	0.01	0.94
AH06	-0.02	0.71	-0.01	0.92	-0.05	0.24	-0.07	0.35

	RelW5				RelW6			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	-0.07	0.18	0.05	0.66	0.00	0.86	**-0.25**	**0.02**
M10	-0.01	0.91	**-0.08**	**0.04**	-0.04	0.36	-0.02	0.77
M17	0.01	0.88	**-0.18**	**0.10**	-0.10	0.24	-0.07	0.51
AH06	0.01	0.94	0.03	0.75	-0.01	0.70	-0.08	0.34

Table 3.6 continued.

	RelW7				RelW8			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	-0.02	0.70	-0.06	0.52	0.00	0.88	-0.12	0.22
M10	0.03	0.25	0.00	0.99	0.03	0.35	0.01	0.88
M17	-0.06	0.39	-0.13	0.20	0.02	0.77	-0.02	0.78
AH06	0.01	0.63	0.00	0.99	**-0.05**	**0.10**	**-0.15**	**0.02**

	RelW9				RelW10			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	-0.03	0.47	0.01	0.90	-0.02	0.55	-0.13	0.17
M10	-0.01	0.61	0.00	0.90	0.01	0.57	-0.01	0.88
M17	0.01	0.84	0.08	0.42	0.01	0.83	**-0.32**	**0.01**
AH06	-0.01	0.80	-0.07	0.36	**-0.08**	**0.02**	-0.02	0.84

	RelW11			
Experiment	β1	*P*	β2	*P*
M1	-0.02	0.63	-0.09	0.34
M10	-0.03	0.27	-0.03	0.37
M17	-0.03	0.63	**0.16**	**0.10**
AH06	0.03	0.49	-0.09	0.16

3.3.3 Selection on Male Body Shape

For males, significant selection associated with body shape variables was stronger and more prevalent. There were 19 significant selection differentials (*S*), absolute values range from 0.08 to 0.4 (Table 3.7). There were 14 significant selection gradients (β), absolute values range from 0.12 – 0.35 (Table 3.8). As for females, I first interpret the selection coefficients associated with RWs that quantify aspects of shape variation for which I had specific *a priori* predictions. Specifically, I predicted that decreased head size and increased caudal peduncle depth would be associated with higher survival, as a result of improved fast start acceleration (see Introduction). Conveniently, male RW4 simultaneously quantifies variation in both head size and caudal peduncle depth, and explains 6.6% of the total phenotypic variation (Table 3.2). Individuals with a high score for RW4 have a thick caudal peduncle and a small head (Figure 3.7). Therefore, I predicted that selection coefficients associated with RW4 should be positive. One selection differential was significantly negative (in contrast to my predictions) in the M10 low-predation experiment ($S = -0.11$, $P = 0.01$) (Table 3.7). No selection gradients associated with RW 4 were significant (Table 3.8). The ANCOVA selection model did not find significant evidence of divergent selection between regimes for RW 4 (trait x regime $P = 0.73$) (Table 3.9). The ANCOVA selection model that included data from all experiments indicated that there was a significant, negative relationship between RW4 and fitness ($S = -0.078$, $P = 0.01$) – in contrast to my predictions.

Table 3.7. Linear Selection Differentials (*S*) for Male RWs 1-11 in the 10 selection experiments. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	RelW1		RelW2		RelW3		RelW4	
Experiment	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*
Low Predation								
M16	**0.09**	**0.09**	0.06	0.23	-0.02	0.74	0.02	0.73
M1	0.10	0.27	0.06	0.52	**0.15**	**0.09**	0.01	0.87
M10	0.03	0.50	0.01	0.84	-0.01	0.78	**-0.11**	**0.01**
AL	0.22	0.14	-0.12	0.42	-0.14	0.36	-0.21	0.18
DL	0.08	0.30	-0.01	0.94	0.08	0.32	6.96	0.33
High Predation								
M15	0.11	0.19	-0.06	0.47	0.09	0.29	-0.08	0.34
M17	-0.18	0.22	-0.01	0.95	-0.14	0.35	0.20	0.18
AH05	0.23	0.24	0.08	0.67	-0.01	0.95	-0.03	0.86
AH06	-0.16	0.11	**0.17**	**0.09**	-0.16	0.12	-0.12	0.24
DH	0.04	0.68	-0.03	0.80	0.08	0.46	0.14	0.17

	RelW5		RelW6		RelW7		RelW8	
Experiment	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*
Low Predation								
M16	0.01	0.90	0.02	0.67	**-0.10**	**0.05**	0.03	0.60
M1	0.01	0.95	**0.15**	**0.09**	**-0.21**	**0.02**	0.05	0.56
M10	**-0.08**	**0.08**	0.02	0.66	**-0.11**	**0.01**	-0.02	0.69
AL	0.03	0.84	**-0.28**	**0.08**	**-0.40**	**0.01**	-0.11	0.46
DL	**-0.15**	**0.05**	-0.05	0.47	0.11	0.16	0.06	0.45
High Predation								
M15	0.07	0.42	-0.01	0.88	-0.10	0.24	-0.07	0.42
M17	0.07	0.63	0.05	0.76	-0.02	0.88	-0.09	0.56
AH05	-0.25	0.22	0.18	0.36	**-0.36**	**0.07**	0.31	0.13
AH06	-0.06	0.57	0.07	0.49	-0.06	0.57	-0.07	0.51
DH	-0.14	0.20	**0.20**	**0.06**	-0.01	0.91	**-0.23**	**0.03**

Table 3.7 continued.

	RelW9		RelW10		RelW11	
Experiment	*S*	*P*	*S*	*P*	*S*	*P*
Low Predation						
M16	-0.04	0.48	-0.03	0.50	0.00	0.97
M1	0.02	0.83	-0.08	0.40	-0.07	0.44
M10	**0.08**	**0.07**	-0.04	0.36	0.02	0.71
AL	**0.34**	**0.03**	-0.19	0.20	**-0.27**	**0.08**
DL	0.06	0.43	-0.08	0.30	-0.02	0.76
High Predation						
M15	0.00	0.97	0.05	0.54	-0.09	0.28
M17	0.20	0.18	0.02	0.90	0.03	0.82
AH05	0.35	0.08	-0.21	0.28	0.12	0.54
AH06	0.14	0.18	0.10	0.30	**-0.18**	**0.07**
DH	0.16	0.13	0.01	0.93	-0.13	0.23

Table 3.8. Linear Selection Gradients (β) for Male RWs 1-11 in the 10 selection experiments. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	RelW1		RelW2		RelW3		RelW4	
Experiment	β	*P*	β	*P*	β	*P*	β	*P*
Low Predation								
M16	0.07	0.40	0.07	0.30	-0.03	0.74	0.03	0.68
M1	0.02	0.81	0.10	0.30	**0.21**	**0.03**	0.11	0.39
M10	0.04	0.46	0.01	0.90	0.06	0.24	-0.07	0.28
AL	0.11	0.51	0.03	0.87	-0.11	0.47	-0.05	0.77
DL	0.04	0.81	-0.03	0.68	0.02	0.86	-0.06	0.41
High Predation								
M15	0.08	0.32	-0.05	0.57	0.15	0.12	-0.04	0.69
M17	-0.12	0.53	-0.06	0.71	-0.08	0.69	0.28	0.13
AH05	0.26	0.19	-0.05	0.79	0.17	0.36	0.16	0.45
AH06	-0.11	0.36	0.17	0.11	-0.13	0.27	-0.08	0.56
DH	-0.02	0.58	0.02	0.80	0.19	0.13	0.05	0.97

	RelW5		RelW6		RelW7		RelW8	
Experiment	β	*P*	β	*P*	β	*P*	β	*P*
Low Predation								
M16	0.06	0.42	0.07	0.15	**-0.13**	**0.10**	0.04	0.53
M1	0.05	0.66	**0.26**	**0.02**	**-0.26**	**0.02**	0.00	0.97
M10	**-0.12**	**0.02**	0.06	0.24	**-0.13**	**0.01**	-0.01	0.88
AL	-0.08	0.65	-0.23	0.16	**-0.29**	**0.09**	-0.12	0.40
DL	-0.13	0.24	-0.09	0.27	0.04	0.70	0.04	0.72
High Predation								
M15	0.03	0.76	-0.02	0.85	**-0.16**	**0.07**	-0.10	0.36
M17	0.13	0.51	0.14	0.38	0.04	0.82	-0.07	0.68
AH05	-0.05	0.93	0.22	0.39	-0.36	0.14	0.19	0.32
AH06	-0.04	0.69	0.04	0.74	-0.02	0.87	-0.05	0.70
DH	-0.09	0.50	**0.26**	**0.04**	-0.05	0.56	**-0.32**	**0.01**

Table 3.8 continued.

	RelW9		RelW10		RelW11	
Experiment	β	*P*	β	*P*	β	*P*
Low Predation						
M16	-0.02	0.76	-0.07	0.13	-0.02	0.52
M1	-0.07	0.45	-0.08	0.36	-0.15	0.13
M10	0.04	0.34	-0.02	0.66	0.01	0.83
AL	0.24	0.14	-0.09	0.83	-0.10	0.60
DL	-0.04	0.55	-0.07	0.44	-0.02	0.72
High Predation						
M15	-0.03	0.72	0.02	0.88	-0.10	0.23
M17	**0.27**	**0.10**	-0.06	0.73	0.01	0.96
AH05	**0.35**	**0.06**	-0.17	0.36	0.01	0.85
AH06	0.12	0.29	0.07	0.53	**-0.22**	**0.04**
DH	**0.24**	**0.03**	0.04	0.37	0.02	0.90

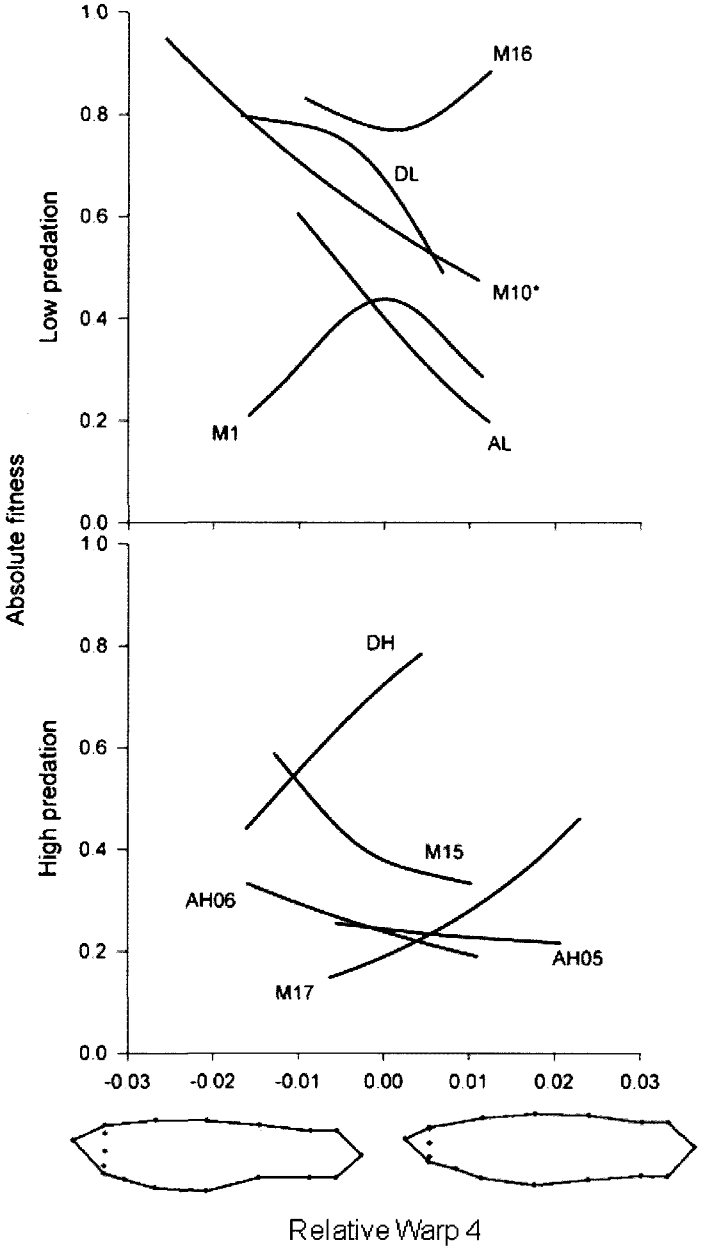

Figure 3.7. Cubic Splines for Male RW4. Cubic splines depict the relationship between phenotypic variation in male RW4 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant (*P* < 0.05) or marginally significant (0.1 > *P* > 0.05).

Table 3.9. Results of the ANCOVA Selection Models for Male RWs 1-11 that (1) tested for significant differences in selection between the two putative regimes (Trait x Regime *P*), (2) estimated selection coefficients separately with low-predation (LP *S*) and high-predation regimes (HP *S*), and (3) estimated universal selection coefficients (Universal *S*) with experiments from both regimes pooled. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	Trait x Regime					Universal	
Trait	*P*	LP *S*	*P*	HP *S*	*P*	*S*	*P*
Cent Size	0.39	**-0.075**	**0.03**	**-0.128**	**0.01**	**-0.104**	**< 0.01**
RelW1	0.70	**0.064**	**0.07**	0.041	0.40	**0.051**	**0.10**
RelW2	0.41	0.003	0.94	0.054	0.26	0.031	0.31
RelW3	0.76	-0.014	0.69	-0.033	0.49	-0.025	0.42
RelW4	0.73	**-0.066**	**0.06**	**-0.087**	**0.07**	**-0.078**	**0.01**
RelW5	0.64	-0.047	0.18	-0.018	0.71	-0.031	0.32
RelW6	0.15	-0.017	0.62	0.073	0.13	0.033	0.28
RelW7	0.98	**-0.118**	**< 0.01**	**-0.116**	**0.02**	**-0.117**	**< 0.01**
RelW8	0.93	-0.020	0.57	-0.026	0.59	-0.023	0.45
RelW9	0.96	**0.092**	**0.01**	**0.089**	**0.06**	**0.090**	**< 0.01**
RelW10	0.47	**-0.065**	**0.06**	-0.021	0.67	-0.040	0.19
RelW11	0.67	**-0.062**	**0.08**	-0.036	0.46	-0.047	0.13

As I did with females, I inspected the remaining selection coefficients, post hoc, in order to determine if other aspects of shape variation were consistently related to fitness. For males, RW 7 explains 2.8% of the total phenotypic variation, and quantifies variation in caudal peduncle thickness. An individual with a high score has a relatively thicker caudal peduncle (Figure 3.8), and thus a positive selection coefficient would be consistent with my original predictions. Unlike RW4, RW7 seems to quantify variation more specifically related to caudal peduncle thickness, without simultaneously describing variation in head size. Male RW7 had the most consistent relationship with fitness of any RW considered in this study, although in all cases significant selection coefficients were in contrast to my original predictions. In 4 low-predation experiments (M16, M1, M10, AL, and DL) and one high-predation experiment (AH05) selection differentials were significant and negative (Table 3.7). In the same low-predation experiments, and a different high-predation experiment (M15) selection differentials were significant and negative (Table 3.8). The ANCOVA selection model found no evidence of divergent selection between regimes (trait x regime $P = 0.98$) (Table 3.9). The ANCOVA selection model that pooled data from all the selection experiments indicated a significant negative relationship between RW7 and fitness ($S = -0.12$, $P < 0.01$). Thus, among the selection experiments considered here, thicker caudal peduncles seem to be consistently associated with lower fitness, in both predation regimes.

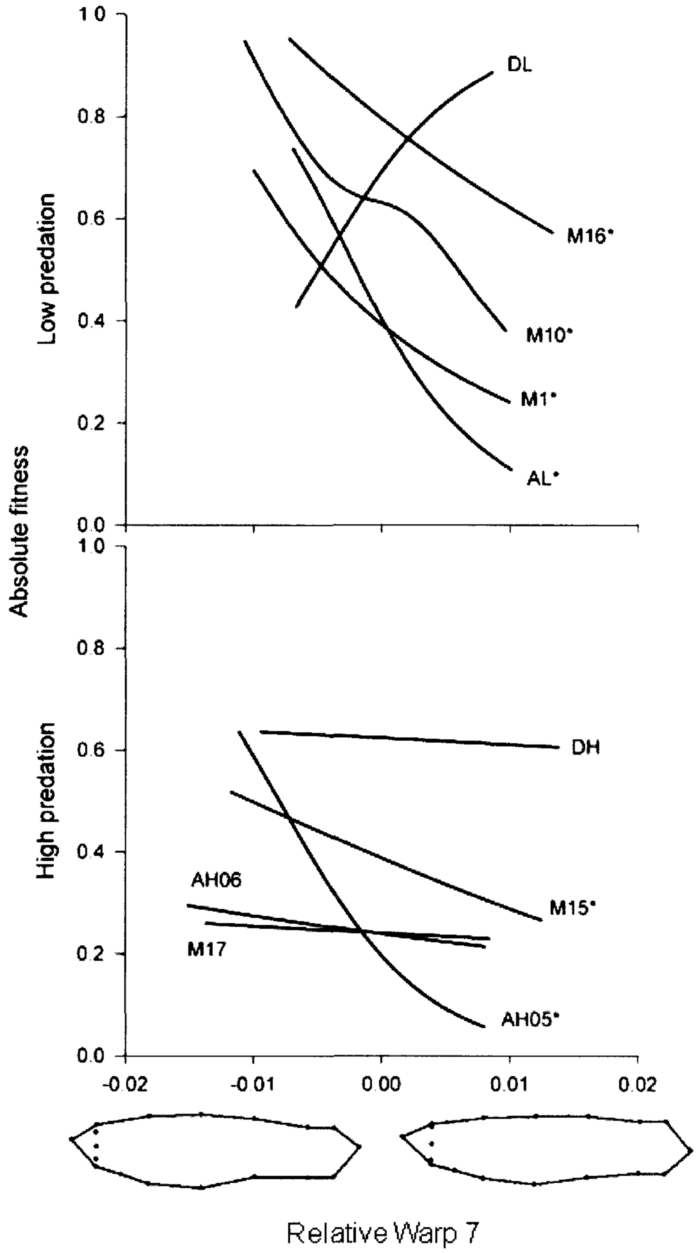

Figure 3.8. Cubic Splines for Male RW7. Cubic splines depict the relationship between phenotypic variation in male RW7 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant ($P < 0.05$) or marginally significant ($0.1 > P > 0.05$).

Male RW9 explains 1.9% of the total phenotypic variation and seems to quantify variation in thickness around the more anterior segments of the caudal peduncle. A higher score indicates an individual was relatively thicker around the more anterior section of the caudal peduncle (Figure 3.9). Therefore, positive selection coefficients would be consistent with my original predictions. In three high-predation experiments (M17, AH05, and DH), selection gradients were significant, positive, and quite large in magnitude (range 0.24 – 0.35) (Table 3.8). The ANCOVA selection model did not find evidence of divergent selection between regimes (Table 3.9). The ANCOVA selection model that pooled data from all experiments indicates that there is a significant, positive relationship between RW9 and fitness ($S = 0.09$; $P < 0.01$).

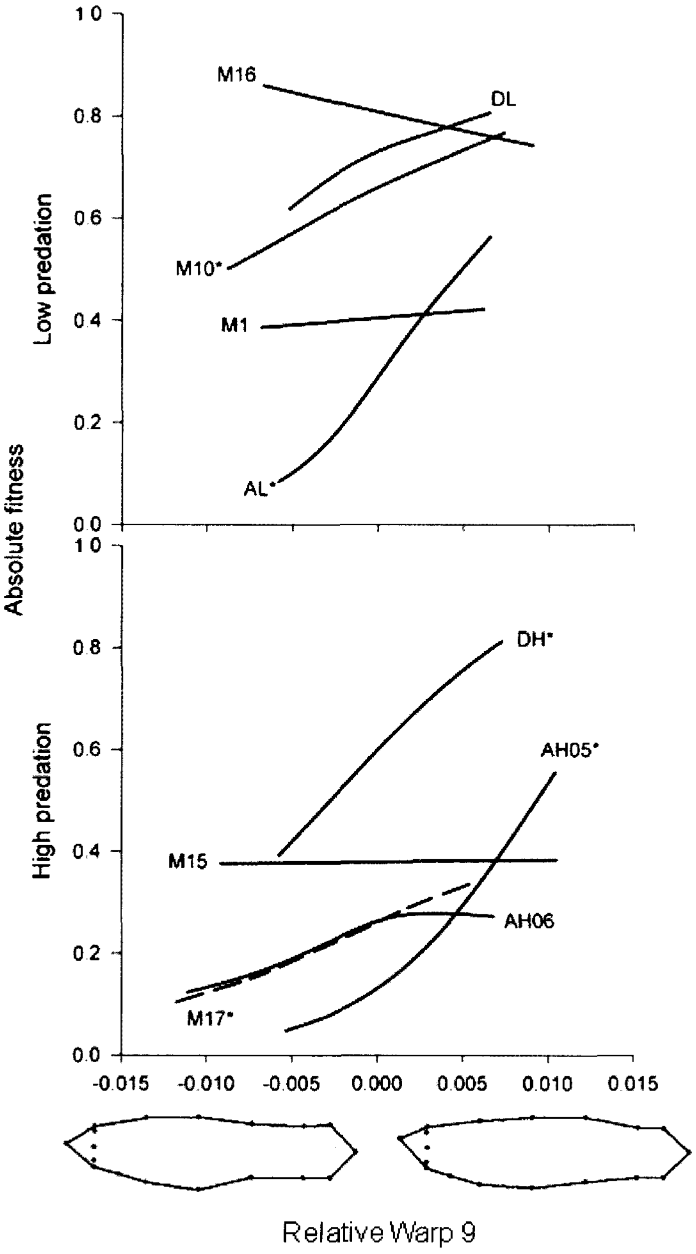

Figure 3.9. Cubic Splines for Male RW9. Cubic splines depict the relationship between phenotypic variation in male RW9 (visualized by deformation grid diagrams on the x-axis) and absolute fitness (y-axis). Asterisks indicate that either the selection differential or gradient quantifying selection for a particular experiment was significant ($P < 0.05$) or marginally significant ($0.1 > P > 0.05$).

As with females, to assess temporal variation in selection I compared the selection gradients associated with early and late recapture episodes, again this approach provided some evidence of fluctuating selection (Table 3.10). For RW4, in two experiments, M1 (low predation) and M17 (high predation), the selection gradient associated with the first episode was insignificant (M1: $\beta = -0.02$, $P = 0.83$; AH06: $\beta = 0.001$ $P = 0.97$); however, in both cases, selection gradients associated with the second recapture episodes were strongly positive (but only marginally significant) (M1: $\beta = 0.31$ $P = 0.07$; AH06: $\beta = 0.39$ $P = 0.1$) – consistent with the prediction that fish with thicker caudal peduncles and smaller heads should have higher fitness (see above). In two high predation populations (M17 and AH06) a similar pattern emerges regarding RW 9. In both cases selection over the first interval was insignificant (M17: $\beta = 0.12$, $P = 0.28$; AH06: $\beta = 0.01$, $P = 0.97$), but over the second interval selection gradients were strongly positive and significant or marginally significant (M17: $\beta = 0.33$, $P = 0.07$; AH06: $\beta = 0.36$, $P = 0.04$) – consistent with the prediction that individuals with thicker caudal peduncles should have higher fitness.

Table 3.10. Selection Gradients for Male RWs 1-11 Calculated Separately for Early (β1) and Late (β2) Recapture Episodes, only experiments with two recapture episodes. Significant ($P < 0.05$) and marginally significant values ($0.1 > P > 0.05$) are in bold.

	RelW1				RelW2			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	-0.01	0.89	0.10	0.45	0.10	0.17	0.03	0.78
M10	-0.02	0.68	**0.12**	**0.03**	-0.03	0.55	0.07	0.15
M16	0.03	1.00	0.06	0.62	0.00	1.00	0.12	0.22
M17	0.01	0.93	-0.22	0.21	0.07	0.53	-0.17	0.18
AH06	-0.04	0.65	-0.13	0.40	**0.14**	**0.10**	0.06	0.74

	RelW3				RelW4			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	0.09	0.22	**0.29**	**0.03**	-0.02	0.83	**0.31**	**0.07**
M10	0.02	0.69	**0.11**	**0.06**	-0.05	0.33	-0.03	0.68
M16	-0.04	1.00	0.01	0.92	0.03	1.00	0.00	0.93
M17	-0.19	0.16	0.08	0.72	0.00	0.97	**0.39**	**0.10**
AH06	-0.06	0.53	-0.24	0.23	-0.14	0.19	0.13	0.49

	RelW5				RelW6			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	-0.05	0.64	0.22	0.16	0.13	0.10	**0.30**	**0.03**
M10	**-0.10**	**0.03**	-0.06	0.27	0.02	0.56	**0.10**	**0.07**
M16	0.03	1.00	0.06	0.62	0.01	1.00	0.09	0.21
M17	0.15	0.23	0.24	0.24	0.14	0.19	0.12	0.53
AH06	-0.06	0.46	0.04	0.75	0.03	0.72	0.07	0.66

Table 3.10 continued.

	RelW7				RelW8			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	**-0.25**	**0.01**	-0.01	0.95	**-0.14**	**0.09**	**0.35**	**0.04**
M10	-0.06	0.15	**-0.17**	**< 0.01**	0.02	0.87	-0.04	0.37
M16	-0.03	1.00	-0.17	0.17	0.02	1.00	0.03	0.66
M17	0.02	0.86	0.00	0.78	0.06	0.64	-0.21	0.39
AH06	-0.03	0.77	-0.09	0.55	-0.03	0.74	-0.04	0.93

	RelW9				RelW10			
Experiment	β1	*P*	β2	*P*	β1	*P*	β2	*P*
M1	0.00	0.95	-0.16	0.21	-0.03	0.71	-0.12	0.32
M10	0.04	0.28	0.01	0.78	0.00	0.88	-0.05	0.33
M16	-0.01	1.00	-0.02	0.81	0.00	1.00	**-0.13**	**0.10**
M17	0.12	0.28	**0.33**	**0.07**	-0.14	0.23	0.10	0.47
AH06	0.00	0.97	**0.36**	**0.04**	0.01	0.97	0.16	0.26

	RelW11			
Experiment	β1	*P*	β2	*P*
M1	**-0.12**	**0.10**	-0.02	0.96
M10	-0.01	0.90	0.05	0.26
M16	0.02	1.00	-0.08	0.28
M17	0.03	0.75	0.03	0.77
AH06	**-0.16**	**0.05**	-0.15	0.33

As with females, I will not specifically interpret data from the remaining male RWs (1, 2, 3, 5, 6, 8, and 10), since I did not predict they would be associated with fitness *a priori*, nor did any strong post hoc patterns emerge. However, the selection differentials and gradients quantifying the relationship between the each remaining RW and fitness are provided in tables 3.7 and 3.8, respectively.

For males, unlike females, the general linear models, implemented in TPSRegr did find evidence of a significant relationship between fitness and multivariate shape variation (shape data represented by all partial warps and uniform components) in some experiments. Significant Wilk's lamba *P*-values were found for the low-predation population M1 (Wilk's lambda $P = 0.04$) and the high-predation population AH05 (Wilk's lamba $P = 0.04$), a marginally significant *P*-value was found for the low-predation population AL (Wilk's lamba $P = 0.1$). In contrast to my original predictions, for all three cases, fish with the highest fitness seemed to have relatively thinner caudal peduncles (Figure 3.10). This result corroborates the consistent and generally well-supported relationships found between male RW7 (which describes variation in caudal peduncle thickness – see above) and fitness in a number of selection experiments (described above).

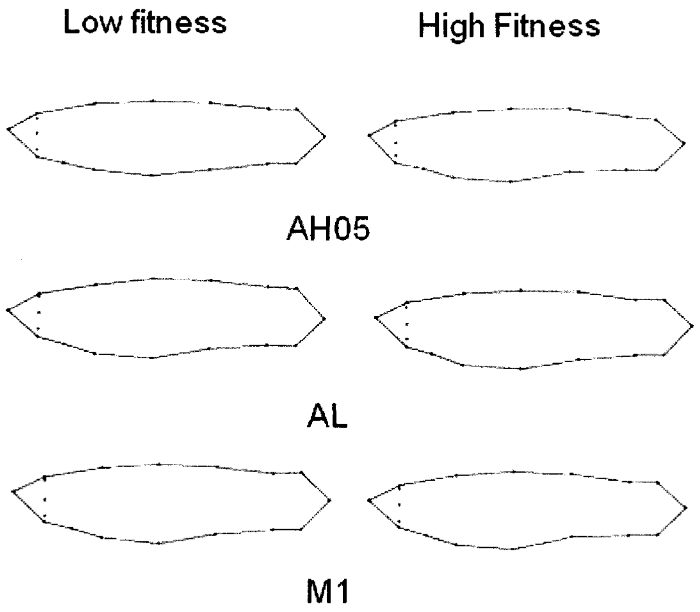

Figure 3.10. Visual Representation of TPSRegr Analyses. In three male experiments (AH05, AL, and M1) the general linear model implemented in TPSRegr found that multivariate shape was significantly influenced by fitness. Deformation diagrams represent the axis shape variation the maximally corresponds to fitness. Due to very subtle morphological differences, the deformation diagrams represent the actual observed phenotypic variation multiplied by three.

3.4 Discussion

My objective was to obtain formal linear estimates of selection on fish body shape from replicated mark-recapture experiments in the wild, and to use those replicate estimates to test *a priori* hypotheses about the fitness consequences of functional trade-offs between different aspects of performance. I predicted that natural selection (based on survival) would favor aspects of shape variation that maximize fast-start swimming performance. The results of the 10 selection experiments described above are variously consistent with this prediction – and suggest some important nuances to current theories of selection on body shape.

3.4.1 Selection Associated with Abdomen Distention in Female Guppies

Selection by dangerous fish predators should result in the evolution of improved fast-start swimming performance by guppies inhabiting high-predation habitats. This prediction is supported by laboratory studies that compared swimming performance between high- and low-predation guppies (Ghalambor et al. 2004). However, elevated risk of mortality in high-predation habitats also selects for increased reproductive allotment (a greater percentage of female mass composed of eggs and developing embryos), and the production of a greater mass of eggs and embryos is predicted to result in decreased swimming performance for four reasons (Ghalambor et al. 2004): 1) the additional reproductive mass (which does not contribute to locomotion) likely decreases acceleration, 2) the increased volume necessary to accommodate eggs may limit axial bending, 3) increased cross-sectional area (abdomen distension) may increase drag, and 4) energy allocation to eggs and embryos may decrease muscle performance. Thus, for female guppies, there is a potential functional trade-off between two important components of fitness, survival and reproduction. Empirical evidence for this functional trade-off is provided by the observation that increasing stages of pregnancy have a much stronger, negative effect on swimming performance for high-predation guppies compared to low-predation guppies (Ghalambor et al. 2004); this pattern lead Ghalambor et al. (2003) to predict that "the selection gradient for increased reproduction in females may be larger than that for predator escape ability". The results of the present study seem to be consistent with this prediction. On the one hand, the ANCOVA selection model for RW4 (describing variation in abdomen distension) provided evidence of divergent selection between predation regimes, selection against abdomen distension was stronger in high-predation sites. On the other hand, it should also be pointed out that selection gradients and differentials associated with RWs quantifying abdomen distension were nearly all insignificant and generally quite weak (see results). Thus, it seems reasonable to suggest that strong selection on life history traits could constrain the evolution of traits that optimize swimming performance and predator evasion (e.g. abdomen distension). Obviously, it would be useful to directly measure selection on life history traits in wild populations of guppies, although such estimates are currently unavailable.

I was surprised that selection associated with abdomen distension was not stronger and more prevalent. There seems to be a considerable amount of phenotypic variation upon which selection could act - RWs describing variation in abdomen distension, RW2 and RW4, explained 24.9% and 8.8% percent of total phenotypic variation, respectively. Also, previous laboratory studies have demonstrated that pregnancy has a strong, negative effect on fast-start swimming performance (Ghalambor et al. 2004), and faster fast-start swimming performance has been shown to increase the probability of evading strikes by predators in a laboratory experiment (Walker et al. 2005). Why was selection associated with abdomen distension so weak and difficult to detect? One possibility, suggested by Ghalambor et al. (2004), is that pregnant guppies alter their behavior so that they are less likely to attract the attention of predators; such behavioral modifications associated with reproductive state have been documented in several taxa (Brodie 1989; Rodewald and Foster 1998; Downes and Bauwens 2002; Frommen et al. 2009; Pruitt and Troupe 2010), and could compensate for the reduced locomotor performance caused by advancing pregnancy in female guppies. Another possibility is that many of the guppies approaching parturition at the start of our experiment may have given birth shortly after they were released back into the experimental sites, and thus the phenotype attributed to these individuals would be substantially skewed. However, I do not think that this potential source of error obfuscates the results of this study for two reasons: 1) our estimates of selection occurred over relatively short periods of time and thus phenotypes measured by my photos are likely accurate for most fish, 2) sample sizes were generally quite large (especially in the ANCOVA selection analyses that pooled data from multiple experiments), which could counteract error introduced by somewhat inaccurate estimates of phenotype. One way to reduce this error in future studies would be to have more frequent recapture intervals, and to take new photographs of each individual during each subsequent recapture event. Of course, this approach would be much more onerous and would potentially compromise the possibility of replicating selection experiments in multiple sites.

3.4.2 Selection Associated with Relative Caudal Depth

Biophysical models predict that fast-start acceleration is maximized by a rear-weighted body shape (thick caudal peduncle and a smaller head) (Webb 1982; Webb and Blake 1985; Law and Blake 1996; Walker 1997; Langerhans et al. 2004; Langerhans 2009a). This shape maximizes the volume of displaced water by the caudal region (which maximally contributes to thrust), and minimizes the amount of drag produced by the anterior region (Walker 1997). However, body shapes that maximize fast-start performance necessarily compromise efficiency (Langerhans 2009a). Thus, relaxed selection on fast-start acceleration is predicted to result in the evolution of traits that minimize energy costs associated with other motivations for locomotion (for example, foraging or searching for mates) (Langerhans 2009a). Variation in these body shape traits within and among species is predicted to be strongly correlated with the distribution of predators, since fast-start swimming is a primary mechanism by which prey species evade capture by predators (Langerhans and Dewitt 2004; Walker et al. 2005). Evolution of traits that influence burst swimming performance is thought to be a major driver of speciation and the evolution of ecological diversity among fishes (Langerhans et al. 2007; Langerhans 2009a).

Extensive support for this ecomorphological paradigm is provided by an enormous number of comparative studies that have tested for consistent, *a priori* predicted patterns of morphological divergence between fishes from high- and low-predation environments (for example, Walker 1997; Langerhans et al. 2003; Langerhans and DeWitt 2004; Alexander et al. 2006; Hendry et al. 2006; Gomes and Monteiro 2008; Burns et al. 2009; Langerhans and Makowicz 2009). Fewer studies, although still quite a few, have experimentally evaluated this paradigm by testing whether or not swimming performance is actually correlated with variation in these traits (for example Blake et al. 2005; Blake et al. 2009; Langerhans 2009a), and whether the ability to evade predators is enhanced by improved burst-swimming performance (Walker et al. 2005). However, no studies have evaluated the prediction that traits that improve fast-start swimming performance are correlated with higher survival in wild populations. My estimates of selection in replicated field experiments support some components of this ecomorphological paradigm, but cast some doubt on whether some tenets are applicable to all systems.

For females, my selection analyses support predictions regarding the fitness consequences of variation in relative head size. The ANCOVA selection model for RW3 (describing variation in head size – see results) detected divergent selection between regimes. In high-predation sites, individuals with larger heads had lower fitness, consistent with the prediction that the increased drag produced by dorsoventral expansion in anterior body segments compromises fast-start swimming and negatively affects predator evasion. However, in low-predation experiments individuals with larger heads had marginally higher fitness, the fitness benefits of having a larger head in low-predation habitats is unclear. In contrast to selection associated with caudal peduncle depth (see Results and below), the relationship between relative head size and fitness is generally consistent with predictions – at least for females.

For males and females, in most cases, the relationship between caudal depth and fitness is the opposite of what I predicted; generally, individuals with deeper caudal peduncles have lower fitness. Female RWs 8 and 10, both quantify variation in caudal depth, and both provide similar results; for both RWs, the ANCOVA selection models that pooled data from multiple experiments found no evidence of divergent selection, and no evidence of a significant relationship between caudal depth and fitness when data from all experiments was pooled. However, for both RW8 and RW10 estimates of selection coefficients from individual experiments were significant, although the direction of selection associated with these significant selection coefficients varied among experiments. In the high-predation experiment DH, fitness was positively associated with increased caudal peduncle depth. For three other high-predation experiments (M15, M17, and AH06), fitness was negatively associated with increased caudal peduncle depth (see results).

For males, natural selection generally favors individuals with narrower caudal peduncles, both RW4 and RW7 quantify variation in caudal depth, and in both cases the ANCOVA selection models found no evidence of divergence between regimes, and a significant, negative relationship between caudal depth and fitness when data from multiple experiments were pooled. Selection coefficients from individual selection experiments and multivariate linear models support this conclusion (see results). Male RW9 suggests some nuances regarding the relationship between fitness and caudal depth, this relative warp seems to specifically describe thickness in the more anterior segment of the caudal peduncle. For RW9, caudal thickness was positively associated with survival. Why this relatively subtle aspect of shape variation is related to fitness is unclear, since biophysical models are fairly unambiguous in predicting that the posterior body segments maximally contribute to thrust during fast-start swimming (Walker 1997). Perhaps, the relationship between this aspect of shape variation and fitness is due to its influence on some other aspect of swimming performance. For example, maneuverability may be improved by a body shape that is dorso-ventrally expanded medially (Webb 1982, 1984).

Complicating matters is the observation that selection associated with relative caudal peduncle depth seems to vary in time. For females in the high-predation experiment M17, strong selection for increased caudal depth (RW10) was only detected over the second recapture interval. Similarly for males, in two experiments, M1 (low predation) and M17 (high predation), strong selection for increased caudal depth was only detected in the second recapture episode (see results for RW4). The causes of such variability in selection are unknown, but could include the effects of transient predators, or fluctuations in environmental variables (for example, water clarity) that modify the strength and pattern of selection temporally.

These inconsistent, and frequently unexpected, results associated with caudal thickness are, however, consistent with the findings of Burns et al. (2009), who emphasize that high-predation populations of Trinidadian guppies occasionally have narrower caudal peduncles. Burns et al. (2009) suggest that these inconsistencies may be the results of spatiotemporal variation in the strength and pattern of selection. Indeed, the results of the present study support this prediction, the pattern and strength of selection associated with relative caudal peduncle depth seems to vary in space and time. Simply, some populations may have narrower caudal peduncles because selection seems to disfavor thicker caudal peduncles in many cases (in both high- and low-predation regimes).

3.4.3 A Role for Sexual Selection?

How do we functionally reconcile these patterns of selection with the results of most comparative and experimental studies which consistently conclude that thicker caudal regions should favor predator evasion? I here consider the possibility that sexual selection influences the evolution of caudal depth, and that functional trade-offs regarding this additional component of fitness may constrain the adaptive evolution of caudal depth. Female guppies generally prefer coloration on males, and Burns et al. (2009) suggest that deeper caudal peduncles could act as larger "billboard" for color signals during courtship. Moreover, larger caudal peduncles might arise in some males to due physical training effects linked to their having relatively large and colorful caudal fins (caudal fins in males vary greatly in size as well as color). Thus, sexual selection could result in the evolution of body shapes with thicker caudal regions. On the surface, this would seem to be a case of functional facilitation rather than functional constraint (Walker 2007), since deeper caudal peduncles would be favored by both sexual and natural selection. However, it is possible that very strong sexual selection could result in the evolution of caudal peduncles that are too thick (from the perspective of survival), if swimming efficiency is massively compromised. In the wild, survival is potentially negatively influenced by energetically inefficient swimming if costs associated with routine activities result in depleted energy reserves, which then compromise fast-start acceleration and predator evasion. Predators in the wild may pursue prey using both sudden strikes (Walker et al. 2005), and drawn-out pursuits (Jablonski 1999), the former likely selects for improved fast-start acceleration (Langerhans 2009b; Walker 2005), while the latter potentially selects for improved efficiency. Such effects may be missed by laboratory experiments that investigate predator prey interactions in confined spaces over relatively short time intervals. Interestingly, the negative relationship between caudal peduncle thickness and survival is stronger for males compared to females, and is stronger in low-predation experiments. Both of these observations are consistent with the possibility that sexual selection may strongly interact with natural selection to influence the evolution of body shape in Trinidadian guppies. First, females chose among displaying males and thus we expect sexual selection to strongly influence male fitness compared to females (Magurran 2005). Second, sexual selection is predicted to be stronger in low-predation habitats (Schwartz and Hendry 2007), and thus may result in a greater degree of "maladaptation" in body shape features for low-predation males (facilitating the detection of selection). Clearly, studies that estimate the strength and pattern of sexual selection associated with shape variables for both high- and low-predation populations are required to evaluate some of these predictions. I concede that the possible effects of sexual selection are somewhat speculative, and that such a role for sexual selection may be a nuance particular to the Trinidadian guppy system.

3.4.4 Summary

Comparative studies and laboratory experiments can be used to test for functional trade-offs between traits but ultimately must be considered means of generating hypotheses regarding the relationship between specific traits and specific components of fitness that must ultimately be tested in wild populations. Here, I used replicated selection experiments to evaluate predictions generated from the consideration of functional trade-offs between body shape traits and various aspects of performance. Consistent with predictions, I found selection against abdomen distension in females; however, this selection was relatively weak, suggesting that strong selection associated with life history traits could constrain adaptive evolution of this trait. Estimates of selection associated with caudal peduncle depth were inconsistent with predictions generated from considering the functional trade-offs between acceleration and efficiency. I suggest that future work needs to consider the possibility that direct selection for predator escape performance may not be the sole or primary mechanism shaping elements of body shape that are commonly linked to fast-start swimming performance. Other aspects of selection, including sexual selection, may significantly constrain the adaptive evolution of body shape, particularly in established populations that may have multiple means of accommodating predation risk. As such trait values often predicted to improve predator escape may often come under negative or balancing selection.

CHAPTER 4: ECO-EVOLUTIONARY EFFECTS ON POPULATION RECOVERY FOLLOWING CATASTROPHIC DISTURBANCE

4.1 Introduction

A key concept within metapopulation theory (Hanski 1999) is that migrants from productive patches (sources) can sustain other populations in harsh habitat patches where population growth is impaired (sinks) (Pulliam 1988). Within such metapopulations, particular demes may persistently function as sources or sinks, or they might switch between these states owing to catastrophic disturbances. Catastrophic disturbances can range from lasting effects on landscape features and selective conditions (for example, volcanic eruptions) to more fleeting influences (for example, occasional floods or droughts). Although often rare, such catastrophic disturbances can have large effects on population dynamics and extinction risk (Shaffer 1981; Lande 1993), and there have been several empirical examples of such effects (reviewed in Sousa 1984; Spiller et al. 1998; Vignieri 2010). Individual survival under such severe and abrupt disturbances may often be dictated by chance, more than adaptive trait variation, providing a distinction from the more subtle disturbances that have often characterize cases of contemporary evolution in the wild (Hendry et al. 2008).

If connected to other populations by individual dispersal, local populations recovering from catastrophic disturbance might receive a critical demographic boost, reducing their risk of extinction (the “rescue effect” - Brown and Kodric-Brown 1977). Alternatively, population recovery following disturbance may primarily be the result of demographic contributions from local surviving individuals (Lindenmayer et al. 2005;

Peakall and Lindenmayer 2006; Peery et al. 2010). The relative influence of these two processes depends largely on the fitness of migrants in their new habitat, which may be reduced compared to residents due to local adaptation (Nosil et al. 2005). In this framework, the nature of rescue effects, like many other problems in conservation biology, is not just ecological or evolutionary, but eco-evolutionary (Kinnison & Hairston 2007).

Uncertainty regarding the demographic benefits of migrants is further hinted by theoretical simulations that variously suggest that migration can impede, prevent, or promote population persistence (Ronce & Kirkpatrick 2001; Kawecki and Holt 2002; Holt et al. 2003; Garant et al. 2007). Unfortunately, little experimental data exist on how local adaptation might modify the relative contributions of local and migrant individuals to population recovery in the wild, albeit some studies have variously suggested ways that selection and dispersal may interact to influence population dynamics (Hanski & Saccheri 2006; Duckworth and Badyaev 2007; Moore and Hendry 2009; Van doorslaer et al. 2009). In this study, I present the results of a series of experiments in wild populations of Trinidadian guppies (*Poecilia reticulata*) that demonstrate the potential for selection on migrants to influence demographic recovery following population collapses resulting from local catastrophic disturbances.

4.1.1 The Trinidadian Guppy System

Trinidadian guppies inhabit streams characterized by waterfalls that prevent large predatory fish species from colonizing upstream sites (Endler 1978; Magurran 2005). These waterfalls have two important consequences for this study. First, adjoining guppy populations above versus below these falls show adaptive divergence in response to the contrasting predator regimes (Endler 1995; Magurran 2005). Traits showing adaptive divergence include shape (Hendry et al. 2006), life histories (Reznick and Endler 1982; Gordon et al. 2009), anti-predator behaviours (Magurran et al. 1992; O'Steen et al. 2002), and body coloration (Endler 1978; Millar et al. 2006). Moreover, these differences are genetically based and evolve on short time scales following experimental translocations between the two predation environments (Endler et al. 1980; Magurran et al. 1992; O'Steen et al. 2002; Gordon et al. 2009). Second, migration and gene flow occur between predation environments, particularly from low-predation sites above waterfalls into high-predation sites below waterfalls (Becher and Magurran 2000; Crispo *et al.* 2006). Thus, within a particular river, the network of Trinidadian guppy populations can be described as an environmentally and phenotypically heterogeneous metapopulation.

Natural guppy populations sometimes experience catastrophic disturbances in the form of very large floods (Grether et al. 2001b; van Oosterhout et al. 2007). A series of these floods occurred during the "dry season" (January to March) in 2005 and 2006, reducing the high-predation population of the Marianne River by several orders of magnitude. For instance, during exhaustive sampling at the focal experimental site (see below), I captured 216 females and 111 males in 2004, but only one female and no males in 2005 and six females and three males in 2006. These same floods did not have a similarly devastating effect on neighboring low-predation populations (that occur in lower order tributaries) or on the abundance of larger fish predators. After the flooding ended, the depleted populations of high-predation guppies were therefore likely experiencing higher proportional rates of immigration from the upstream low-predation habitats, particularly if low-predation fish were more likely to be distributed over barriers during high water. I here ask how these migrants might influence population recovery. As noted above, the answer is not straightforward because although the numerical effect should enhance recovery, strong selection on migrants (Nosil et al. 2005) might reduce this benefit.

I addressed two specific research objectives. First, I quantified selection against migrants by testing for potential differences in both survival and reproductive success between high-predation and low-predation guppies. Using equal numbers of both ecotypes, I established experimental populations (in two years) at a focal high-predation site, and tested for differential survival using mark-recapture techniques. Based on phenotypic differences presumed to reflect adaptation to predation regimes (see above), I predicted that the low predation ecotype would have lower survival compared to the high predation ecotype. I also tested for sexual selection on low-predation males relative to high-predation males using predator-free enclosures outside of the focal experimental site. Whether or not this sexual selection would act for or against the low-predation ecotype was not clear *a priori*. On the one hand, female guppies commonly prefer to mate with colorful males (Endler and Houde 1995), and so might preferentially mate with the more colorful low-predation migrants. On the other hand, high mortality rates of migrants and migrant phenotypes could select for positive assortative mating by ecotype (Schluter 2000), in which case the low-predation males may have relatively low mating success with high-predation females.

My second objective was to quantify the demographic contributions of local and migrant individuals to population recovery in the focal high-predation site. To do this, I used population genetic assignment techniques to test for ecotypic differences in the number of offspring contributed to subsequent generations of the experimental populations established at the focal high-predation site. While low-predation fish are sure to make an initial numeric addition to the experimental populations, their contribution to population growth (recovery) in subsequent generations will be strongly dependent upon their ability to survive and reproduce in the high-predation environment. Therefore, I predicted that the demographic contributions of the migrant (low-predation) guppies would be somewhat less than the local (high-predation) guppies.

4.2 Methods

4.2.1 Study Site and Mark-Recapture Techniques

All experiments were conducted in the Marianne River system, which flows from Trinidad's northern mountain range. Within the Marianne River drainage three source populations were used for the experiments: the high-predation mainstem (HP) source and two low-predation sources (LP1 and LP2 respectively) (Figure 4.1). The high-predation section of the Marianne River contains several species of potential predatory fishes including: several species of goby: *Eleotris pisonis*, *Gobiomorus dormitor*, and *Dormitator maculatus* (Gobiidae); and a river "mullet", *Agonostomus monticola* (Mugilidae). The low-predation tributaries of the Marianne River drainage contain less dangerous predators including a killifish (*Rivulus hartii*) and several species of predatory prawns (*Macorbrachium spp*). Additional information describing the location of these tributaries, and their environmental characteristics, can be found in a series of publications describing the color (Millar et al. 2006), shape (Hendry et al. 2006), and population genetic structure (Crispo et al. 2006) of the guppies inhabiting this river.

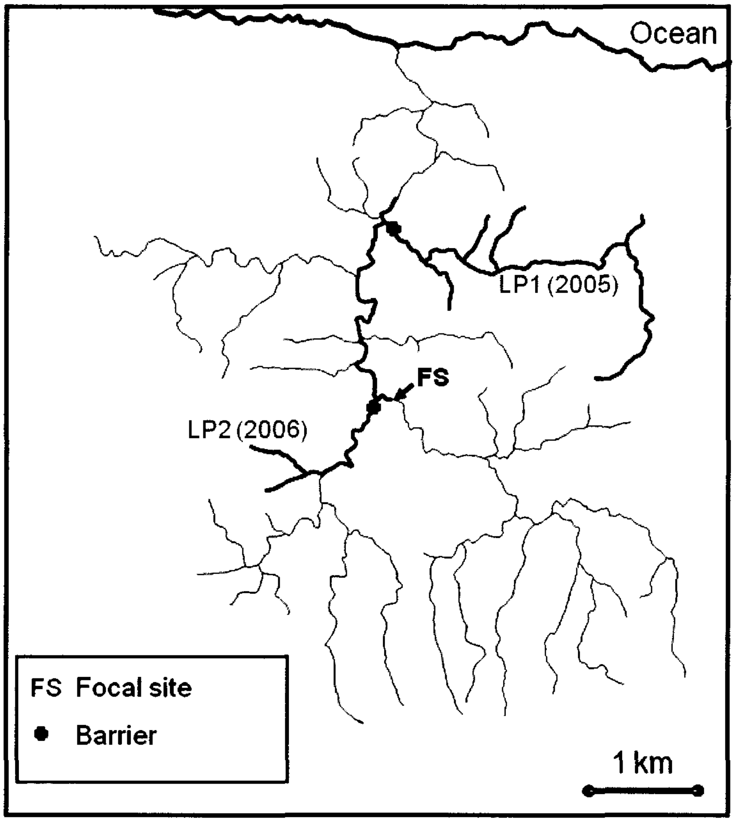

Figure 4.1. Map of the Marianne River Drainage. The focal site (FS) is where experimental populations were established. LP1 and LP2, shown in blue, indicate the locations of the two low-predation source populations used in 2005 and 2006, respectively. I have also indicated the location of barriers that are thought to have prevented the colonization of these low-predation tributaries by predatory fish. Shown in red is the section of the river where I observed that the guppy population had been decimated by floods in 2005 and 2006. I have confirmed the presence of predatory fish throughout the red section. The high-predation guppies introduced into the focal site originated from a series of localized side-channels, within the red section (but well below the focal site), where some guppies had resisted the floods. Thus, since none of the guppies originated from the focal site, there is no potential for a home-site advantage.

To study differential survival of high-predation and low-predation ecotypes in the high-predation habitat, I introduced approximately equal numbers of marked guppies from each ecotype into a focal high-predation site (Figure 4.1) and recaptured the fish and their offspring every two weeks for approximately four months (Table 4.1) using standard mark-recapture techniques for guppies (Rodd and Reznick 1991; Reznick et al. 1996; Olendorf et al. 2006; Van Oosterhout et al. 2007; Gordon et al. 2009). Two separate experimental introductions were implemented using different low-predation sources, one in 2005 using LP1 guppies, and one in 2006 using LP2 guppies. High predation fish came almost entirely from mainstem river sections well below the study reach, eliminating the potential for a home-site advantage (Figure 4.1).

Table 4.1. Genotypes of Experimental Guppies. Parents and recruits assigning to high-predation population cluster (HP), low-predation population cluster (LP1 or LP2), or inferred to have an admixed genotype (Hybrid) throughout the duration of both introduction experiments (2005 and 2006). Recapture episodes occurred approximately every two weeks. Numbers in parentheses are the numbers of unmarked guppies captured during a particular recapture episode (assumed to be offspring of introduced guppies).

Year	Genotype	Release	Recap 1	Recap 2	Recap 3
2005	HP	85 (0)	62 (1)	85 (40)	117 (49)
	LP1	83 (0)	18 (0)	18 (6)	12 (3)
	Hybrid	0 (0)	0 (0)	0 (0)	1 (1)
2006	HP	99 (0)	72 (0)	63 (0)	67 (13)
	LP2	98 (0)	55 (0)	29 (0)	8 (0)
	Hybrid	0 (0)	0 (0)	0 (0)	0 (0)

Table 4.1 continued.

Year	Genotype	Recap 4	Recap 5	Recap 6	Recap 7
2005	HP	133 (52)	95 (49)	73 (16)	-
	LP1	11 (5)	11 (7)	13 (5)	-
	Hybrid	1 (1)	5 (5)	10 (7)	-
2006	HP	79 (24)	116 (45)	34 (7)	28 (4)
	LP2	9 (3)	6 (1)	0 (0)	0 (0)
	Hybrid	0 (0)	0 (0)	0 (0)	0 (0)

Before release, each guppy was individually marked with two sub-cutaneous injections of elastomer dye (Northwest Marine Technology). Using a combination of six different colors and (up to) six different anatomical locations, two sub-cutaneous injections provided 540 individually identifiable marking codes for each sex per year. The focal site (Figure 4.1) was a series of 5 pools located just downstream from a steep and extensive set of cascades and upstream of another rapids and a small but deep gorge. These "barriers" discouraged guppy emigration out of the site. Moreover, these barriers and the severely reduced abundance of guppies in habitats outside the study largely precluded any significant immigration. Each recapture episode occurred over two days. On the first day I sampled through the entire study site until no fish were apparent. I then returned the next day to capture any remaining fish that might have been missed during the first attempt. During each recapture episode, I sampled for guppies in the pools immediately above the upstream barrier, but never encountered any. I also sampled all downstream pools within 500 m of the gorge that delimited the focal site. Very few experimental guppies were encountered downstream and emigrants were not included in my analyses because they were presumed to play not significant role in local population recovery. Neither ecotype showed a greater tendency for leaving the site.

The program MARK (White & Burnham 1995) was used to simultaneously estimate recapture and survival probabilities from mark-recapture data. I predicted that high-predation ecotypes would have higher survival than low-predation ecotypes, and thus the most likely mark-recapture model would produce ecotype-specific estimates of survival. I performed separate MARK analyses for each sex and year (total of four). The data did not show evidence of overdispersion ($P > 0.05$); thus, I compared the candidate models using Akaike's Information Criteria (AICc). For each analysis, the suite of candidate models variously included separate parameter estimates (survival and recapture probability) for different recapture episodes, and different source populations (ecotypes) (Table 4.2).

Table 4.2. Mark-Recapture Model Selection. Results of four separate MARK analyses for each combination of sex and year. For each analysis, rows represent particular candidate models, which each estimate survival (Φ), and recapture (p) probability. Each candidate model variously estimates regime (reg), or recapture-episode (ti) specific parameter values as well as interactions between these effects. Thus, models vary in the number of parameters they estimate (K). The most likely candidate model has the lowest Akiake's information criteria score (AICc).

Model	AICc	Δ AIC	*w*	Likelihood	K	Dev
2005 females						
{Φ(reg) p(.)}	426.39	0.00	0.333	1.000	3	89.17
{Φ(reg*ti) p(.)}	426.56	0.18	0.304	0.915	15	62.97
{Φ(reg*ti) p(reg)}	427.65	1.27	0.177	0.531	16	61.71
{Φ(reg) p(reg)}	428.37	1.98	0.123	0.371	4	89.08
{Φ(reg) p(ti)}	430.79	4.40	0.037	0.111	9	80.78
{Φ(reg*ti) p(ti)}	431.62	5.23	0.024	0.073	20	56.03
{Φ(ti) p(.)}	437.24	10.85	0.001	0.004	8	89.42
{Φ(ti) p(reg)}	439.42	13.04	0.000	0.002	9	89.42
{Φ(ti) p(ti)}	441.88	15.50	0.000	0.000	13	82.91
{Φ(reg*ti) p(reg*ti)}	445.68	19.29	0.000	0.000	27	52.18
2005 males						
{Φ(reg) p(ti)}	246.31	0.00	0.508	1.000	9	32.61
{Φ(reg) p(.)}	248.36	2.05	0.182	0.358	3	47.81
{Φ(reg) p(reg)}	249.17	2.86	0.122	0.239	4	46.51
{Φ(reg*ti) p(ti)}	249.39	3.09	0.109	0.214	16	18.83
{Φ(reg*ti) p(reg*ti)}	251.49	5.19	0.038	0.075	17	18.37
{Φ(reg*ti) p(.)}	252.60	6.29	0.022	0.043	11	34.26
{Φ(reg*ti) p(reg)}	252.82	6.51	0.020	0.039	12	32.11
{Φ(ti) p(reg)}	269.98	23.67	0.000	0.000	9	56.28
{Φ(ti) p(ti)}	274.90	28.59	0.000	0.000	13	51.78

Table 4.2 continued.

{Φ(ti) p(.)}	278.64	32.34	0.000	0.000	8	67.22
2006 females						
{Φ(reg) p(ti)}	578.93	0.00	0.843	1.000	10	107.39
{Φ(reg*ti) p(reg)}	583.63	4.70	0.081	0.096	16	98.93
{Φ(reg*ti) p(.)}	584.97	6.04	0.041	0.049	15	102.50
{Φ(reg*ti) p(ti)}	585.44	6.51	0.032	0.039	21	89.35
{Φ(reg*ti) p(reg*ti)}	590.62	11.69	0.002	0.003	25	85.11
{Φ(ti) p(reg)}	611.66	32.73	0.000	0.000	10	140.11
{Φ(reg) p(.)}	618.00	39.07	0.000	0.000	3	161.13
{Φ(ti) p(.)}	618.10	39.17	0.000	0.000	9	148.69
{Φ(reg) p(reg)}	618.57	39.63	0.000	0.000	4	159.64
{Φ(ti) p(ti)}	619.85	40.92	0.000	0.000	15	137.38
2006 males						
{Φ(reg) p(.)}	401.14	0.00	0.293	1.000	3	58.71
{Φ(reg) p(ti)}	401.20	0.07	0.283	0.966	7	50.33
{Φ(reg) p(reg)}	402.32	1.19	0.162	0.552	4	57.82
{Φ(reg*ti) p(reg)}	402.42	1.29	0.154	0.525	12	40.49
{Φ(reg*ti) p(.)}	403.63	2.50	0.084	0.287	11	43.96
{Φ(reg*ti) p(ti)}	406.60	5.46	0.019	0.065	14	40.08
{Φ(reg*ti) p(reg*ti)}	411.25	10.11	0.002	0.006	18	35.27
{Φ(ti) p(reg)}	411.29	10.16	0.002	0.006	7	60.42
{Φ(ti) p(.)}	413.26	12.12	0.001	0.002	6	64.53
{Φ(ti) p(ti)}	417.25	16.11	0.000	0.000	9	62.02

4.2.2 Enclosure Experiment

I performed an enclosure experiment to isolate the effects of sexual selection from viability selection. To do this I first collected immature guppies from the high-predation section of the Marianne River (Figure 4.1), and maintained females as virgins until they reached maturity. I then constructed a barrier across the mouth of a side channel downstream from the focal site, and removed all potential predators and guppies. To test for differences in reproductive success between low- and high-predation males I placed virgin high-predation female guppies into the enclosed side channel along with a mixture of high-predation and low-predation males from the source populations (Table 4.3). Males from the LP1 and LP2 populations were assessed against the same HP source in independent trials. Before release, each fish was marked (see above), and provided scale samples for DNA. These fish were left in the enclosure for 2 days, after which guppies were recaptured from the enclosed side channel. A flash flood allowed some guppies to escape from the LP2 vs HP experiment while I was removing the guppies from the enclosure. This reduced the sample of females for this comparison (Table 4.3), but not males since I had collected scale samples from males (from which I extracted DNA), prior to introducing them into the enclosure. For both experiments, recaptured females were returned to the field station; and after two weeks they were dissected and four embryos were haphazardly selected for parentage analyses.

Table 4.3. Numbers, and Origins, of Guppies in Enclosure Experiment. Numbers of experimental high-predation females, high-predation males, and low-predation males in a predator-free side channel of the Marianne River, and the total number of offspring that were sired by each male ecotype. Sample sizes differ between trials (LP1 vs HP and LP2 vs HP) because a flash flood allowed some guppies to escape from the LP2 vs MS experiment while I were removing the guppies from the enclosure. This reduced the sample of females, but not males since I had collected scale samples from males (from which I extracted DNA), prior to introducing them into the enclosure.

Trial	N females	N HP males	N LP males	HP offspring	LP offspring
LP1 vs HP	25	12	12	29	15
LP2 vs HP	8	16	16	14	2

Mothers, candidate sires, and offspring were genotyped at 6 microsatellite loci: Pre15, Pre53, Pre8, Pre9, Pre46, and Pre 32. Details of extraction and amplification methods are provided elsewhere (Paterson et al. 2005; Crispo et al. 2006). I assigned paternity using the program Cervus 3.0 (Kalinowski et al. 2007), which uses a likelihood-based approach to estimate the difference in log-likelihood scores between multiple candidate sires. I was conservative in my assignments and only further considered offspring whose father was known with greater than 95% confidence - 44 out of 94 offspring in the LP1 vs HP trial and 16 out of 32 in the LP2 vs HP trial. These data were then analyzed in a general linear model where the dependent variable was the number of confidently assigned offspring sired by individual males, and the independent variables were predation regime, trial (LP1 versus HP; LP2 versus HP), and the interaction term between regime and trial. Despite the highly conservative nature of my paternity assignments, I do not suspect a bias in the probability of assigning paternity to males of one ecotype or the other, because results were qualitatively similar in a supporting analysis where I assigned a much larger proportion of offspring to parental ecotype as opposed to individual sires.

4.2.3 Population Assignment of Wild Recruits

DNA was extracted from the scale samples of all guppies initially released (see above) in the focal site (Figure 4.1), and all individuals were genotyped at 11 microsatellite loci: Pre9, Pre13, Pre15, Pre26, Pre32, Pre38, Pre39, Pre46, Pre53, Pre72, and Pre80 – details of microsatellite amplification are provided elsewhere (Patterson et al. 2005; Crispo et al. 2006). The program STRUCTURE 2.2 (Pritchard et al. 2000) was then used to assign (separately for each year) individuals to either the high-predation or low-predation source population. STRUCTURE uses a Bayesian clustering approach to estimate the number of populations in a data set (K), and can probabilistically assign individuals to one of the identified populations, or indicate if an individual has an admixed genotype. I performed a K = 2 model in STRUCTURE to identify the two major population genetic clusters in the allelic data set. In each year, the two major clusters corresponded very closely to the different source populations (see Results). Unmarked guppies sampled during the recapture episodes of the experimental populations were assumed to be the offspring of the originally introduced individuals because so few local fish were present at the start of the experiment. Individual offspring were assigned a Q-value which represents the probability that an individual's parents were from the high-predation source population, the low-predation source population, or were the result of hybridization between the two ecotypes (Q = 0.5). This analysis allowed us to measure the genetic and demographic contribution of each ecotype to the subsequent generation of the experimental population.

4.3 Results

4.3.1 Differential Survival of Ecotypes

Our mark-recapture experiment (performed at the focal site), found that low-predation guppies experienced very high mortality, compared to the high-predation guppies, when the two were tested together in a novel high-predation habitat (Tables 4.1 and 4.2, Figure 4.2). This conclusion is well-supported because the most likely candidate models for all four MARK analyses had ecotype-specific estimates of survival, while the least-likely candidate models typically did not (Table 4.2). All models lacking an ecotype-specific survival estimate have a delta AIC value of at least ten. For the 2005 females and 2006 males the most likely candidate model estimated an ecotype-specific term for survival, and neither an ecotype-specific nor a recapture-episode-specific term for recapture probability (Table 4.2). For the 2005 males and 2006 females, the most likely candidate model included an ecotype-specific survival term and a recapture probability term that depended on the recapture episode (Table 2), indicating that my ability to sample all guppies in the focal site differed between recapture episodes. This result is possibly the because of variability in environmental conditions (water level or clarity). Consistent with most other guppy mark-recapture studies, females had much higher survival than males (Tables 4.1 and 4.2, Figure 4.2).

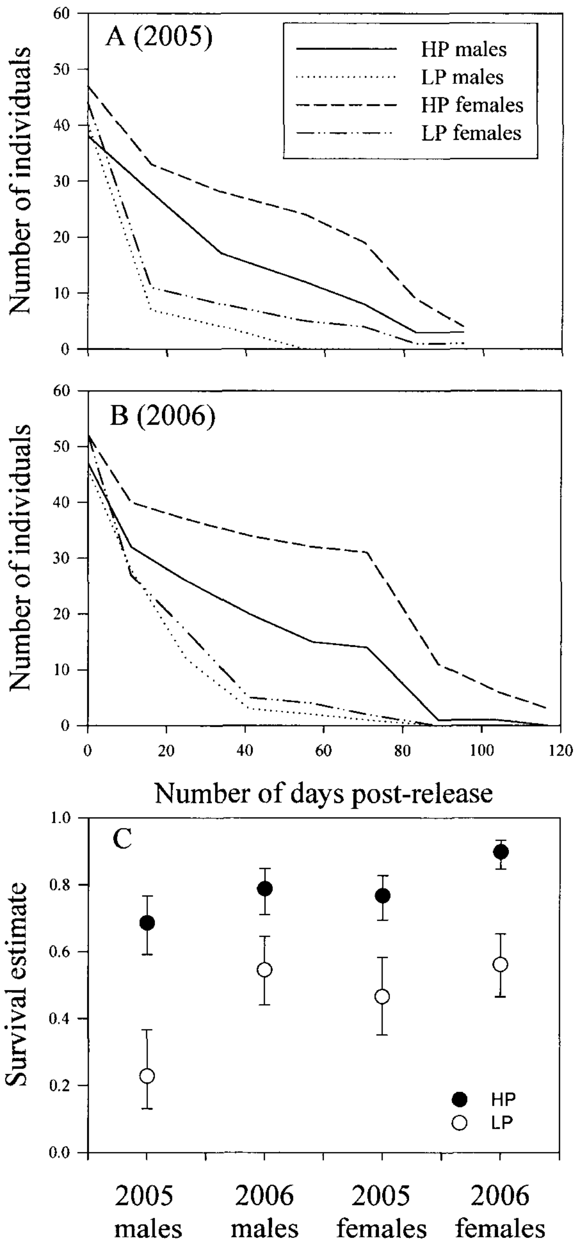

Figure 4.2. Survival of Guppies Introduced to the Focal Site. Numbers of the high- and low-predation guppies originally introduced into the experimental site for 2005 (A) and 2006 (B) plotted against number of days post-release. Probability of survival over a recapture interval (Ψ) was formally estimated using the program MARK (C), errors are 95% confidence intervals.

4.3.2 Differential Mating Success of Ecotypes

In all enclosure experiments, high-predation males sired more offspring than their low-predation counterparts (Tables 4.3 and 4.4), despite equal numbers of both ecotypes in the enclosures. For the 56 male guppies used in these experiments, reproductive success ranges from 0-6 offspring. In the LP1 vs HP trial, there were 29 offspring with high-predation fathers and 15 offspring with low-predation fathers. Differences in reproductive success were more dramatic in the LP2 vs HP trial where 14 offspring were sired by high-predation fathers, whereas only two offspring had low-predation fathers. Overall, the least-squares mean number of offspring sired by high-predation males was more than twice the mean number of offspring sired by low-predation males (2.42:1, $P = 0.017$) (Table 4.4). There was also a significant effect of trial in this analysis (Table 4.4), which is due to the reduced number of females from the LP2 vs HP trial (see Methods).

Table 4.4. Results of Enclosure Experiment. Results of a general linear model that tested for a difference in reproductive success (offspring sired) between high- and low-predation male guppies from the 2 separate trails of the enclosure experiment (see supplemental methods). A total of 56 male guppies, whose reproductive success ranged from 0-6, were included in the analysis. From this analysis, the least-squares mean number of offspring sired by high-predation and low-predation candidate sires was 1.65 and 0.68, respectively.

Factor	DF	F-ratio	P-value
Regime	1	6.1	0.017
Trial	1	11.9	0.0011
Trial x Regime	1	0.3	0.5

4.3.3 Differential Demographic Contributions of Ecotypes

In both years, the experimental populations at the focal site initially declined, which was expected because I did not consider offspring as having recruited to the population until they reached maturation (about 30-50 days after birth) (Table 1, Figure 4.3). Also in both years, secondary floods (starting approximately 65 days after introduction) caused population declines preceding the end of the experiments (Figure 4.3). After these initial declines, population size increased again, and in both years, the majority of these recruits were from the high-predation ecotype (Figure 4.4). In 2005, 207 recruits were assigned to the high-predation population, 26 were assigned to the LP1 population, and 17 were identified as hybrids. In 2006, 93 recruits were assigned to the high-predation population, only 4 assigned to the low-predation (LP2) population, and none were identified as hybrids. Thus, although low-predation ecotypes did contribute to population recovery in a high-predation environment in both years, the overwhelming majority of recruitment was from the high-predation ecotype.

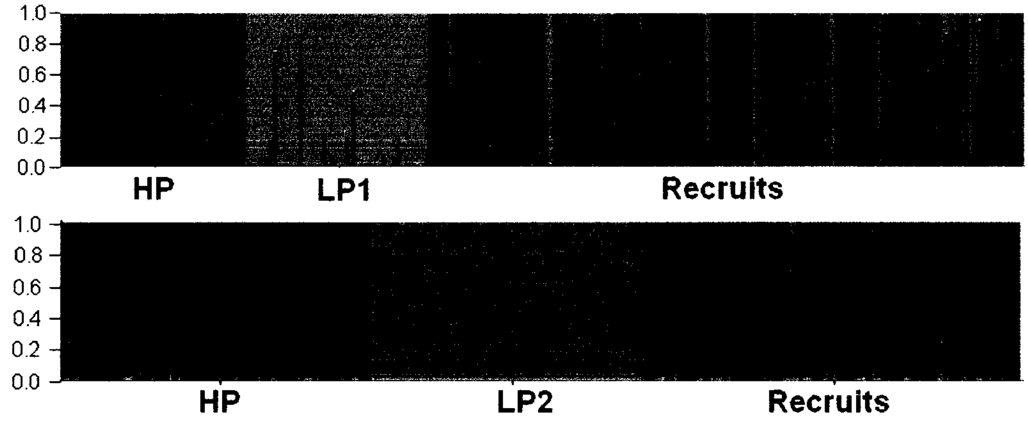

Figure 4.3. Genetic Structure of Experimental Populations. Output of STRUCTURE analyses for K = 2 model. Each experimental individual (parents and recruits) is represented by a single vertical line. These lines are partitioned into two colored segments which represent that individual's estimated membership fraction in either the high-predation (red) or low-predation (blue) (LP1 in 2005; LP2 in 2006) population cluster.

To assess how selection on migrants may have influenced the population dynamics of recovery I must consider how local populations would have responded in the absence of migrants or in the absence of contemporary evolution. In Figure 4.4, I plot the observed size of the experimental populations through time, along with the relative numbers of individuals whose genotypes assigned to either the high-predation or low-predation (including hybrids) populations. I also present the expected size of the experimental population under a "null selection model" – which assumes ecological equivalence between ecotypes (calculated by applying the local high-predation birth and death rates to the total population size at the previous recapture interval, see figure 4.4). To quantify the demographic benefit of migrants, I can compare the observed population size to the number of individuals with pure high-predation genotypes. When the experimental population size was maximal, this benefit amounted to 15 recruits (10% of the population) in 2005 and 6 recruits (5% of the population) in 2006. To estimate the demographic cost of contemporary evolution in the form of selection on migrants, I can compare the observed population size to that estimated under the null selection model. The latter exceed the former by 115 individuals (a 44% cost compared to the null selection model) in 2005 and 108 individuals (a 47% cost) in 2006.

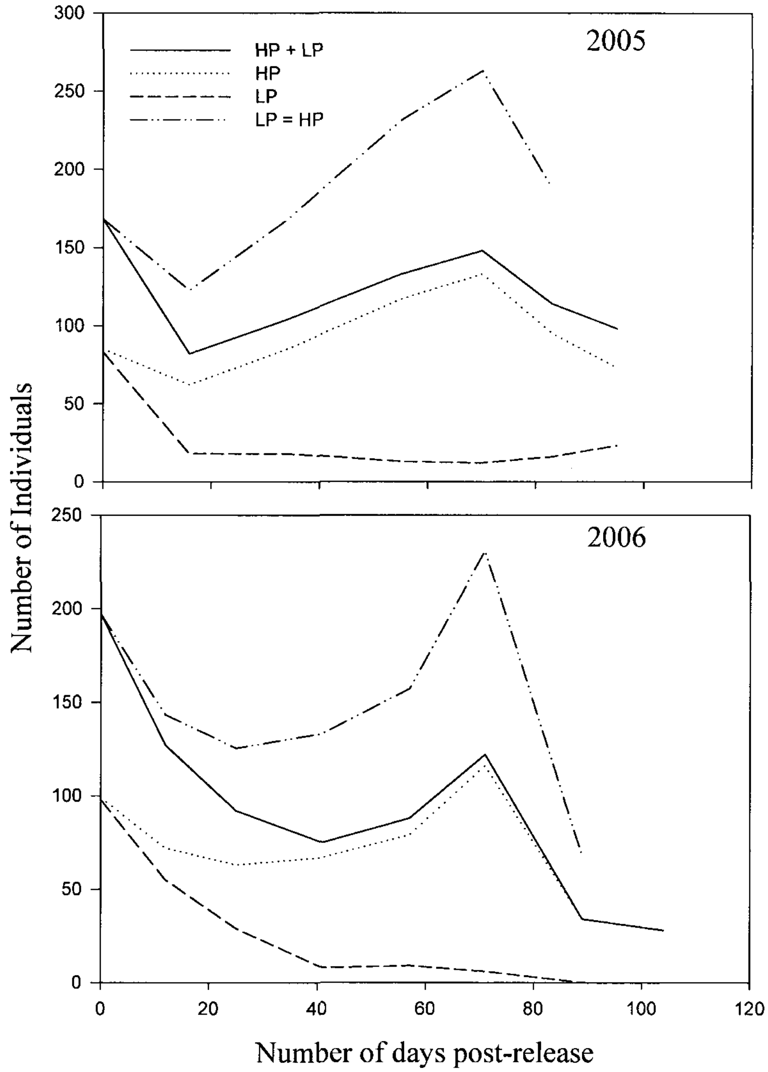

Figure 4.4. Population Size at the Focal Site. The numbers of guppies (parents and offspring) whose genotypes assign to either the high- (HP) or low-predation (LP) populations, and the total number of guppies in the experimental population (HP + LP) plotted against the number of days post-release. Also included is predicted population size assuming selective equivalence between the HP and LP ecotypes (LP = HP). This last line was generated by applying the high-predation birth rate and death rate to the total population size at the previous recapture episode ($N_t = N_{t-1} - (N_{t-1}(\text{HP deathrate})) + (N_{t-1}(\text{HP birthrate}))$).

4.4 Discussion

I combined natural catastrophes with controlled experiments to assess the combined roles of contemporary evolution and demographic rescue on population recovery following a catastrophic disturbance. A series of massive floods decimated guppy population in the high-predation section of the Marianne River. I predicted that population recovery might be accelerated by demographic contributions from neighboring migrant sources into remnant populations. However, I also predicted that, due to local adaptation, the low-predation ecotype would have higher mortality in the high-predation environment compared to the local high-predation ecotype; and that selection against migrants would constrain the demographic benefit of any population "rescue". Ultimately, selection against low-predation guppies was even stronger than I anticipated and thus played a major role in constraining population recovery in the focal high-predation site. At the same time, such selection also assured that the overwhelming majority of individuals in subsequent generations were offspring of the local ecotype, thus maintaining the long-term fitness of the population.

4.4.1 Differential fitness of high- and low-predation ecotypes

Consistent with my predictions, high-predation guppies had much higher survival rates than low-predation guppies in the focal high-predation site. This result is unequivocal, and applies to both males and females, and both sources of low-predation guppies (LP1 and LP2). My head-to-head comparison of ecotype survival is particularly instructive because such assessments quantify the net effects of multifarious selection on comprehensive phenotypes. Differences in survival rates appear to be much stronger than the relatively subtle phenotypic divergence among Marianne River populations in shape (Hendry et al. 2006) and color (Millar et al. 2006) thought to reflect adaptation to divergent predation regimes. Compared to these findings, studies that have estimated contemporary patterns of selection associated with particular phenotypic traits for guppies have produced more equivocal results. For selection associated with body size (Reznick et al. 1996), and color (see chapter two) the pattern and strength of selection seems to be similar in both high- and low-predation sites, inconsistent with predictions distilled from phenotypic differences. Strong survival effects have been noted in another experimental introduction of guppies (Gordon et al. 2009), and in studies of salmon introduced to New Zealand (Kinnison et al. 2008). Taken together, these findings reinforce the idea that many individual traits interact to determine overall adaptation and that assessment based on single characters will often be insufficient.

The ultimate demographic contributions of migrant versus local males to a recovering population will depend not only on viability selection but also on the nature of sexual selection. Thus, using predator-free enclosures, I also tested for relative mating success of migrants relative to residents. Again, the high-predation ecotype seemed to have much higher fitness than the low-predation ecotype. The average number of offspring per male was nearly three times higher for high-predation males. Because predators were not present in the enclosures, this dramatic difference in reproductive success was the result of sexual selection, not viability selection. Because multiple males and females were in each field enclosure, the differences reflect some unknown combination of overt female choice, coercive (i.e., sneak) mating by males, male-male aggression, sperm competition, and female sperm sorting (Magurran 2005). My use of multiple fish, field enclosures, and genetic assignment of offspring make these results more integrative and realistic than most previous studies of sexual selection in guppies.

Thus, owing to both viability and sexual selection, low-predation guppies have lower fitness in a high-predation environment than do high-predation guppies, or in other words, there is profound selection against migrants even given the close geographic proximity of migrant sources and evidence that gene flow does occur (Crispo et al. 2006). Lower fitness does not by itself preclude a demographic "rescue effect" – that is, these migrants might still have a positive effect on population growth following a disturbance. I therefore specifically quantified the potential rescue effect by monitoring the demographic contributions (offspring recruitment) of each ecotype to the experimental population after the introduction in each year.

4.4.2 Demographic Consequences of Selection Against Migrants

I predicted that, due to local adaptation, the demographic contribution of the migrants (low-predation) would be reduced compared to the contribution of the local (high-predation) guppies. However, I was surprised by the magnitude of the difference of the demographic contribution made by locals versus migrants. Compared to the expectations of the "null selection model", the observed population size at the focal experimental site was drastically reduced; this comparison is heuristically informative in showing how ongoing contemporary evolution, in the form of selection against migrants, can play a potentially dominant role in the dynamics of wild populations. Such eco-evolutionary dynamics might easily be overlooked in nature, where they could be considered "*cryptic*" in the sense that they occur in the absence of any apparent change in selective conditions and without overt trait changes generation-to-generation. Importantly, although high-predation populations may benefit less from an immediate rescue effect, selection appears to be very effective in limiting genetic loads that might otherwise impair mean local fitness and rates of rebound during subsequent generations or future disturbances (Ronce and Kirkpatrick 2001). It remains to be seen whether eco-evolutionary effects ultimately place particular populations at higher or lower risk of extinction.

4.4.3 Conservation Implications

The metapopulation concept is fundamental to modern conservation biology, including efforts to preserve biodiversity (Damshen et al. 2006) and to predict biological responses to climate change (Loarie et al. 2009). Furthermore, interactions between divergent selection, adaptive divergence, and gene flow are fundamental to evolutionary theory (Hendry et al. 2001; Kawecki and Holt 2002). Few empirical studies, however, have specifically linked evolutionary and metapopulation theory to evaluate the eco-evolutionary dynamics associated with selection against migrants (Hanski and Saccheri 2006; Duckworth and Badyaev 2007; Moore and Hendry 2009), much less the role of such dynamics in population recovery from catastrophic population disturbance. My experimental assessment supports prior theoretical work (Boulding and Hay 2001; Kinnison and Hairston 2007; Garant et al. 2007; Ronce and Kirkpatrick 2001; Kawecki and Holt 2002; Holt et al. 2003) in suggesting important interactions between selection, migration and demography in nature and places those interactions in a pressing conservation context – population recovery following catastrophe. Whereas prior studies of contemporary evolution in conservation contexts have tended to emphasize modest but persistent disturbance and directional trait change (Visser 2008; Darimont et al. 2009), such conditions are not prerequisite for eco-evolutionary conservation concerns. I have shown that eco-evolutionary dynamics may be a consideration even where disturbance is fleeting, selection patterns persist largely unchanged, net evolution is limited, and populations exchange migrants. The potential for eco-evolutionary dynamics to limit the efficacy of natural rescue effects or human restoration efforts should be considered carefully in light of evidence that humans may be accelerating both the incidence of catastrophic disturbance and the fragmentation of metapopulations into more physically isolated and ecologically divergent populations.

CHAPTER 5: CONCLUSION

My dissertation research addressed two complimentary topics: 1) spatiotemporal variation in natural selection in high- and low-predation populations of Trinidadian guppies, and 2) the demographic consequences of contemporary evolution in the form of selection against migrants. I would now like to summarize the major results of my dissertation research and their broader relevance, discuss the limitations of my research, and suggest possibilities for future work. Below, I will first consider the major components of my dissertation separately, before synthesizing them on a broader scale.

5.1 Spatiotemporal Variation in Selection

Divergent natural selection between ecologically variable habitats is widely acknowledged to be an extremely important process in adaptive divergence. Few studies, however, have actually tested for selection in replicated natural populations (Siepielski et al. 2009), much less implemented statistical approaches that explicitly evaluate adaptive hypotheses for common or divergent elements of selection within and among putative selective regimes using replicated selection experiments. A major contribution of my dissertation was to directly measure natural selection in high- and low-predation populations of Trinidadian guppies, and to pool data from multiple selection experiments to test *a priori* hypotheses for selection within and between divergent predator regimes. Until now, the evidence for divergent natural selection in this classic study system has mostly come from comparative studies of guppy phenotypes between high- and low-predation sites (for example, Endler 1978), laboratory experiments (for example, Godin and McDonough 2003), and experimental introductions (for example, Endler 1980).

These previous studies have strongly suggested the presence of functional trade-offs between different aspects of performance from which I distilled predictions regarding the anticipated relationship between fitness and traits in high- and low-predation selection experiments. For male color (chapter two) and body shape (chapter three), my *a priori* predictions for contemporary selection, predictions that have been stated directly or held implicitly for decades, often turned out to be overly simplistic, if not totally wrong. The most general case of this observation is that, contrary to predictions from the literature, natural selection can be quite strong in low predation sites, despite generally lower mortality rates compared to high-predation sites.

The discrepancies between common predictions and my results have been useful in generating novel hypotheses of the true nature of functional trade-offs in this system, and by extension studies of role of predators in shaping the traits of prey in general. For example, given my result that the pattern and strength of natural selection associated with male color seems to be similar in both high- and low-predation regimes, I developed a new hypothesis that I believe has considerable merit for future study, that male guppies in high-predation populations are more colorful because of the indirect effect predators have on reducing the strength of sexual selection, rather than the direct effects of divergent patterns of prey consumption. Importantly, this hypothesis would be considered far less credible if no directed study of selection in nature were performed and we were allowed to continue assuming that natural selection is weaker in low-predation sites. Thus, I suggest that explicit tests of divergent selection between putative selective regimes are an under-applied but powerful tool that could profitably be employed in many study systems. A number of interesting possibilities for future research are suggested by my results.

My assessments of spatiotemporal variation in selection led me to suggest that covariation in behavioral traits strongly influences the relationship between morphological traits and fitness. Thus, I suggest that behavioral traits may be universally important in modifying relationships between morphological traits and fitness, and recommend that future studies should explicitly evaluate the strength of selection associated with behavioral traits in wild populations. Such studies are relatively rare (Kingsolver et al. 2001), but have high value for the effort, especially if such estimates could be directly assessed alongside selection on the morphological traits of individuals. For example, in guppies an especially interesting possibility is directly measuring the fitness costs associated with predator inspection behavior, which is thought to be an anti-predator adaption in high-predation guppies (Dugatkin 1992). An extension of this type of research would be to measure selection directly on performance (i.e. fast start swimming speed and swimming endurance), which potentially integrate morphological, behavioral, and physiological traits, such selection studies are also relatively rare (Irshick et al. 2008), but fill and important gap that is implicit in most studies assessing the trait – performance – fitness pathway (Ghalambor et al. 2003; Walker 2007).

The maintenance of trait variation in the wild is a topic of general interest in evolutionary biology, and the guppy system has become a model system for investigating this topic (see chapter two). The spatiotemporal variation in selection I have documented for both color and shape strongly suggest that variability in natural selection should not be considered mere "slop", but the partial basis for mechanisms generating and maintaining trait variation in this system. However, my selection studies were not ultimately designed to evaluate the proximate causes of variability in natural selection. In future studies, it would be interesting to experimentally manipulate potential causative agents in order to determine if fluctuating selection is related to variability in specific environmental conditions (for example, water clarity). Additionally, the maintenance of trait variation is likely strongly influenced by the mode of selection. Theoretically, stabilizing selection is anticipated to reduce trait variation, while disruptive selection is predicted to increase trait variation (Lande and Arnold 1983). My dissertation research did not specifically consider non-linear selection because the predictions I tested were more characteristically about directional selection. Future studies, however, could take advantage of the phenotype and fitness datasets I have accumulated to study non-linear selection. Such studies could potentially provide additional insights into the maintenance of trait variation in this system.

For both color and shape, my selection studies suggest that trait variability among populations may be strongly influenced by interactions between natural and sexual selection. For color, this result was anticipated *a priori*, because I explicitly predicted that the evolution of male color in this system represent a balance between survival and mate attraction. However, for shape, potential interactions between sexual and natural selection were not anticipated. Nonetheless, it seems possible that stronger sexual selection in low-predation habitats may influence the evolution of body shape in this system (see chapter three). Selection studies that simultaneously estimate sexual and natural selection for both color and shape would be extremely beneficial in evaluating some of the predictions generated by my estimates of natural selection (for example, Hamon and Foote 2005).

5.2 Eco-Evolutionary Effects of Selection Against Migrants

In my fourth chapter I combined field experiments with natural catastrophic events to show that ongoing evolution is a major determinant of migrant contributions to population recovery. I can see at least three reasons why these results are highly relevant to the conservation of populations and species suffering from acute stressors. First, the prevalence of drastic disturbance is likely increasing as a result of human activities and global climate change (for example, Post et al. 2009). Second, several recent studies and growing body of theoretical work, have revealed the ecological importance of evolutionary processes under non-equilibrium conditions (for example, Hanski and Saccheri 2006). Third, restoration and recovery of endangered populations frequently relies on supplementation from neighboring populations, or domestically-reared individuals (for example, Araki et al. 2007). Taken together, the above points stress the need for theoretical and empirical studies that consider evolutionary mechanisms in attempts to predict whether natural immigration or supplementation may, or may not, aid the recovery of threatened populations.

While I maintain that my field experiments were a heuristically useful exercise documenting strong eco-evolutionary effects, much work remains. I here suggest two improvements that could be implemented in future studies. First, a major limitation of this component of my dissertation research is that I was unable to directly determine if migrants, in an absolute sense, benefited or impeded local population recovery on the near- and long-term. Migrants did numerically contribute a small portion of offspring to total population growth; however, that was to be expected. A more intriguing question is the degree to which such "successful" migrants ultimately influence phenotype distributions and the fitness of populations in subsequent generations. A first step to this would be to understand how introgression of non-local genes might influence the survival and reproduction of hybrid offspring (Ronce and Kirkpatrick 2001). In truth, I had hoped to assess this issue, but simply did not have the resources available to do so. It would also be fascinating to compare the rate of population recovery between numerous replicate populations that had either received a demographic boost from migrants, or that had not received any migrants, over several generations. However, the effort and resources to undertake such a project would be immense.

A second limitation of this component of my dissertation research is that no direct link was established between genetic variation, selection and demographic effects. This does not undermine the basic premise that natural selection can mediate the contributions of migrants, but it again has relevance to the potential long-term consequences of gene flow. The allele frequencies of the experimental populations certainly changed over time, and this evolutionary change was the result of strong selection against migrants. However, I do not know if fitness differences between high- and low-predation guppies were the result of trait differences resulting from environmental effects on phenotypes associated with the different rearing environments of source fish, heritable differences between populations, or both. Regardless, the ultimate evolutionary outcome is the same, migrant genotypes are filtered out of the local population. However, in the same way that it is intellectually satisfying to indentify the ecological agents of selection (Endler 1986); I argue it would also be intellectually satisfying to identify the proximate cause of trait variability that results in strong ecological consequences. An exciting possibility for future work would be to experimentally manipulate genetic variation in functionally important traits, and assess ecological consequences in wild populations. In a way, this approach is analogous to laboratory studies that have assessed the link between evolvability and population dynamics (for example, Yoshida et al. 2003, 2007).

5.3 Integrated Summary

Considering the results of these two research components together I would like to highlight two strong conclusions. First, it seems that contemporary patterns of viability selection are not always consistent with geographical patterns of trait variation in this system. For at least three traits: size (Reznick et al. 1996), color (chapter two), and shape (chapter three) estimates of selection in the wild are wildly variable and only occasionally consistent with predictions based on biophysical first principles and patterns of trait divergence between regimes. Interestingly, the somewhat equivocal results from the selection studies are in sharp contrast with the results of the introduction experiment where two different source populations of low predation guppies each had very low fitness compared to the locally-adapted high-predation source (also see Gordon et al. 2009). The logical inference here is that selection is potentially strong and pervasive, but rarely attributable to the overwhelming effects of a single critical trait. Stated differently, multiple traits contribute to overall fitness, and the absence of a predictive relationship between individual traits and fitness by no means implies that contemporary adaptive processes are not acting or weak. The complexity suggested by these results is not to be disparaged, but like so many aspects of my dissertation emphasize the importance of multiple methodological approaches to study evolution in its full complexity. Without such variable approaches there is a worrisome risk of stereotyping particular study systems in ways that may be heuristically appealing but intellectually stagnating.

Second, taken together, the two components of my dissertation research suggest that eco-evolutionary effects are likely common in nature. In chapter four I documented strong interactions between selection and demography even though the environmental disturbance was fleeting and there was no net change in phenotype. In comparison with many earlier studies of eco-evolutionary dynamics that focused on non-native species that have recently colonized a novel habitat (for example, Kinnison et al. 2008; Palkovacs and Post 2009), the results of my field experiments highlight the possibility that eco-evolutionary effects can be important even in the absence of large-scale, persistent changes in selective regime. Furthermore, if we accept that natural selection can have ecological effects, it follows that the general importance and relevance of such effects to contemporary ecological process will be strongly related to variability in the strength and pattern of natural selection. Indeed, natural selection is implicit in any complete view of true eco-evolutionary dynamics, not only for its role in driving evolution that might influence ecological conditions, but also in the role that such altered ecological conditions may play in reshaping selection, in what has been termed an eco-evolutionary feedback loop (Post and Palkovacs 2009). The variation in selection documented in chapters two (color) and three (shape), suggest that eco-evolutionary effects owing to the direct effects of selection may occur on very fine spatiotemporal scales, and thus are likely to be common in nature. However, in recognition of the aforementioned feedback loop, it must also be recognized that the selection we see today may not be fully characteristic of the selection that initially started the eco-evolutionary process down its partly self-cut path. It is even possible that the variation in selection so prevalent in my studies owes in part to concurrent eco-evolutionary dynamics.

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Dylan Jones Weese was born in Regina, Saskatchewan, Canada on November 5th, 1977 to Robert and Menna Weese. He later moved to Ottawa, and then Toronto (both in the province of Ontario). After graduating from Humberside Collegiate Institute, Dylan moved to Guelph, Ontario to study zoology at the University of Guelph. While attending the University of Guelph, he undertook an undergraduate research project studying the lake trout of Great Bear Lake (Northwest Territories, Canada) with Dr. David Noakes. This experience convinced him to follow a career in biology, and under the mentorship of Dr. Beren Robinson, Dylan studied the evolutionary ecology of pumpkinseed sunfish and obtained a MSc degree from the University of Guelph. Dylan then came to the University of Maine to start a PhD project with Dr. Michael Kinnison in the School of Biology and Ecology, where he studies the evolutionary ecology of the Trinidadian guppy. Dylan is a candidate for the Doctor of Philosophy degree in Biological Sciences from the University of Maine in August, 2010.