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INTRA- AND INTERPOPULATION VARIATION IN MORPHOLOGY AND BEHAVIORS IN THE SAILFIN MOLLY, *POECILIA LATIPINNA* (POECILIIDAE: *POECILIA: MOLLIENESIA*)

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BEHAVIORS IN THE SAILFIN MOLLY, *POECILIA LATIPINNA*
(POECILIIDAE: *POECILIA*: *MOLLIENESIA*)

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ABSTRACT

Understanding factors that contribute to population differences can provide

insight into the process of speciation, yet population level studies seldom take

into account variation among individuals within a population. Such

intrapopulation variation may influence the degree to which interpopulation

variation in suites of traits can arise. My research focused on characterizing

intra- and interpopulation variation in male morphological and behavioral traits in

the sailfin molly, *Poecilia latipinna*. First, I characterized the overall body shape

of several populations of *P. latipinna* collected from north Florida in two different

years. I used linear morphological measurements to examine shape and found

there to be population differentiation within each sampling year, but some of the

traits that explained this variation differed between sampling years. Males were

more often correctly classified back into their original populations in 2005 (72%

correctly assigned) than in 2007 (67% correctly assigned), suggesting variability

between years in the degree of morphological differentiation. Second, I

generated unique behavioral profiles for males from three of these populations in

three distinct behavioral contexts: mating, activity, and inspection. I tested males

in two situations per context: mating (with a receptive vs. a non-receptive

female); activity, (after viewing a social group vs. a predator); and boldness,

(inspecting a conspecific social group vs. a predator). I found that male sailfin

mollies showed (1) strong positive associations between situations within a

context, (2) strong positive associations between courtship display rates and level of boldness in predator inspection, and (3) no significant differences in behaviors between populations. Male size at maturity (known to have a Y-linked genetic basis) was strongly positively associated with courtship display rates and boldness but not activity. These findings suggest that mollies may possess a behavioral syndrome where larger males are bolder toward predators, court females more vigorously and have proportionately larger dorsal fins. Thus, variation among individuals within populations in these associated traits may be slowing the degree of differentiation in behavior among populations despite interpopulation variation in body shape.

DEDICATION

This thesis is dedicated to my daddy, Andrew H. Beck (1952-2003), and to my

dear friend, Lara Relyea-Fraley. Daddy, your quiet love of nature inspired me

from an early age to respect and enjoy everything the natural world has to offer.

Lara, you taught me so much more than biology! You taught me to be strong

and independent and to value my education and myself. You shared with me

your love of science and enthusiasm for learning. Your strength and

encouragement gave me the confidence to leave home and pursue my dreams

and educational goals. You have both helped me, in so many ways, to become

the person I am today. Thank you.

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thank all of my family, especially my mother-in-law and close friend, Ms. Dawn,

for believing in me and encouraging me to take this journey.

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CHAPTER ONE

UNDERSTANDING THE INFLUENCE OF MORPHOLOGY ON BEHAVIOR
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INTRODUCTION

Variability among conspecific populations is a common, widespread, natural phenomenon that occurs in a vast array of different taxa (reviewed by Foster 1999; Foster and Endler 1999). Populations can vary in behaviors and morphological traits that are used in signals for attracting mates, defending territories, or advertising social status. Such variability among populations in signaling traits and female preferences for these traits can promote divergence and lead to premating reproductive isolation (Ptacek 2000). For example, bower birds (Family: Ptilonorhynchidae) are a group of 14 different species of small to medium-sized birds that occurs throughout Indonesia, Australia, and New Zealand (Borgia 1985; Diamond 1986; Borgia et al. 1987; Borgia 1995a, b). The males of these species build elaborate bower structures of various colors and designs, upon which males display to attract the attention of females (Borgia 1985; Borgia et al. 1987; Borgia 1995a, b; Albert et al. 2000). Male plumage varies in color and conspicuousness by species and population, and the more drab the male is, the more colorful and elaborate the bower he builds (Diamond 1986; Albert et al. 2000). Variation in bower structure is paralleled by female

preferences for particular bower characteristics of males from their own populations (Borgia 1985; Borgia et al. 1987; Borgia 1995a, b). Hence, divergence in bower mating signals between bower bird populations has played an important role in reproductive isolation and speciation in these birds (Borgia 1985; Borgia et al. 1987; Borgia 1995a, b).

Interpopulation variation has the potential to promote speciation, particularly when male mating signals are under strong natural or sexual selection leading to reproductive isolation. Environmental variation between habitats can promote divergence in male signaling traits, which can then be reinforced by sexual selection through divergence in female mating preferences between environments (Schluter 2001). For example, in threespine stickleback (*Gasterosteus* spp.) populations, males differ in nuptial throat coloration by foraging habitats, such that limnetic males are red, and benthic males are black (Boughman 2001). Male coloration has evolved as a result of natural selection to take advantage of the signal transmission of color through the waters of their varying habitats. Waters vary along a gradient from clear in limnetic habitats to brownish (tannin enriched) in benthic habitats.   Red wavelengths transmit as a high contrast color in clearer water, but are washed out by the red-shifted background of the more brownish water in benthic environments (Boughman 2001). Males in tannin enriched benthic habitats have evolved black coloration, as black transmits better (higher contrast) through the brownish water. Females possess greater optic sensitivity to, and attraction for, red color when they are

from clearer water limnetic habitats, and thus, strongly prefer to mate with males from their own populations, enhancing divergence between environments in nuptial coloration, which promotes the speciation process (Rundle et al. 2000; Boughman 2001).

Although population variation has the potential to promote speciation, it is not an absolute outcome. For example, populations of the Trinidad guppy (*Poecilia reticulata*) vary in intensity of predation, nutrient richness, and degree of geographic isolation from one another (Endler 1983; Houde 1997). As a result, natural selection has favored differences between populations in male behaviors (e.g., courtship display and predator inspection behavior) and morphological traits (e.g., body size and standard length, male body coloration; Houde 1997; Magurran 1998). Reciprocal transplant studies have shown that guppies have a high rate of evolution in these traits in response to the different environments; approximately seven orders of magnitude faster than the rate of evolution in morphology estimated from the fossil record (Reznick et al. 1997). Under similar environmental conditions, the African rift lake cichlids (Family: Cichlidae) rapidly speciated into hundreds of different species (Meyer et al. 1990; Meyer 1993), yet the Trinidad guppy populations show very little evidence of reproductive isolation (Magurran 1998). Magurran (1998) argues that one reason for this lack of speciation among guppy populations may be attributed to the morphological variation seen between the sexes that is associated with differences in resource allocation for reproduction. Females are naturally larger in size and mass than

males, allowing them to feed in deeper, more benthic areas, while males are largely restricted to open water feeding and, in some cases, shallow benthic areas. Trinidad guppies, unlike the African cichlids, are less able to diverge as a result of naturally occurring feeding morphs in populations because the morphs are a result of sexual dimorphism rather than trophic specialization by both sexes (Magurran 1998). Hence, variation within populations impedes divergence between them. Magurran (1998) also proposes increased gene flow, as a result of males continually searching for receptive females, as another explanation for the lack of reproductive isolation observed among guppy populations. Males within these populations have a high degree of mobility, and compared to females that tend to remain in established schools, males swim from school to school in search of mating opportunities, often through sneaky copulations, which undermines female choice (Magurran and Seghers 1994a, b; Magurran 1998). Such male-biased dispersal increases gene flow between schools and populations and inhibits interpopulation divergence and speciation.

Variability among individuals within a population is also a common and widespread phenomenon in many taxa, yet such intrapopulation variation has been largely ignored by behavioral researchers for more than fifty years (Sih et al. 2004a). More recently, the role of variation among individuals, especially in behavior, in the persistence of seemingly non-adaptive traits in populations has been explored (Sih et al. 2004a; Bell 2007). For example, in some populations, individuals have been observed foraging in the presence of a predator (bold

behavior type), a potentially maladaptive behavior, while the other members of the population have all fled or hidden for their safety (shy behavior type; McElreath and Strimling 2006). When such behaviors are correlated across different situations within an ecological context (e.g., foraging in the presence versus the absence of a predator) or across different contexts (e.g., mating, foraging, predator avoidance), these correlated suites of behaviors are referred to as a behavioral syndrome (Sih et al. 2004b; Bell 2007). Behavioral syndromes have been described in a growing number of taxa including: insects (Sih et al. 2002; Pruitt et al. 2008; Logue et al. 2009; Walling et al. 2009; Wilson et al. 2010), fish (Godin and Dugatkin 1996; Budaev 1997; Coleman and Wilson 1998; Bell and Stamps 2004; Brown and Braithwaite 2004; Bell 2005; Brown et al. 2005; Moretz et al. 2007; Wilson and Godin 2009), amphibians (Richardson 1993; Storfer and Sih 1998), reptiles (Stapley and Keogh 2004, 2005; Carter et al. 2010), birds (Dingemanse et al. 2003; Dingermanse et al. 2004) and mammals (Hessing et al. 1994; Gosling 1998; Réale et al. 2000). The presence of a behavioral syndrome may help to explain why potentially maladaptive traits, in certain contexts, may persist (Sih et al. 2004a, b).

Behaviors that are correlated can produce trade-offs, which can potentially carry over across different contexts, thereby, having a major effect on the evolutionary process (Sih et al. 2004a). According to Sih et al. (2004a), these tradeoffs produce three important general implications that should be considered by behavioral researchers. First, the fact that behaviors are correlated within a

behavioral syndrome implies that they evolved as a complete package and should, therefore, not be studied in isolation of each other, but rather, as a single unit. Second, trade-offs that carry over across contexts can result in individuals that do not behave optimally in some situations. Finally, this potential for suboptimal behavior in some contexts, allows for some individuals to do well in certain situations or contexts, while they do poorly in other situations or contexts, which could help to explain the maintenance of individual variation in behavior (Sih et al. 2004a, b). For example, all individuals exhibit some level of aggression, which they can alter across situations and contexts, but certain individuals may always be more aggressive than others within a given population. Individuals that possess this heightened level of aggression may be at an advantage in defending territory, holding nest sites, or fighting for potential mates, but they may be at a considerable disadvantage when it comes to courting mates or providing parental care (Sih et al. 2004a,b). So a syndrome (e.g., boldness) that has little to do with a specific context (e.g., mating systems) may actually be linked to it, as a result of its fitness advantage in a different context (Stapley and Keogh 2005).

There is now a push within the behavioral community, and a growing body of evidence, to establish a strong foundation for behavioral syndrome research. The majority of this work has looked at behavioral syndromes among individuals within a population; far fewer studies have examined behavioral syndromes among different populations (Bell 2005, Bell and Stamps 2004, Brown and

Braithwaite 2004, Brown et al. 2005, Pruitt et al. 2008). Bell (2005) argues that behavioral syndromes can remain stable or vary across populations as a result of two opposing hypotheses. The 'constraint' hypothesis can be used to describe syndromes where the behaviors are tightly coupled and the underlying evolutionary mechanisms are difficult to modify. Here behaviors may be strongly genetically correlated as a result of pleiotropy or strong linkage disequilibrium. We would expect to find that such syndromes remain stable across populations. However, if behaviors in a syndrome are easily decoupled or highly influenced by environmental pressures, then we would predict populations to be more variable, as described by the 'adaptive' hypothesis.

Álvarez and Bell (2007) observed the behavior of sticklebacks from three pond and three stream populations. They found that stickleback populations varied significantly in risk-taking behavior (i.e., willingness to forage following a simulated, aerial predator attack), with stream populations exhibiting bolder behavior than pond populations. A previous study examined stickleback behavior from two populations: Navarro River and Putah Creek (Bell and Stamps 2004, Bell 2005). The sticklebacks from the Navarro population were under intense predation pressure from fish, bird, and snake predators, while the sticklebacks from the Putah population were under less intense predation pressure having fewer, primarily fish, predators to avoid. Bell and Stamps (2004) found that although the Putah sticklebacks were overall more bold, more aggressive, and more active than the Navarro population, there was no evidence

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of a behavioral syndrome in the Putah population. A bold/aggressive behavioral

syndrome was, however, described in the Navarro population, which remained

stable in correlations of these behaviors across ontogeny. In both of these

studies, populations differed significantly in behavior, which appears to be

strongly influence by an environmental pressure (i.e., degree of predation

pressure), and in the study that observed fish behavior in several different

contexts (Bell and Stamps 2004) a behavioral syndrome was described in one,

but not the other population, providing additional support for the 'adaptive'

hypothesis (Bell 2005).

Within populations, differing selective pressures (e.g., natural and sexual

selection) often compete with each other, limiting the degree to which

populations can diverge from each other. Godin and Dugatkin (1996) reported

that female guppies, *P. reticulata*, have strong preferences for bolder males.

This suggests that males are under sexual selection pressure to be bolder (i.e.,

willing to approach novel objects and predators and remain active in novel

situations and environments). Due to the nature of their increased risk taking

behavior, bolder males are more likely to be injured or killed than shier males that

avoid such risky, bold behavior. Shier males are favored by natural selection and

rewarded with a potentially longer lifespan, increasing their lifetime reproductive

success. Since natural and sexual selection favor and help to maintain different

behavioral types within these populations, there is a diminished opportunity for

variation between populations to become so great that reproductive isolation can evolve (Schuster and Wade 2003).

Across a wide range of taxa, it has been observed that males within a species, and even within a single population, can have strikingly different behavioral profiles, and these behavioral polymorphisms are often associated with distinct morphological types, leading to alternative male mating behaviors or strategies (reviewed in Gross 1996; Schuster and Wade 2003). For example, in the marine isopod, *Paracerceis schulpta*, three distinct male morphs have been described (Shuster 1987; Shuster and Wade 1991). The alpha males have an enlarged body with uropods and telsons that they use to actively exclude other males from their spongocoels and, therefore, their harems. Beta males are female mimics, and gamma males, the smallest of the males, are juvenile mimics. Both beta and gamma males attempt to sneak copulations with females when the alpha male is distracted. Similar mating systems of territoriality/courtship versus satellite/sneakers have been described in other systems as well (Gross 1982, 1985; Lank and Smith 1987; Gross 1991a, b; Sinervo and Lively 1996).

This relationship between behavioral and morphological polymorphisms can be genetically fixed, as in the marine isopods, or more evolutionarily plastic and adaptive (Eberhard 1982; Ehlinger and Wilson 1988; Walling et al. 2009 and cited references). In song birds, for example, courtship song is an important male mating signal, but the song a male bird can sing is constrained by the size

and shape of its beak. In a number of song bird species, birds with smaller beaks can open and close their beaks more rapidly than a larger beaked bird, producing a very different song (reviewed in Nowicki and Podos 2004). A speciation event has been described between populations of the swamp sparrow, *Melospiza georgiana*, due to just such a morphological adaptation (Ballentine 2006). Birds in coastal populations (*M. georgiana nigrescens*) have larger beaks than inland populations (*M. georgiana georgiana*), which is thought to be an adaptation allowing the birds to feed on benthic invertebrates in the coastal marshes. The larger beaks limit the coastal birds' ability to produce rapid, broad band trills, thereby, reducing their overall vocal performance, a component of bird song known to be important in mate choice (Ballentine et al. 2004; Nowicki and Searcy 2005; Ballentine 2006). This divergence in courtship song is thought to have been important in the speciation of these swamp sparrow races. Similar byproduct divergence has been described in Darwin's finches (*Geospiza* spp.) of the Galapagos Islands, where beak size and shape have been shown to be under strong natural selection to take advantage of novel food sources and ecological niches. In turn, such ecological divergence has had a strong influence on male courtship song and, therefore, the speciation of Darwin's finches (Podos 2001, Podos and Nowicki 2004).

Understanding the relative roles of variability in morphology, behavior and associations between them at the level of both individuals and populations is important in determining the causes and consequences of population divergence

and speciation. My thesis research focused on measuring levels of variation in morphology and behaviors in mating, activity and social contexts at both the individual and population levels in the sailfin molly, *Poecilia latipinna*. I used these fish as a model system to investigate the interaction between individual and population level variation and how they might promote or inhibit divergence in mating signals and the speciation process.

THE STUDY SYSTEM

The livebearing fishes commonly known as mollies are an ideal group in which to study mating signal divergence because enormous variation in behavior and morphology associated with mating signals exists at all hierarchical levels: between species, among populations within a species, and among individual males within a single population (Ptacek 2005; Hankison and Ptacek 2007). There are four species within the sailfin molly clade (*Poecilia: Mollienesia*): *P. latipinna, P. petenensis, P. latipunctata*, and *P. velifera* that range from the southeastern United States into Mexico along the Gulf and Atlantic coasts (Figure 1.1; Ptacek and Breden 1998). The sailfin molly of the southeastern US, *P. latipinna*, can naturally tolerate a wide range of salinities from fresh water to full strength seawater (Travis and Trexler 1987), allowing it to occupy the widest geographic range of the four sailfin molly species (Figure 1.1). *Poecilia latipinna* can be found in inland freshwater springs and ponds and coastal waterways

along the Gulf of Mexico, around the tip of Florida, and along the Atlantic coast

as far north as Georgetown, South Carolina (Travis and Trexler 1987).
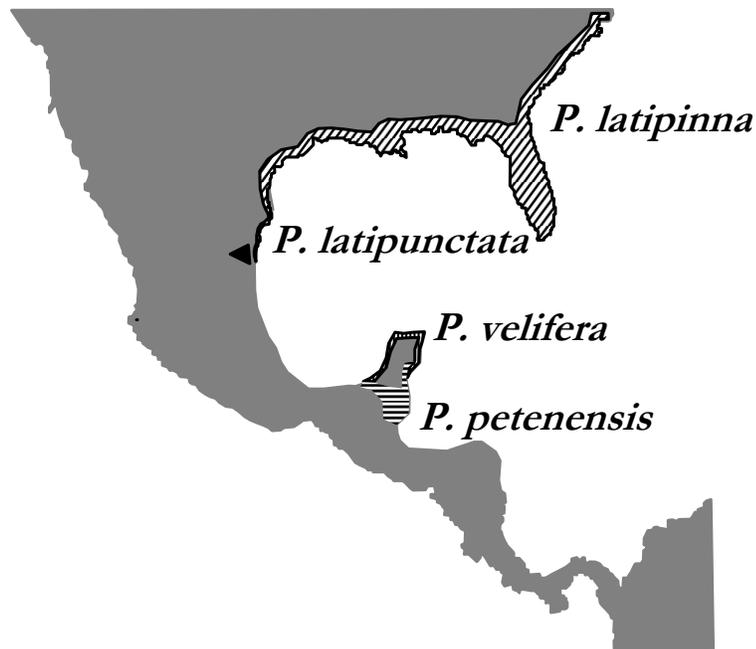
Figure 1.1. Map of North and Central America showing the distribution and range of four described species of sailfin mollies. (Figure modified from Ptacek and Breden 1998).

Male sailfin mollies exhibit enormous variation in male body length

(standard length (SL) measured from the tip of the snout to the insertion of the

caudal fin) both between and within populations (Farr et al. 1986; Travis 1989;

Ptacek and Travis 1996). In sailfin mollies, as in many poeciliids, male size is

fixed at maturity (i.e., growth ceases following complete formation of the

gonopodium, the fused anal fin used in internal fertilization) and inherited

patriclinally (Travis 1994a, b) in a fashion similar to that of the swordtail genus

Xiphophorus, where size is controlled by a single locus at the *P* gene on the Y

chromosome (Kallman 1989). Sons mature at a similar size as their fathers

(within a few millimeters; Travis 1994a, b; Figure 1.2), and since little growth

occurs after sexual maturity, a small male can never grow to the size of a large

male (Figure 1.3).
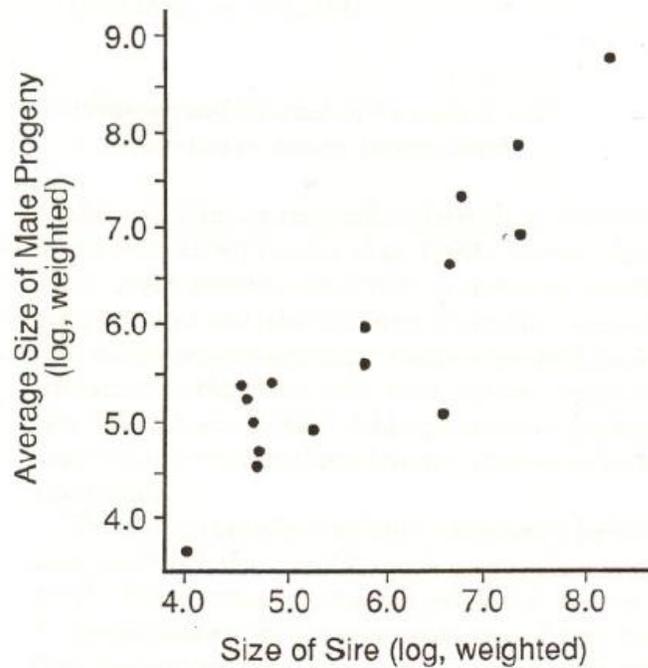
Figure 1.2. Scatterplot of mean SL of male progeny (sons) in a paternal half-sib family and the SL of their sires. (Figure from Travis 1994a, b).

SL = 50 mm

SL = 25 mm

Figure 1.3. A large male (SL = 50 mm; top) and a small male (SL = 25 mm; bottom) collected from Steve's Ditch, Wakulla County, Florida.

Sailfin mollies are sexually dimorphic with males possessing an enlarged dorsal fin (i.e., sailfin) that they raise and lower in a courtship display to elicit female cooperation in the mating process (Farr 1989). Males also possess a copulatory organ called the gonopodium, which is formed from the fusion of the last anal fin rays (Constanz 1989). The gonopodium is used to transfer sperm into the female's gonopore (behavior termed a gonopodial thrust) for internal

fertilization. Females give birth to a brood of live young approximately every 28 days (Trexler 1989). For 24 - 48 hours after giving birth, females are receptive to male courtship behavior and can be enticed by male courtship displays to cooperate in the mating process by holding a stationary position to allow for more successful sperm transfer (Farr and Travis 1986; Farr 1989). Females advertise their receptivity to males during this narrow window of time through the release of pheromones in their urine, and sailfin males have been shown to perform a higher number of display behaviors to receptive females as compared to non-receptive females (Travis and Woodward 1989; Sumner et al. 1994). Males will still, however, court and mate with non-receptive females because female mollies store sperm that can remain viable for up to four months (Constanz 1989).

Males of *P. latipinna* populations show considerable variation among populations in many behavioral and morphological traits (Ptacek 2005). Studies have shown that males vary among populations in their rates of different courtship and mating behaviors (Farr et al. 1986; Trexler 1986; Travis and Woodward 1989; Travis 1994b; Ptacek and Travis 1996, 1997) and in morphological traits that are known to be under natural and sexual selective pressures (Farr et al. 1986; Trexler 1986; Farr 1989; Trexler et al. 1994; Ptacek and Travis 1997; Gabor 1999; Gabor and Page 2003; MacLaren et al. 2004). Correlations between morphology and courtship behavior have been found, with varying degrees of strength, in some, but not all populations (Farr et al. 1986; Ptacek and Travis 1996).

Populations of *P. latipinna* exhibit a wide distribution of male and female

standard body sizes, which vary within and among populations (Kallman 1989;

Travis 1989; Travis 1994b; Ptacek and Travis 1996). Sexually mature males can

naturally vary in standard length from 15 – 65[+] mm (Travis 1994b), and females

from 20 – 70[+] mm (Travis, unpublished data; Ptacek and Seda, pers. obs.). A

balance of natural and sexual selection pressures likely maintains this wide

range of sizes seen between different populations (Ptacek and Travis 1997;

Ptacek 2005). Due to positive associations between certain morphological traits

and SL (Ptacek 2005) and behavioral traits and SL (Farr et al. 1986; Ptacek and

Travis 1996), large males (> 40 mm) possess more of the exaggerated male

sexual traits associated with sailfin mollies, including an increased sailfin size

(MacLaren et al. 2004), and higher courtship display rates (Farr et al. 1986;

Ptacek and Travis 1996) than do small males (< 30 mm), which are drab by

comparison and rely primarily on forced insemination attempts through

gonopodial thrusts (Figure 1.3). Females can exert strong sexual selection

pressure, via female preference, for these exaggerated male traits (Ptacek and

Travis 1997; Gabor 1999; Gabor and Page 2003; MacLaren et al. 2004), and,

when given the choice, they choose to mate with the largest male (Ptacek and

Travis 1997; Gabor and Page 2003; MacLaren et al. 2004). Males can make

themselves appear larger to females by extending their dorsal and caudal fins,

which increases their overall lateral projection area (sum of body, dorsal fin and

caudal fin area; MacLaren et al. 2004; MacLaren 2006). Larger males may also

gain a mating advantage in male-male competition by preventing smaller males

access to receptive females (Travis 1994b). It is often the case, however, that a

receptive female will be chased through the waters of her habitat by many

different males (of all sizes) thrusting at her from every direction (Travis 1994b;

Schlupp et al. 2001), which may circumvent female choice and allow smaller

males to gain mating opportunities.

Males of *P. latipinna* mature within a wide range of ages (50 - 200[+] days;

Travis 1989), with small males (< 30 mm) maturing more quickly (some within 30

days; Ptacek and Seda, pers. obs.) than large males (> 40 mm), which can take

over eight months to reach full sexual maturity (Ptacek and Seda, pers. obs.).

Age and size at sexual maturity of males is also strongly, positively correlated

with body mass. A small, 20 mm male will have a dry body mass around 60 mg,

compared to a large, 58 mm male that has a dry body mass over 1600 mg

(Travis 1989). Since smaller males mature earlier, more of their total lifespan is

spent at a sexually mature status, allowing them the potential for increased

reproductive success. Smaller males are, therefore, favored by fecundity

selection.

The habitats of *P. latipinna* are known to vary widely in area, water depth

and clarity, amount of vegetation cover, salinity, tidal influence, and many other

variables (Travis and Trexler 1987). Previous studies have shown that molly size

distributions (male and female; within a population) cannot be attributed to any

one or particular combination of these environmental factors in all habitats

(Trexler 1986; Travis and Trexler 1987). In sailfin molly populations, the greatest contributor to variation in male size distribution was attributed to the presence or absence of large males (> 40 mm) (Travis and Trexler 1987; Trexler et al. 1994; Ptacek and Travis 1996). Populations with larger males tend to have greater variance in male SL at maturity than do those with predominantly smaller males (Ptacek and Travis 1996). Trexler et al. (1994) examined wading bird predation as a natural selection mechanism for maintenance of the large variation seen in interpopulation size distribution of sailfin mollies. They reported that great egrets (*Casmerodius alba*) preferentially ate large males, especially when there was no vegetative cover obstructing their view of the fish. Snowy egrets (*Egretta thula*) showed a preference for large mollies, as well, and were better at catching large males under vegetative cover than the great egrets. As wading birds are visual predators, water depth and clarity and vegetation cover will limit the habitats where natural selection as a result of bird predation will impact the size distribution of mollies, favoring smaller males (< 30 mm) in shallower, clearer water habitats with little vegetative cover (Trexler et al. 1994). Natural and sexual selective pressures act together to maintain a wide distribution of male body sizes and shapes across different north Florida populations of the sailfin molly, *P. latipinna* (Ptacek 2005). Natural selection favors small males because they are able to more easily hide and escape from avian predators (Trexler et al. 1994) and because they mature more quickly than large males, allowing small males to spend more of their total lifespan seeking successful reproductive

opportunities (Travis 1989; Travis 1994b). Large males, however, despite their considerably longer time to reach sexual maturity, are strongly favored by sexual selection through female mating preferences for larger size (Ptacek and Travis 1997; Gabor and Page 2003; MacLaren et al. 2004). The interplay between these two forces of evolution contributes to the widespread variability observed in male size distributions between sailfin molly populations (Travis 1989; Travis 1994b; Ptacek 2005).

GOALS AND OBJECTIVES OF MY STUDY

The primary goal of my thesis research was to quantify the degree of intra- and interpopulation variability in morphology and behaviors in the sailfin molly, *Poecilia latipinna*. In addition, I assessed the degree to which morphological characters are correlated with the behavioral repertoires of male mating, social and inspection behaviors, and how such correlations may influence the degree of intra- and interpopulation divergence in behavioral profiles of north Florida *P. latipinna* populations (Mounds Pond, Steve's Ditch, and Fiddlers Point). The importance of my research is in its ability to make connections between an organism's life history and its expression of behavioral and morphological traits. Recent studies have demonstrated that certain suites of characters do not evolve independently of one another and often result in evolutionary trade-offs such as those described in the guppy, *P. reticulata* (Godin and Dugatkin 1996; Piyapong

et al. 2010; Smith and Blumstein 2010). My thesis research had two primary objectives: 1) to describe the overall shape of a number of north Florida *P. latipinna* populations collected from several years and to identify which, if any, morphological characters best predict the level of population variation observed within or among these populations through time; 2) to describe the overall repertoire of mating behaviors, activity levels, and inspection behaviors for three north Florida *P. latipinna* populations and to identify any suites of correlated behaviors (i.e., behavioral syndromes) that exist within or among these populations.

To address my first objective, I made morphological measurements of 15 linear traits from digital pictures of males collected from 9 populations in two years: 2005 and 2007. This objective focused on measuring morphological variation within and between populations and testing whether morphological variation is similar between different sampling years and among populations within each year.

To address my second objective, I tested 32 males, in a range of sizes, from each of three north Florida populations to determine their overall repertoire of mating behaviors, activity levels, and inspection behaviors. These data allowed me to investigate potential correlations between male mating behavior profiles and other temperament behaviors important to the life history of these fish. While interpopulation variation is known to exist in male mating behaviors (Farr et al. 1986; Ptacek and Travis 1996), no studies have examined population

level activity or inspection behavior profiles or the degree of variation at the level of the individual within these populations in their mating, activity, or inspection behaviors. Nor have these studies tested the impact that this intrapopulation variation may have on variation observed between populations.

Results of my thesis research will show the degree to which morphology and behavior can evolve independently of one another and whether suites of correlated behaviors (i.e., behavioral syndromes) that vary between individuals within a population have evolved, which may decrease the rate of population divergence in mating signals in sailfin mollies. If I find that *P. latipinna* morphology and mating, activity, and inspection behavior profiles are not correlated at the level of the population or the level of the individual within a population, my results would suggest that natural and sexual selection have the ability to influence behavioral traits independently of the constraints of morphological traits. Previous studies have shown a consistent influence of male size (i.e., standard length and body area) and dorsal fin morphology (e.g., fin area, fin length, length of the first and last dorsal fin rays) on mating behavior profiles (Farr et al. 1986; Loveless et al. 2009, 2010). Little is known, however, about the degree to which males from different populations vary in morphological features and whether such variation influences population divergence in their behavioral repertoires.

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CHAPTER TWO

THE SHAPE OF SELECTION: PATTERNS OF MORPHOLOGICAL
DIVERGENCE IN THE SAILFIN MOLLY, *POECILIA LATIPINNA*

ABSTRACT

Understanding how evolutionary mechanisms contribute to population

divergence in morphology provides insight into how adaptations arise and are

maintained in natural populations. I examined patterns of divergence based on

15 morphological traits in nine populations of males of the sailfin molly, *Poecilia*

latipinna, between two sampling years (2005 and 2007). I found significant

population divergence in morphology for both years, especially as a result of

differences among some populations in caudal fin shape and head shape. In

addition, in 2007, changes in sexual traits, (i.e., shape of the dorsal fin and length

of the gonopodium), contributed significantly to population differences. Specific

patterns of population differentiation were not consistent within or between

sampling years for sexual traits or swimming traits. These results suggest that

natural selection and sexual selection both contribute to population divergence in

morphology in male mollies, but vary both spatially and temporally with respect to

their targets of shape.

INTRODUCTION

Examining patterns of morphological differentiation among conspecific populations can illuminate how divergent selective regimes can generate and maintain phenotypic diversification (Endler 1977, 2000; Rice and Hostert 1993; Reznick and Travis 1996; Schluter 2000; Langerhans and DeWitt 2004). Local populations may exhibit variation in the optimal value of a trait due to differences in environmental conditions, and selection may shift population means toward these optima, while maintaining genetic variation among populations as a result of migration-selection balance (Slatkin 1975, 1978). While numerous studies have described local adaptations in response to varying natural and sexual selection regimes between populations (e.g., see reviews by Schluter 2001; Ptacek 2000; Panhuis et al. 2001), far fewer have examined the degree of population differentiation with respect to varying levels of gene flow and how gene flow may actually maintain genetic variability within and between natural populations across space and time.

Morphology is relevant to nearly all aspects of an organism's biology and is often subject to strong natural and sexual selection that may vary across a species' geographic distribution (Arnold 1983; Bels et al. 2003; Kingsolver and Pfennig 2007). Because natural and sexual selection may affect morphological traits differently, comparing patterns of divergence between populations in traits that are known targets of either natural or sexual selection can lend insight into

the role that these selective forces potentially play in shaping population diversification in ecological and mating signal traits (Kirkpatrick 2001; Panhuis et al. 2001; Schluter 2001; Kirkpatrick and Ravigne 2002; Nosil et al. 2007). Comparing the degree of divergence in ecological and mating signal traits among populations with varying levels of gene flow can assess the strength and stability of these selective forces on morphology through time.

The sailfin molly (*Poecilia latipinna*) is a common fish of salt marshes, brackish impoundments, and specialized freshwater habitats throughout the southern Atlantic and Gulf coasts of the southeastern US (Lee et al. 1980). A member of the livebearer family Poeciliidae, *P. latipinna* has been the subject of numerous studies of population differentiation in life history traits (Trexler 1989; Travis 1994a), male mating behaviors (Farr et al. 1986; Ptacek and Travis 1996), and allozyme variation (Trexler 1988). Sailfin mollies are an ideal system in which to compare intra- and interpopulation variation in body size and associated morphological traits for several reasons. First, male sailfin mollies exhibit enormous variation in male body length (standard length (SL) measured from the tip of the snout to the insertion of the caudal fin) both within and between populations (Snelson 1985; Farr et al. 1986; Travis 1989; Ptacek and Travis 1996). In sailfin mollies, as in many poeciliids, male size is fixed at maturity (i.e., no further growth occurs following complete formation of the gonopodium, the fused anal fin used in internal fertilization) and inherited patriclinally (Travis 1994a, b), presumably through a Y-linked genetic mechanism similar to the *P*

locus in the related swordtail genus *Xiphophorus* (Kallman 1989). Males with allelic variants on the Y chromosome have similar juvenile growth rates but initiate sexual maturation at different ages, producing a strong genetic correlation between age and size at maturity (Travis 1994b). Small males (20 mm SL) mature much sooner (3-4 weeks) than large males (50 mm SL; 8-9 months) (Travis 1994b; M. Ptacek pers. obs.). This natural selection advantage to small males in earlier maturation and potentially greater lifetime reproductive success is offset by strong sexual selection through female mating preferences for larger males (Ptacek and Travis 1997; Gabor and Page 2003; MacLaren et al. 2004).

Second, males of *P. latipinna* show considerable variation among populations in many behavioral and morphological traits (Ptacek 2005). Studies have shown that males vary among populations in rates of different mating behaviors (Farr et al. 1986; Trexler 1986; Travis and Woodward 1989; Travis 1994b; Ptacek and Travis 1996, 1997) and in morphological traits that are known to be under natural and sexual selective pressures (Farr et al. 1986; Trexler 1986; Farr 1989; Trexler et al. 1994; Ptacek and Travis 1997; Gabor 1999; Gabor and Page 2003; MacLaren et al. 2004; Ptacek 2005). In addition, positive associations between certain morphological traits (e.g., dorsal fin size and shape) and courtship display behavior rates have been found, with varying degrees of strength, in some, but not all populations examined (Farr et al. 1986; Ptacek and Travis 1996).

Finally, the habitats of *P. latipinna* are known to vary widely in area, water depth and clarity, amount of vegetation cover, salinity, tidal influence, temperature, and types of predators (Travis and Trexler 1987; Trexler et al. 1994). Previous studies have shown that molly size distributions (male and female; within a population) cannot be attributed to any one or particular combination of these environmental factors in all habitats (Trexler 1986; Travis and Trexler 1987). In sailfin molly populations, the greatest contributor to variation in male size distribution was attributed to the presence or absence of large males (> 40 mm) (Travis and Trexler 1987; Trexler et al. 1994; Ptacek and Travis 1996). Populations with larger males tend to have greater variance in male SL at maturity than do those with predominantly smaller males (Ptacek and Travis 1996). Natural and sexual selective pressures act together to maintain a wide distribution of male body sizes among different north Florida populations of the sailfin molly, *P. latipinna* (Travis 1994a, b; Ptacek 2005). The degree to which these forces maintain interpopulation variation in body shape is less well known.

In addition to variability in the biotic and abiotic features defining the habitats typical of sailfin molly populations, the degree of spatial isolation and potential gene flow among populations may also contribute to morphological differentiation. Trexler (1988) found that allozyme variation was greater between regions (i.e., north Florida, south Florida, and Georgia) than it was between demes (i.e., populations) within a region. He also found that allozyme variation

best conformed to an isolation by distance model, with isolation occurring over a long distance (> 50 km), suggesting a high rate of gene flow. These results suggest that local populations regularly exchange migrants at fairly high rates and the high levels of heterozygosity reported from allozymes (5-9% per locus) suggest large effective population sizes and little evidence that genetic drift plays a lasting significant role in diversification between populations at smaller spatial scales (Trexler 1988; Travis 1994b). Thus, variability among north Florida populations in body size and mating behavior rates suggests that local selective forces are strong in the face of gene flow (Ptacek 2005).

In this study, I addressed three specific questions. First, do north Florida populations of the sailfin molly, *P. latipinna*, differ in overall shape, and is the degree of variation observed among populations consistent between different sampling years that may vary in their degree of population connectivity? Second, how do populations differ in morphological traits influenced by natural selection (body depth and caudal fin shape) versus morphological traits influenced by sexual selection (dorsal fin and gonopodium shape), and is the pattern of variation observed among populations consistent between sampling years? Third, what role does male size distribution within a population play in explaining the degree of population variation observed in naturally selected or sexually selected morphological traits? For example, do populations with mostly smaller males have relatively larger values of sexually selected traits than do populations with larger-sized males? To answer these questions, I quantified the degree of

morphological variation among males using linear and area measurements of 15

morphological traits from nine different north Florida populations across two

different sampling years, one immediately following a hurricane (Hurricane

Dennis, August, 2005) where gene flow and connectivity between populations

should be high and a second sample, two years later (2007) following a drought,

where gene flow and connectivity was predicted to be low.

METHODS

Collection of Fish

The fish measured in this study were wild-caught males of *P. latipinna* collected

in 2005 and 2007 from nine different locations across northern Florida, USA:

Bald Point (BP), Fiddlers Point (FP), Lighthouse (LH), Live Oak (LO), Marine Lab

(ML), Mounds Pond (MP), Wacissa River (WR), Pinhook (PH), and Steve's Ditch

(SD) (Table 2.1, Figure 2.1). Fish were collected by pulling a 2.8 x 1.2m seine

numerous times across the entire area of the pond or creek being sampled. This

method has been shown previously to successfully collect a random sample of

the size distribution of males and females of *P. latipinna* within a population

(Travis and Trexler 1987). A total of 92 males was collected from seven different

populations in 2005, and a total of 224 males was collected from four different

populations in 2007; two populations, FP and MP, were sampled in both years
(Table 2.1).

Table 2.1. Summary of collection data.

Population	Collection Year	Sample Size	Site Co-ordinates
Mounds Pond	2005	12	N30º05.178', W084º09.665'
Wacissa River	2005	9	N30º08.799', W083º58.421'
Lighthouse	2005	11	N30º04.366', W084º10.643'
Bald Point	2005	12	N29º56.823', W084º20.477'
Fiddlers Point	2005	12	N29º58.379', W084º20.700'
Live Oak	2005	19	N30º04.224', W084º16.579'
Marine Lab	2005	17	N29º58.839', W084º23.008'
Pinhook	2007	22	N30º07.678', W084º01.127'
Mounds Pond	2007	87	N30º05.178', W084º09.665'
Steve's Ditch	2007	74	N29º58.379', W084º23.357'
Fiddlers Point	2007	41	N29º58.379', W084º20.700'

The two sampling years were chosen to represent potential variation in the
level of population connectivity among these north Florida populations. Fish in
the 2005 samples were collected in the five months following Hurricane Dennis
(August 10, 2005, making land fall near Live Oak Island, Wakulla County,
Florida; J Travis pers. obs.), a major climatic event that potentially affected these
P. latipinna populations by increasing gene flow and connectivity between
previously isolated populations as a result of widespread flooding in the region.
We resampled in May and August of 2007, during a period of two years of
drought conditions in north Florida (< 2134 mm rainfall 2006-2007; NOAA,

National Climatic Data Center, www.ncdc.noaa.gov), where the degree of

connectivity between sites was likely much less than in 2005. I attempted to

resample from as many populations as possible, but certain populations were

extinct due to drying completely (LO, BP) or changes in salinity following the

hurricane event (LH), resulting in only two populations being sampled in both

years (FP and MP). I added two additional populations (PH, SD) to the 2007

collections (Table 2.1).

Figure 2.1. Map of Florida illustrating the location of the nine sample populations. Fish were collected in Franklin, Wakulla, and Jefferson counties. ● Indicates populations sampled in 2005; ■ indicates populations sampled in 2005 and 2007; ▲ indicates populations sampled in 2007.

Morphological Measurements

Euthanized (buffered 0.5% MS-222) or anesthetized live fish (buffered 0.1% MS-222) were placed on a dissecting mat with the dorsal and caudal fins spread fully and the gonopodium positioned away from the body using insect mounting pins, then photographed for morphological analyses. I took digital photographs of the left side of each male using a Sony Cyber-shot (DSC-F707) digital camera at 2560 x 1920 resolution (Sony Electronics, Inc., San Diego, CA, USA). Live fish were revived and returned to their holding tanks until they were shipped to Clemson, SC, USA, where they were maintained in 600 liter stock tanks for additional study. Using NIH Image J (version 1.37) software (developed at the National Institute of Mental Health, Bethesda, Maryland, USA, and publicly available on the internet at http://rsb.info.nih.gov/nih-image/), I measured 11 linear and 3 area morphological traits from each male's photograph (Figure 2.2). Area measurements were determined by tracing the outline of the fin or body from the digital photograph and using the program's estimate of area. Dorsal fin ray number was also counted.
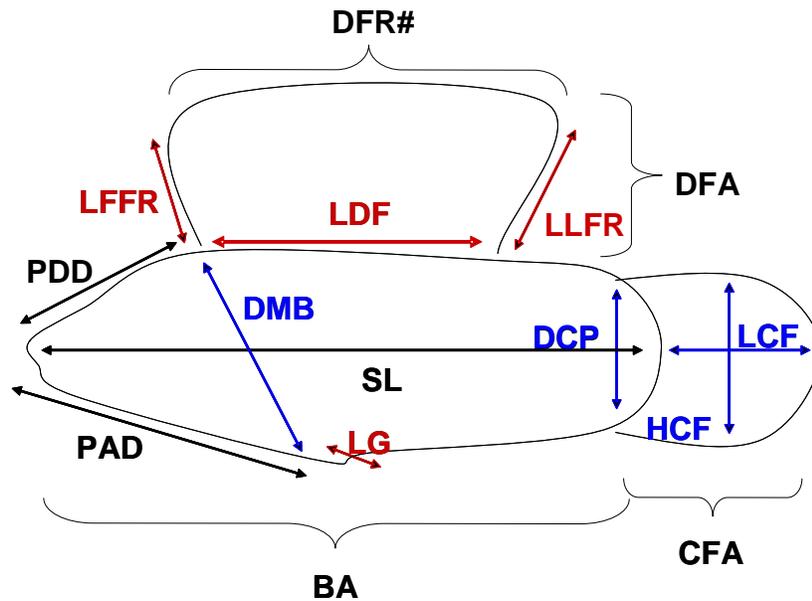
Figure 2.2. Linear measurements made on male *Poecilia latipinna*: PAD, pre-anal distance; PDD, pre-dorsal distance; LFFR, length of first fin ray; LDF, length of dorsal fin; LLFR, length of last fin ray; DMB, depth at mid-body (from the anterior insertion point of the dorsal fin to the anterior insertion point of the gonopodium); SL, standard length; LG, length of gonopodium; DCP, depth at caudal peduncle; LCF, length of caudal fin; HCF, height of caudal fin; BA, body area (left side); DFA, dorsal fin area (lateral image of left side of fin); CFA, caudal fin area (lateral image of left side of fin). Dorsal fin ray number was also recorded. Traits highlighted in red are 'sexual traits' used in male courtship displays and are strongly influenced by sexual selection. Traits highlighted in blue are 'swimming traits' used in locomotion and are strongly influenced by natural selection.

Statistical Analysis

All measurements and fin ray counts were first natural log-transformed (ln) to obtain linearity in trait/body size relationships and insure normality among samples. To determine whether population differences existed in shape independently of body size, I size-adjusted each morphological trait by regressing the ln trait value on ln SL (or ln BA for area measures) in a regression analysis that included all males from all populations in both years pooled.   By pooling all populations and years, I could calculate the deviation of each trait from the "global" male SL or BA for north Florida *P. latipinna*. Residuals from these regressions for all 13 morphological traits used in population comparisons were used as the dependent variable.

To test for the main effect of population differences, I performed a multivariate analysis of variance (MANOVA) for each year separately since different populations were sampled between years. Once a significant effect of population had been demonstrated, I performed a one-way analysis of variance (ANOVA) on each morphological trait followed by a Tukey-Kramer HSD post-hoc test for those traits showing significant differences in the ANOVAs in order to determine which populations (within each sampling year) were different from each other in morphological traits. I then used canonical discriminant function analysis (DFA) to find the combination of shape variables that best described morphological differences between populations for each sampling year. These

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analyses also provided an estimate of the amount of total morphological variation explained by each discriminant axis (i.e., factor), and the degree of misclassification to population of origin provided a measure of the degree of population differentiation in each year.

Morphological traits were then divided into two sets (Figure 2.2). "Sexual" traits were those used in either intersexual or intrasexual displays: length of the dorsal fin along the base (LDF), height of the first dorsal fin ray (LFFR), height of the last dorsal fin ray (LLFR), length of the gonopodium (LG). "Swimming" characters were traits not used in those displays; these were chosen either as indicators of general body shape (body depth at base of the caudal fin (DCP) and midbody depth (DMB)) or measures of dermal bone growth (length of the median caudal fin ray (LCF) and maximum height of the caudal fin perpendicular to the median caudal fin ray (HCF)) that might be correlated with the expression of the median fins (which are also of dermal origin). In other fishes, these traits often contribute to differences among populations in swimming performance (e.g., Webb 1982; Walker 1997; Ghalambor et al. 2003). I performed DFAs on these two types of traits separately for each year to compare the degree of population divergence between naturally selected and sexually selected traits within and between sampling years. All statistical analyses were performed with JMP (version 8) software (Cary, North Carolina, USA).

RESULTS

Population Differences in Morphology

The non-size adjusted averages for each morphological trait are reported in

Appendix A and populations showed considerable variation in most traits in both

2005 and 2007. Results of the MANOVA showed significant population

differentiation in both years (2005: $F_{72, 392}$ = 4.12; P < 0.0001; 2007: $F_{36, 618}$ =

6.67; P < 0.001). Populations differed significantly in both years in SL and BA

(Table 2.2) with some populations having primarily small males (2005: LH, MP

and WR; 2007: PH) while others had extremely large males (2005: ML; 2007:

FP) (Figure 2.3). There was considerable overlap between the two years in

shape variables that contributed significantly to population differences (Table 2.2)

and most traits were significantly different between some populations in both

sampling years (Table 2.3).
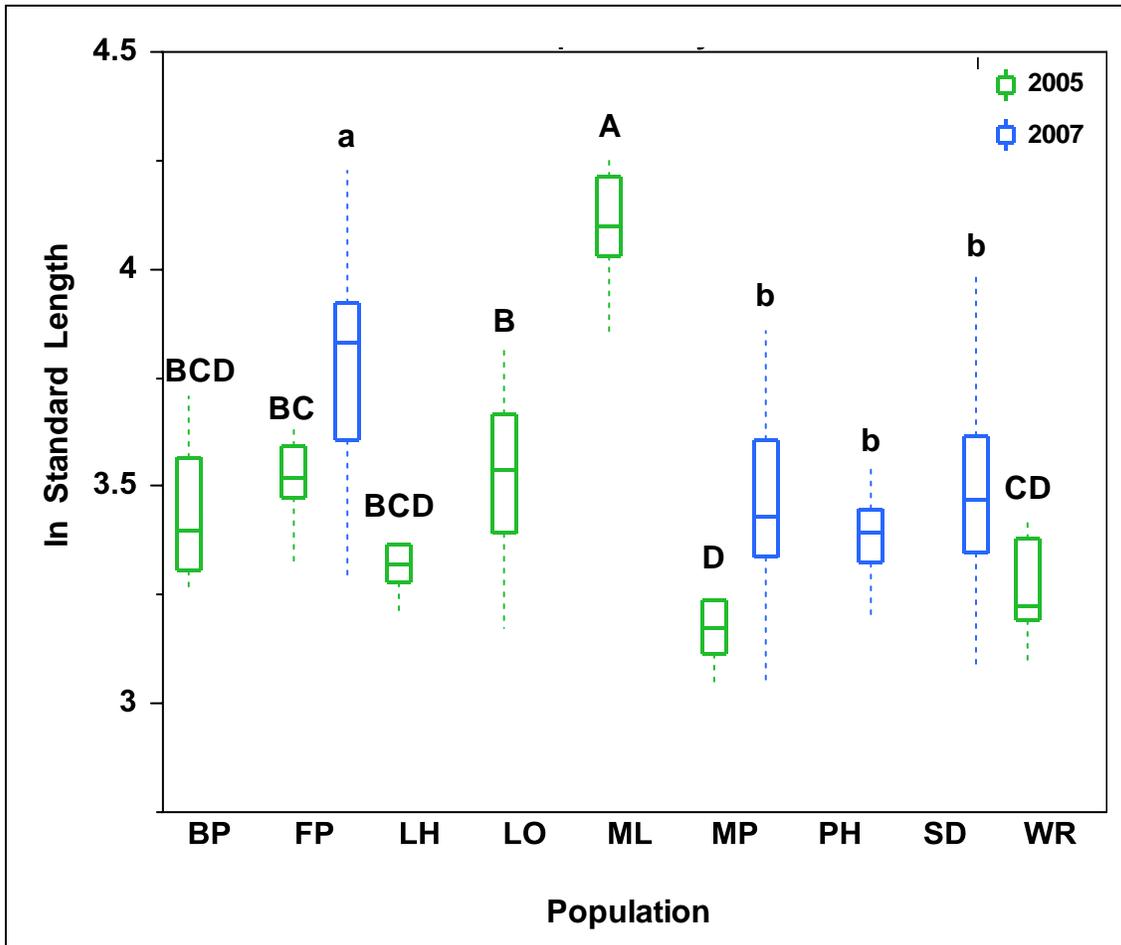
Figure 2.3. Boxplots of male standard length by population within each sampling year. The upper and lower horizontal lines of the box represent the first and third quartiles, and the middle horizontal lines represent the median. Dashed lines above and below the box represent the range. Tukey's post-hoc test: upper case letters (ABCD) indicated populations collected in 2005; lower case letters (ab) indicated populations collected in 2007. Populations not connected by same letter (within each year/letter case) are significantly different.

Table 2.2. One-way ANOVA of population means for 15 morphological traits over two years. Significant values are bolded.

Year	Trait Type	Trait	r^2	df	F	P
2005	Body	**SL[1]**	**0.696**	**6, 85**	**35.69**	**0.0001**
		BA[1]	**0.688**	**6, 85**	**34.29**	**0.0001**
	Sex	**LDF[2]**	**0.121**	**6, 85**	**3.083**	**0.0089**
		LFFR[2]	**0.188**	**6, 85**	**4.514**	**0.0005**
		LLFR[2]	0.045	6, 85	1.722	0.1256
		LG[2]	0.058	6, 85	1.942	0.0832
		DFA[3]	**0.083**	**6, 85**	**2.379**	**0.0357**
	Swim	**DMB[2]**	**0.198**	**6, 85**	**4.752**	**0.0003**
		DCP[2]	**0.409**	**6, 85**	**11.49**	**0.0001**
		LCF[2]	**0.090**	**6, 82**	**2.454**	**0.0312**
		HCF[2]	**0.460**	**6, 82**	**13.52**	**0.0001**
		CFA[3]	**0.353**	**6, 82**	**8.990**	**0.0001**
	Other	**PDD[2]**	**0.266**	**6, 85**	**6.503**	**0.0001**
		PAD[2]	**0.187**	**6, 85**	**4.500**	**0.0005**
		DFR#[4]	0.023	6, 85	1.362	0.2394
2007	Body	**SL[1]**	**0.210**	**3, 220**	**20.74**	**0.0001**
		BA[1]	**0.237**	**3, 220**	**24.04**	**0.0001**
	Sex	LDF[2]	0.013	3, 220	1.983	0.1174
		LFFR[2]	0.009	3, 220	1.659	0.1767
		LLFR[2]	**0.026**	**3, 220**	**2.973**	**0.0326**
		LG[2]	**0.051**	**3, 220**	**4.976**	**0.0023**
		DFA[3]	**0.078**	**3, 220**	**7.318**	**0.0001**
	Swim	DMB[2]	0.008	3, 220	1.607	0.1888
		DCP[2]	**0.081**	**3, 220**	**7.547**	**0.0001**
		LCF[2]	**0.195**	**3, 220**	**18.97**	**0.0001**
		HCF[2]	**0.078**	**3, 220**	**7.307**	**0.0001**
		CFA[3]	0.018	3, 220	2.366	0.0718
	Other	**PDD[2]**	**0.112**	**3, 220**	**10.37**	**0.0001**
		PAD[2]	**0.162**	**3, 220**	**15.37**	**0.0001**
		DFR#[4]	**0.157**	**3, 220**	**14.82**	**0.0001**

[1] Transformed using natural log transformation.
[2] Transformed and size adjusted using the residuals from lnTrait vs. lnSL regression.
[3] Transformed and size adjusted using the residuals from lnTrait vs. lnBA regression
[4] Transformed and size adjusted using the residuals from Trait vs. lnSL regression.

Table 2.3. Tukey-Kramer HSD post-hoc test of population means for 15 morphological traits over two years. Populations are listed from smallest (left) to largest (right) trait values.

Year	Trait Type	Trait	Population						
2005	Body	SL[1]	MP	WR	LH	BP	FP	LO	ML
		BA[1]	MP	WR	LH	BP	FP	LO	ML
	Sex	LDF[2]	ML	LH	BP	LO	MP	FP	WR
		LFFR[2]	LO	FP	LH	ML	BP	MP	WR
		LLFR[2]	ML	FP	LO	MP	LH	BP	WR
		LG[2]	LH	BP	MP	FP	ML	LO	WR
		DFA[3]	LH	BP	ML	FP	MP	LO	WR
	Swim	DMB[2]	WR	BP	LH	ML	MP	LO	FP
		DCP[2]	LH	WR	ML	FP	LO	MP	BP
		LCF[2]	BP	LH	ML	LO	WR	MP	FP
		HCF[2]	LH	WR	ML	FP	LO	MP	BP
		CFA[3]	LH	WR	MP	BP	ML	LO	FP
	Other	PDD[2]	MP	WR	BP	LH	LO	FP	ML
		PAD[2]	WR	BP	MP	ML	LO	FP	LH
		DFR#[4]	WR	LH	BP	LO	MP	ML	FP

Table 2.3. cont.

Year	Trait Type	Trait	Population			
2007	Body	SL[1]	PH	MP	SD	FP
		BA[1]	PH	MP	SD	FP
	Sex	LDF[2]	SD	PH	MP	FP
		LFFR[2]	SD	PH	MP	FP
		LLFR[2]	SD	MP	FP	PH
		LG[2]	FP	MP	SD	PH
		DFA[3]	SD	FP	PH	MP
	Swim	DMB[2]	FP	SD	MP	PH
		DCP[2]	PH	MP	SD	FP
		LCF[2]	MP	PH	FP	SD
		HCF[2]	PH	SD	MP	FP
		CFA[3]	PH	MP	SD	FP
	Other	PDD[2]	FP	SD	MP	PH
		PAD[2]	FP	SD	PH	MP
		DFR#[4]	FP	SD	PH	MP

[1] Transformed using natural log transformation.
[2] Transformed and size adjusted using the residuals from lnTrait vs. lnSL regression.
[3] Transformed and size adjusted using the residuals from lnTrait vs. lnBA regression
[4] Transformed and size adjusted using the residuals from Trait vs. lnSL regression.

Discriminant analyses of all shape traits showed clear distinctions between some populations in both years (Figure 2.4) and similar traits contributed to population separation in each year (Table 2.4B). In 2005, some populations showed clear separation, especially along DF1 (Figure 2.4A), and 72% of males were correctly classified to their population of origin. DF1 (explaining 41.3% of the total variation among males from different populations) primarily differentiated populations based upon caudal fin shape (HCF, DCP, CFA) with males from LH and WR having smaller caudal fins than other populations (Figure 2.4A, Table 2.4). This result was further confirmed in Tukey's post-hoc comparisons showing LH and WR having significantly smaller values for HCF, DCP and CFA than other populations (Table 2.3). DF2 (explaining 23.3% of the total variation among males from different populations) primarily differentiated populations based upon head and body depth (PAD, PDD, DMB) and dorsal fin height (LFFR) with males from WR having shallower heads and bodies and taller dorsal fins than other populations (Figure 2.4A, Table 2.4). This result was further confirmed in Tukey's post-hoc comparisons showing WR having significantly smaller values for DMB than other populations (Table 2.3).

In 2007, populations were also clearly differentiated (Figure 2.4B) with 67% of males correctly classified to population of origin. DF1 (explaining 67.9% of the total variation among males from different populations) primarily differentiated populations based upon dorsal fin shape (DFA, DFR#) and caudal

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fin length (LCF) with males from FP and SD having smaller dorsal fins with fewer

dorsal fin rays and longer caudal fins than other populations (Figure 2.4B, Table

2.4). Tukey's post-hoc comparisons also showed FP and SD males had

significantly lower DFR# and smaller DFA and significantly larger LCF than MP

males (Table 2.3). DF2 (explaining 23.8% of the total variation among males

from different populations) separated populations by head depth (PDD, PAD) and

gonopodium length (LG) with FP and MP having shallower heads and shorter

gonopodia than SD and PH (Figure 2.4, Table 2.4). These results were further

confirmed by Tukey's post-hoc comparisons showing FP males had significantly

smaller values of PDD and PAD than the other three populations and significantly

shorter gonopodia than males from SD and PH (Table 2.3).

Table 2.4. Canonical discriminant correlation scores for the first two discriminant factors for all morphological traits by sample year. Significant values are bolded.

Trait	2005		2007	
	DF1	DF2	DF1	DF2
LDF	-0.052	**-0.349**	0.019	**-0.328**
LFFR	-0.053	**-0.538***	**0.132**	**-0.211**
LLFR	-0.084	**-0.340**	**0.169**	**-0.234**
PDD	-0.157	**0.525***	**0.253**	**0.612***
PAD	**-0.357**	**0.631***	**0.481***	**0.505***
LG	0.093	0.121	0.024	**0.507***
DMB	**0.215**	**0.731***	**0.188**	0.066
DCP	**0.799***	0.082	**-0.324**	**-0.426**
LCF	-0.038	**0.252**	**-0.648***	0.110
HCF	**0.845***	0.157	0.014	**-0.451**
DFA	0.143	-0.078	**0.433***	-0.046
CFA	**0.693***	**0.281**	-0.177	**-0.225**
DFR#	0.058	**0.312**	**0.589***	0.127

* Indicates traits with the highest loading values in the first and second factor of the discriminant function analysis.
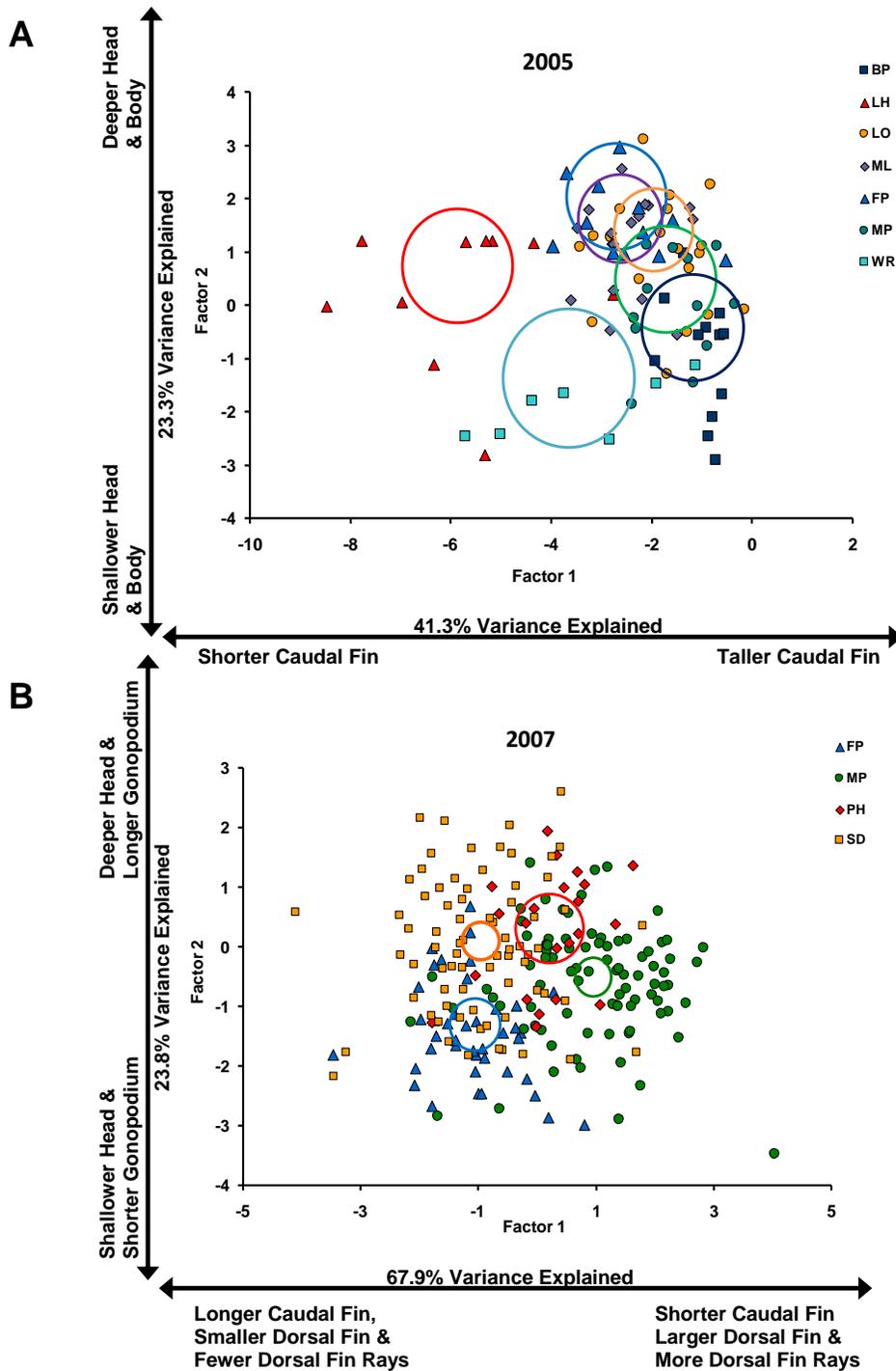
Figure 2.4. Discriminant scores one and two for all morphological traits among populations collected in A) 2005 and B) 2007. Circles represent 95% confidence intervals about the means.

Naturally Selected Versus Sexually Selected Traits

Comparing population divergence in naturally selected versus sexually selected morphological traits suggests some interesting differences in how these two forces of evolution may contribute to population differentiation. For sexually selected traits (LDF, LFFR, LLFR, and LG) populations in 2005 showed the greatest separation based upon average male size (SL) differences among them. DF1 (explaining 48.4% of the total variation among males from different populations and correctly classifying 43.5% of males to population of origin) showed males from the population with the smallest average SL, WR, had the largest relative size of sexual traits, i.e., longer, taller dorsal fins and longer gonopodia (Figure 2.5A, Table 2.5). This pattern was not as clear in 2007, although DF1 (explaining 78.9% of the total variation among males from different populations and correctly classifying 32.1% of males to population of origin) separated FP somewhat from the other three populations and males from FP had longer, taller dorsal fins, but shorter gonopodia (Table 2.5). The FP population had the largest average SL in 2007 and no populations in this sampling year were composed primarily of small males (average SL < 30 mm) (Appendix A).

Populations in 2005 showed considerable overlap based upon naturally selected traits (DMB, DCP, LCF, HCF) with DF1 (explaining 76.6% of the total variation among males from different populations and correctly classifying 48.3% of males to population of origin) only clearly separating ML from other

populations (Figure 2.6A). The ML population had the largest average SL (60

mm, Appendix A) among the seven populations sampled and males from this

population were considerably deeper bodied than males from other populations

(Table 2.5). In 2007, DF1 (explaining 68.4% of the total variation among males

from different populations and correctly classifying 53.6% of males to population

of origin) separated MP from the other three populations (Figure 2.6B) and males

from MP were deeper bodied with shorter, but taller caudal fins (Table 2.5).

Table 2.5. Canonical discriminant correlation scores for the first discriminant factor for 'sexual' and 'swimming' morphological traits by sample year. Significant values are bolded.

Trait	2005	2007
	DF1	DF1
Sex		
LDF	**0.596**	**0.509**
LFFR	**0.852**	0.383
LLFR	**0.264**	0.444
LG	**0.419**	-0.747
Swim		
DMB	**0.215**	-0.219
DCP	0.127	**0.338**
LCF	0.026	**0.843**
HCF	0.202	**-0.137**

A

2005

◇ ML
● LO
△ FP
□ BP
▲ LH
■ WR
○ MP

Discriminant - Sex Traits (mean +/- 95% CI)

2007

B

△ FP
□ SD
○ MP
◇ PH

Discriminant - Sex Traits (mean +/- 95% CI)
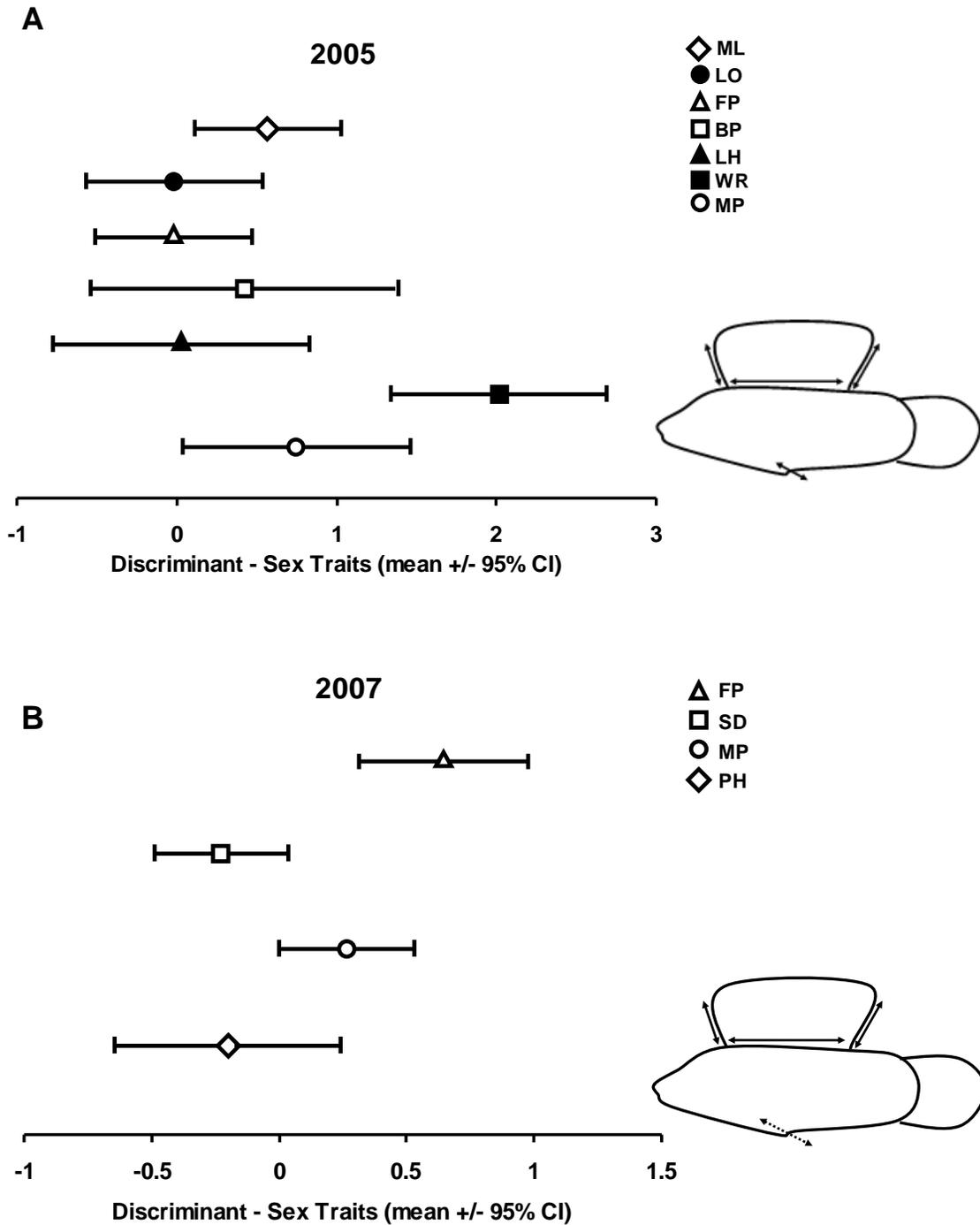
Figure 2.5. Discriminant score, factor 1 (mean ± 95% confidence intervals) for 'sexual' morphological traits (LDF, LFFR, LLFR, LG) for populations sampled in A) 2005 and B) 2007.   Fish diagrams show the traits:↕, positive correlations; ⋮, negative correlations.
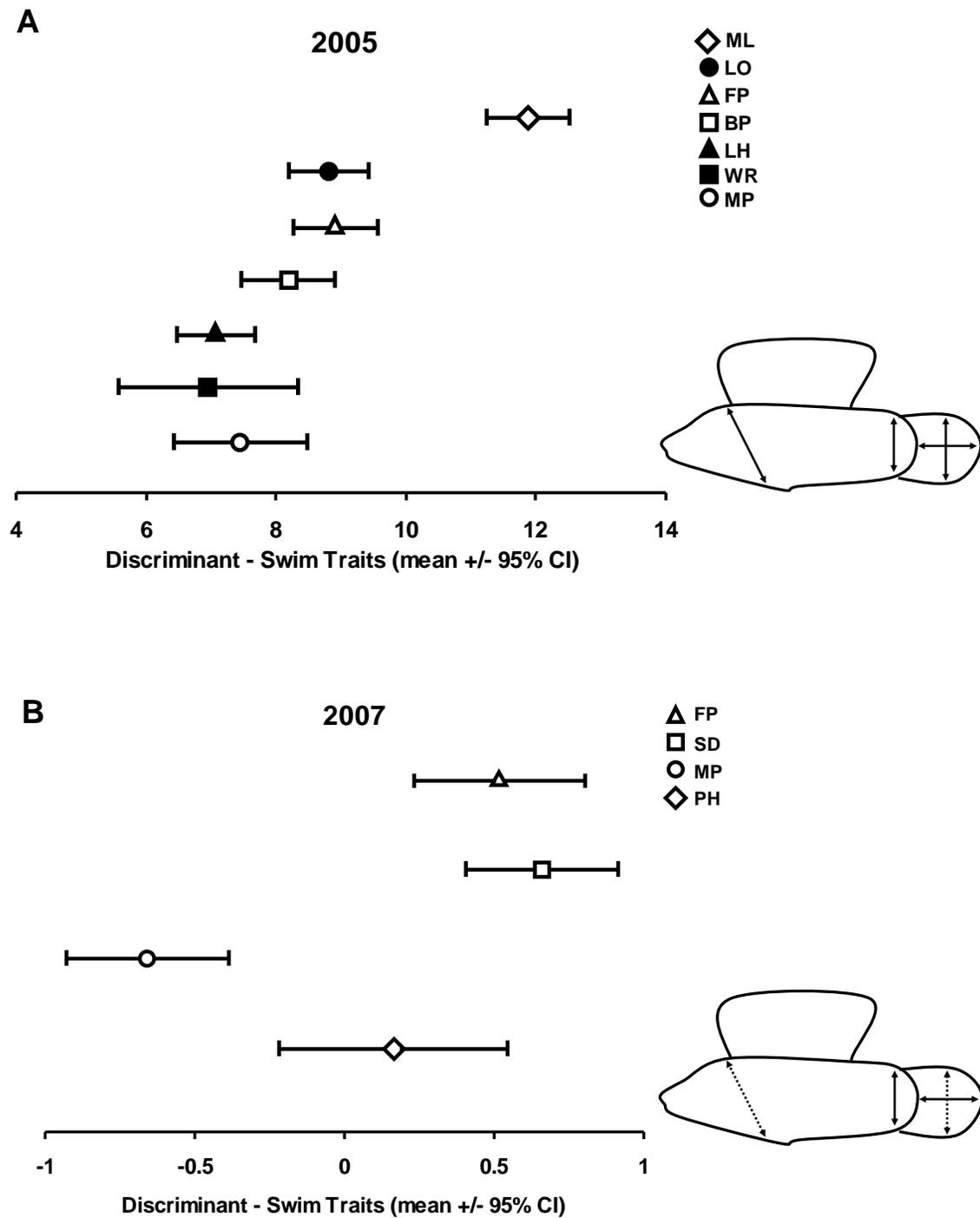
Figure 2.6. Discriminant score, factor 1 (mean $\pm$ 95% confidence intervals) for 'swimming' traits (DMB, DCP, LCF, HCF) for populations sampled in A) 2005 and B) 2007. Fish diagrams show the traits: ↓, positive correlations; ↕, negative correlations.

DISCUSSION

Male populations of *P. latipinna* in north Florida are clearly differentiated based upon morphology. In both sampling years, male populations could be distinguished from one another based upon differences in caudal fin shape and head depth. In 2007, male populations differed in sexual traits as well, i.e., size of the dorsal fin and length of the gonopodium. The degree of population differentiation was similar between sampling years, with 72% of males correctly classified to their population of origin in 2005 and 67% correctly classified to their population of origin in 2007. Thus, despite potentially higher gene flow in 2005 following widespread flooding as a result of hurricane Dennis, populations still showed considerable divergence.

Interestingly, in 2005, the two freshwater populations, LH and WR showed the greatest separation based on shape from the other populations found in brackish or seawater. Males from these two freshwater populations had, on average, smaller male SL and previous studies have shown that both males and females are smaller from freshwater populations (Travis and Trexler 1987; Travis 1994b). Freshwater environments are osmotically challenging for mollies and individuals have higher respiration rates, less precise ionic regulation, lower growth rates, longer times to maturation, and generally, lower condition in freshwater habitats (Trexler 1989; Trexler et al. 1992). Males from LH and WR had smaller caudal fins, potentially contributing to poorer swimming performance

in freshwater environments as well (Webb 1982; Walker 1997; Ghalambor et al. 2003). A similar pattern was seen in 2007, where males from MP and PH populations, both found in brackish to freshwater tidal creeks, had shorter caudal fins than males from FP and SD, both saltmarsh populations.

Differences in caudal fin shape and head shape contributed to population separation in both sampling years. Traits more likely to be influenced by sexual selection (dorsal fin shape, gonopodium length) only contributed to population separation in 2007. Widespread flooding following hurricane Dennis may explain why traits more likely under natural selection were more important in separating populations in 2005. Extreme climatic events (e.g., typhoons, hurricanes, droughts) provide brief episodes of strong natural selection, especially if these events are rare, fairly quick, occur within a well-defined area, and far exceed the normal environmental conditions of the habitat (Endler 1986; Brown and Brown 1989). The 100 mm of rain that fell in the short span of hours accompanied by 3-4 meter coastal storm surges associated with hurricane Dennis (Beven 2005; NOAA, National Climatic Data Center, www.ncdc.noaa.gov) would have potentially created a strong episode of selection on swimming performance in these molly populations. Sampling of these populations following the hurricane would have collected the 'survivors' of such a selective event. Changes in naturally selected traits have been shown to be of considerable magnitude following episodes of extreme environmental challenge (Brown and Brown 1989; Grant and Grant 1993; Blob et al. 2008, 2009; Maie et al. 2009).

In 2007, following two years of drought conditions in north Florida (< 2134 mm rainfall 2006-2007; NOAA, National Climatic Data Center, www.ncdc.noaa.gov), connectivity between populations would have been greatly reduced. Indeed, several of these populations were extinct (LO, BP) due to complete drying of the habitat. Less gene flow between populations allows for sexual selection through female mating preferences to promote population divergence in male traits. Such a pattern has been shown for interpopulation divergence in male mating behaviors in these populations of *P. latipinna*, where females prefer males from their native population to males from other, foreign populations (Ptacek and Travis 1997). Stronger divergence among male populations based on differences in shape of the dorsal fin and length of the gonopodium in 2007 may be explained by increased sexual selection pressures. Both dorsal fin and gonopodium size are known targets of female mating preferences in mollies and other poeciliid fishes (MacLaren et al. 2004; Langerhans et al. 2005; Kozak et al. 2008).

Patterns of divergence between populations in sexual versus swimming traits were not as clear. Populations were not ordered with respect to their degree of divergence between the two types of traits or between years within a type of trait. For instance in 2005, the population with the smallest average male size, WR, had the largest values of sexual traits, but the population with the largest average male size, FP, had the largest values of sexual traits in 2007. Dorsal fins exhibit positive allometry with male SL (Farr et al. 1986; Hankison and

Ptacek 2007), while the gonopodium shows negative allometry (Ptacek and Travis 1998). Hubbs (1942) first noted a "counter-gradient" phenomenon in males of *P. latipinna*, whereby, larger males have relatively larger values of dorsal fin size and relatively smaller values of gonopodium length. Such disproportionately larger dorsal fins have also been reported in small-sized male populations in north Florida (Ptacek 2005), a pattern seen in 2005 but not in 2007 in this study. Interestingly, in 2007, in both populations that were also sampled in 2005 (FP and MP), average male SL had increased considerably from the previous sampling. Thus, as a result of migration events, populations that are small at one point in time may become large male populations at another point in time, potentially confounding selection for the countergradient phenomenon.

Overall, male sailfin molly populations show considerable morphological divergence. Divergence occurred in both morphological traits that are targets of natural selection and those that are targets of female mating preferences. While some traits contributed to population divergence in both sampling years, others had stronger influence on population differentiation in only one of the two years. This study adds to a growing body of evidence that suggests that population variation in morphology, behavior and life history in the sailfin molly, *P. latipinna*, results from a balance between natural selection and sexual selection, which vary in their direction and magnitude both spatially and temporally (Travis 1994a; Ptacek 2005). Future studies should concentrate on quantifying the contributions

of environmental variation and predation regimes to predictable patterns of

population differentiation in male body size, shape and mating behaviors.

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CHAPTER THREE

LARGE, ACTIVE, BOLD & SEXY: INDIVIDUAL VARIATION
IN MALE SIZE AND BEHAVIOR IN THE SAILFIN MOLLY

ABSTRACT

Variation among individuals in different behaviors and associations of behaviors in different contexts can lead to the maintenance of behavioral polymorphisms. In addition, such variability in behavioral types or syndromes can slow the rate at which populations diverge in behavioral phenotypes. I investigated the potential for behavioral syndromes to exist in the sailfin molly, *Poecilia latipinna*, by comparing the behavior of individual males in different situations within a behavioral context (e.g., mating context: receptive vs. non-receptive females) and across three different contexts (mating, activity and inspection). I found that male mollies show strong positive associations between situations within a context. Certain males showed high courtship display rates in response to both types of females, high activity levels following either inspection of conspecifics or inspection of a predator, and high levels of boldness in response to social groups and predators. Evidence for a behavioral syndrome in sailfin mollies came from the strong positive association between courtship display rates and boldness in predator inspection. Male size at maturity (known to have a Y-linked genetic basis) was strongly positively associated with courtship display rates and levels

of boldness. Activity levels were independent of male size. These findings suggest that individual variation exists among male mollies, with certain males being more active and larger males being bolder and courting more vigorously. Such variation among males in behavioral associations within and between different contexts may slow the rate at which populations of *P. latipinna* diverge in individual behaviors.

INTRODUCTION

Natural and sexual selection frequently maintain a mix of behavioral phenotypes or mating strategies within a single population (Endler 1992; Schuster and Wade 2003) and such variation among individuals can slow down the rate at which populations diverge from one another and, thus, inhibit speciation (Magurran 1998; Ptacek 2000). A number of evolutionary mechanisms have been proposed to maintain individual variation in mating signals, including negative frequency dependent sexual selection (Sinervo and Lively 1996; Punzalan et al. 2005; Rios-Cardenas et al. 2007) and trade-offs between female and predator preferences for attractive male traits (Endler 1983; Rosenthal et al. 2001; Basolo and Wagner 2004). Although fitness differences among individuals for phenotypic traits including male mating strategies (Schuster and Wade 2003; Evans et al. 2003), size variation (Gross 1996; Ptacek and Travis 1997), and color polymorphisms (Endler 1992; Houde 1997; Godin and McDonough 2003; Bourne et al. 2003; Lindholm et al. 2004) have been documented, individual variation in behaviors across a variety of different contexts has only recently received similar attention (Wilson 1998; Dall et al. 2004).

Like other types of traits, behavioral traits have the potential to show phenotypic and even genotypic correlations, with suites of behavioral types occurring among individuals. Such behavioral syndromes (Sih et al. 2004a; Bell 2007) arise when particular behaviors or temperaments (e.g., shy vs. bold

behaviors) are correlated within an individual and expressed between multiple situations within a context (e.g., mating interactions with different types of females) or across contexts (e.g., mating and foraging). For example, in male guppies (*Poecilia reticulata*), a bold/shy behavioral phenotype has been described where bolder males have a higher willingness to approach and inspect predators and novel food sources, and resume normal foraging behavior more quickly after a disturbance as compared to shier males (Godin and Dugatkin 1996; Piyapong et al. 2010). Bold behavioral types are often, but not always, associated with brighter male coloration (Godin and Dugatkin 1996). Females have been shown to prefer more colorful males (Houde and Endler 1990; Endler and Houde 1995), but also, bolder males regardless of their coloration (Godin and Dugatkin 1996). By preferentially mating with colorful males and bolder males, female guppies are, thus, choosing on average, relatively bold, and perhaps more viable, individuals. Variability among males in the strength of association between color and bold behavior may maintain polymorphism in both traits in guppy populations.

Sailfin mollies (genus *Poecilia*, subgenus *Mollienesia*) present an interesting system in which to explore individual variation in behavior as males vary both within and between populations in male size at sexual maturity and rates of certain mating behaviors (e.g., courtship displays and gonopodial thrusts; Farr et al. 1986; Ptacek and Travis 1996). The polymorphism in male size at maturity is inherited patriclinally (Travis 1994a, b) and is presumably genetically

controlled in a similar fashion to the Y-linked *P* locus described for the swordtail

genus *Xiphophorus* (Kallman 1989). Different *P* alleles control differences in the

time to reach sexual maturity, with small males (18 – 25 mm SL) reaching

maturity in four to eight weeks, while large males (> 50 mm SL) may take over

one year to mature (Ptacek 2002; Loveless et al. 2010). For at least one sailfin

molly species, *P. latipinna*, size at maturity is correlated with rates of certain

mating behaviors. On average, larger males perform higher rates of courtship

displays than smaller males, while smaller males perform higher rates of sneak

copulations termed gonopodial thrusts (Farr et al. 1986; Ptacek and Travis 1996).

Thus, within *P. latipinna* populations, variation exists in both male size and

mating behavior repertoire, and variability in both, is likely maintained by a

balance between natural selection and sexual selection favoring different sizes

and mating behaviors in different environments (Ptacek and Travis 1997; Ptacek

2005).

      While population variation in male mating behaviors has been described in

the sailfin molly *P. latipinna* (Farr et al. 1986; Ptacek and Travis 1996), far less

attention has been paid to the degree of variability among individual males within

a population, or whether individual variation in male behaviors may obscure

differences between sailfin molly populations and slow the rate of population

divergence. In addition, the correlation of male mating behavior with other

behavioral phenotypes such as boldness or activity has not been previously

explored in sailfin mollies. Correlations of behaviors across contexts such as in

mating interactions and exposure to predators may also be important in generating and maintaining variability among males in behavioral phenotypes.

In this study, I quantified levels of expression of mating, activity, and inspection behaviors in males of the sailfin molly *P. latipinna*. I asked whether behavioral associations exist within a context (e.g., mating, activity, inspection) between different situations (e.g., consistent behavioral types during mating interactions with receptive vs. non-receptive females), and also, whether behavioral types exist between different contexts (e.g., mating vs. inspection). Because of the known influence of male size on mating behaviors in sailfin mollies, I also asked whether male size at maturity (a fixed genetic trait) was positively or negatively associated with certain behavioral types across different contexts (mating, activity, and inspection). I examined associations between different behaviors and between behaviors and male size within and between three different populations in order to determine if behavioral syndromes occur in sailfin mollies and whether different populations vary in their degree of expression of particular behavioral associations.

METHODS

Collection and Housing of Fish

The fish used in this study were first generation, wild-caught *Poecilia latipinna*

collected in May and August, 2007 from three locations in Wakulla County,

Florida, USA: Mounds Pond (N30º05.178', W084º09.665'), Steve's Ditch

(N29º58.379', W084º23.357'), and Fiddlers Point (N29º58.379', W084º20.700').

Fish were collected by pulling a 2.8 x 1.2m seine numerous times across the

entire area of the pond or creek being sampled. This method has been shown

previously to successfully collect a random sample of the size distribution of

males and females of *P. latipinna* within a population (Travis and Trexler 1987).

All fish were transported to Clemson University, Clemson, South Carolina,

USA and housed in a climate-controlled greenhouse (Biomedical Research

Center Greenhouse, Clemson University; Animal Research Protocol No.

AUP2008-040). Each population was housed in a single, large Rubbermaid

stock tank (600 liters; Fairlawn, Ohio, USA) with ample filtration and aeration to

support a density of up to 200 adult fish. Stock tanks contained conditioned

(AmQuel, NovAqua, and Seachem marine buffer; Beavercreek, Ohio, USA) city

tap water maintained at a salinity of 12 parts per thousand (ppt). Fish were

housed at a temperature of 25°C and with an ambient photoperiod. Fish were

fed once daily with commercial flake food (Ocean Star International Freshwater

Flake (60%) with Brine Shrimp Flake (38%) and Spirulina Flake (2%) mixture; Burlingame, California, USA) and dried algae wafers (Hikahi tropical algae wafers; Beavercreek, Ohio, USA).

After several weeks of acclimation, fish from each population were moved to a laboratory animal facility (Aquatic Animal Research Laboratory, Clemson University; Animal Research Protocol No. ARC2007-026) and housed for behavior trials. Stimulus and test fish were kept in 37.9 liter group (by population) aquaria with a maximum density of 10 individuals of both males and females for at least a week prior to behavioral observation. Each test male was then removed from the group tank and housed with a single female companion (from the same population) in separate 18.95 liter aquaria. All fish were housed in identically conditioned, 12 ppt water, at a temperature of 25°C, and with a photoperiod of 14:10h light:dark cycle, provided by Sylvania Gro-lux fluorescent bulbs (20-W full spectrum 350-750 nm, with spectral peaks at 400, 440, and 540 nm; Rochester, New York, USA). Fish were fed once daily with commercial flake food and supplemented weekly with dried algae wafers; the tanks and filters were cleaned every other week accompanied by a 50 percent water change.

Selection of Test Males

In order to capture the full range of male sizes from each population, males were not chosen randomly as test subjects (Ptacek and Travis 1996). Instead, the 8

largest, the 8 smallest, and 16 intermediately-sized males were chosen for a total

of 32 test males per population: Fiddlers Point, small 26.6 – 31.3 mm,

intermediate 39.8 – 49.7 mm, large 52.4 – 64.1 mm; Mounds Pond, small 25.4 –

29.3 mm, intermediate 31.2 – 45.7 mm, large 46.1 – 55.9 mm; Steve's Ditch,

small 23.9 – 29.4 mm, intermediate 30.5 – 42.1 mm, large 47.7 – 63.7 mm.

Mating Behavior Trials

In order to assess mating behavior profiles, males were tested with a single

female (unfamiliar, but from the same population) in a direct contact trial. In

order to maximize each male's sexual response, their female companions were

removed from the housing tank 24 hours prior to testing (Ptacek and Travis

1996), and stimulus females were generally chosen within 10 mm SL of the test

males' size (Hankison and Ptacek 2007). Fish were tested in an 18.95 liter

aquarium that was externally covered on the front with one-way film (SOPUS

Products, Moorpark, California, USA) and on the three remaining sides with black

paper to minimize observer effects. The male was placed in the test tank and

allowed to acclimate for 10 minutes, at which point the female was added, and an

additional 10 minutes was provided for acclimation. I then observed the male's

behavior during the 10 minute observation period and recorded all courtship

displays using a Dell Latitude laptop computer and event recorder software (The

Observer, version 5, Noldus Information Technology, Leesburg, Virginia, USA).

Each male was tested once with a receptive female (< 48 h post-partum; more likely cooperative during copulation) and once with a non-receptive female (already gravid and more likely uncooperative during copulation; Ptacek and Travis 1997). I randomized whether males were tested first with a receptive or non-receptive female, and trials were performed at least 24 hours apart. I found no significant effect of trial order on courtship display rates ($F_{1,202}$ = 2.258, P = 0.135). Similar experimental designs have also detected no carry-over effects between trials on rates of male mating behaviors (Ptacek and Travis 1997; Ptacek et al. 2005).

Activity and Inspection Behavior Trials

The same 32 males from each population were observed in a 25 minute activity and inspection behavior trial. These trials were performed in a 75.8 liter, 3-chambered dichotomous choice tank (Figure 3.1). A conspecific social group (2 males and 2 females from the same population but unfamiliar to the test male) was placed in one of the end chambers of the test tank, and a single, natural predator, the gulf killifish (*Fundulus grandis*), was placed in the other end chamber. Gulf killifish have been collected in two of the three male populations tested in this study: Steve's Ditch and Fiddlers Point. A thin, flexible, opaque piece of vinyl was placed in each of the end chambers against the plexiglass divider to prevent the test male from viewing either stimulus at the start of the

trial. The test male was placed in the middle of the neutral zone and given a 5 minute acclimation period. Then, the first opaque divider was carefully removed revealing the stimulus (social group or predator), and the test male was observed for 5 minutes. The opaque divider was then replaced, and the test male was observed for another 5 minute (post-stimulus) period. The opaque divider was then removed from the opposite end chamber to allow the test male to view the other stimulus; the test male was observed for another 5 minutes. The opaque divider was then replaced, and the test male was observed for an additional 5 minute (post-stimulus) period. Each trial (25 minutes total) was recorded using a digital camcorder (Sony Handycam, DCR-HC96, Sony Electronics, Inc., San Diego, California, USA), and all activity and association time data were collected from the recorded videos. A male was considered to be approaching a stimulus if he was within the 100 mm preference zone directly in front of the stimulus (Figure 3.1). I recorded the total time that a male spent in the preference zone with either the predator or conspecific social group as a measure of inspection (boldness) behavior. I randomized whether males were tested first with a social group stimulus or first with a predator stimulus. I found no significant effect of trial order on levels of inspection behavior ($F_{1,189} = 3.384$, $P = 0.067$). In order to estimate overall activity level for each male, the number of squares (indicated by the grid drawn on the back of the test tank, Figure 3.1) that the male moved into during each five minute period of the trial after presentation of the predator (post-predator activity) and after presentation of the social group (post-social activity)

was also recorded from the trial videos. I did find an effect of trial order on levels of activity; males that viewed the predator first had a decreased overall activity level (average activity between both situations) compared to males that viewed the social group first ($F_{1,188}$ = 8.754, P = 0.004). Thus, for this behavioral measure, when testing for associations between post-social and post-predator activity levels, I first divided males into a group that viewed the predator stimulus first, and a second group that viewed the social group first (see results below).
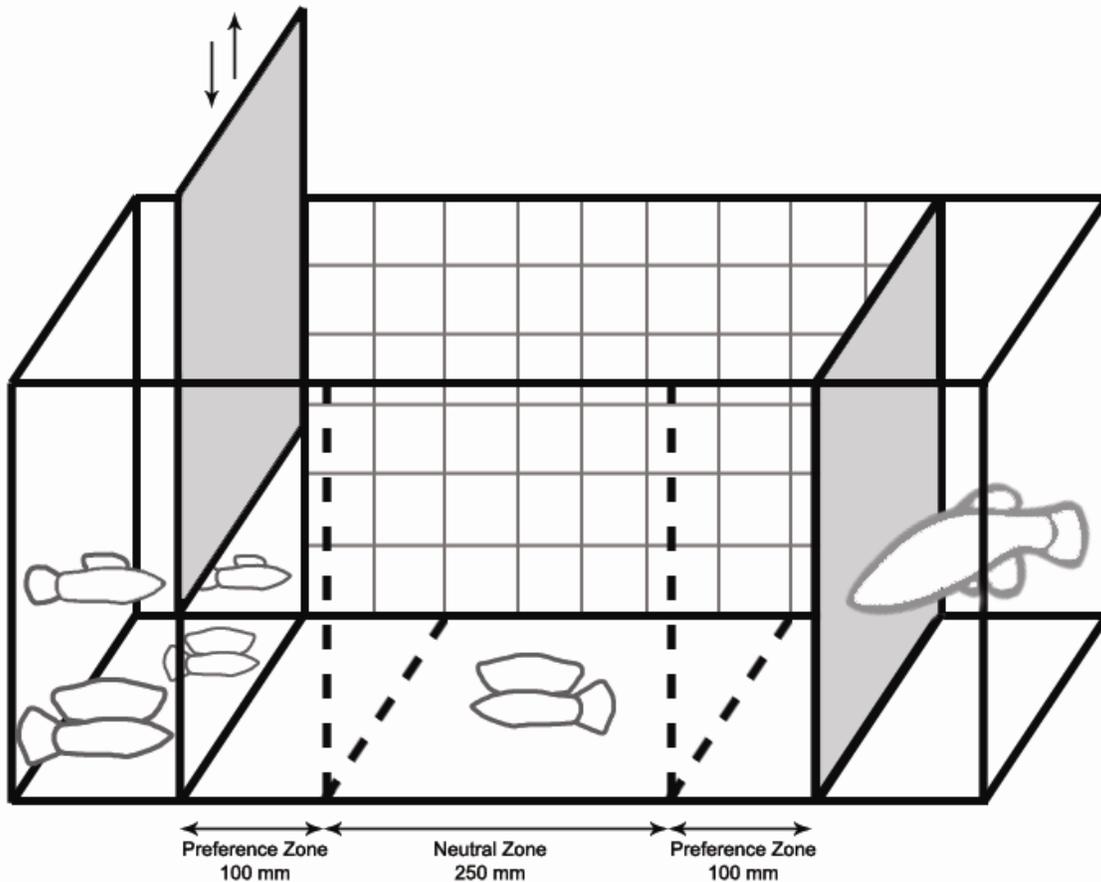
Figure 3.1. Three chambered dichotomous choice tank used in activity and inspection behavior trials. The two end compartments (14 liters each) were partitioned with plexi-glass and sealed to insure no exchange of water from the end compartments into the center test compartment, thus, inspection behaviors were based on visual cues to the test male only. The center compartment was covered by a grid of squares (50x50 mm each) and divided into three zones (2 preference zones; 1 neutral zone) by lines drawn on the back of the tank. The preference zones were 100 mm in width and located to the far right and the far left of the neutral zone, directly in front of each stimulus compartment, leaving the 250 mm in the direct center of the test tank as the neutral zone. Dashed lines indicate preference zones.

Male Size

After a male had completed all behavioral trials (both mating trials and the

activity/inspection trial), I measured the standard length (SL: tip of lower lip to

caudal peduncle) of each test male to the nearest mm.

Statistical Analysis

I analyzed square-root transformed number of courtship displays per 10 minute

observation as my mating measure, square-root transformed number of grid

squares crossed per 5 minute post-stimulus observation as my activity measure,

and untransformed total time (seconds) in the preference zone as my inspection

measure. To test for the main effects of population differences and situations

within each context in behavior rates or approach time scores, I performed an

analysis of covariance (ANCOVA) with male SL as the covariate. After finding no

population level effects (see results below) on behaviors for any of the three

behavioral contexts, I pooled data for all males, regardless of population, to test

for associations between behaviors within contexts, but in different situations

(e.g., receptive and non-receptive females in mating context) and between

contexts (i.e., mating, activity, inspection). To estimate the magnitude of pairwise

relationships between mating, activity and inspection behaviors, I calculated the

Pearson product-moment correlations for all possible comparisons and adjusted

the significance level using a sequential Bonferroni correction method (Rice 1989). To estimate the magnitude of pairwise relationships while accounting for the influence of male size, residuals of all mating, activity and inspection behaviors corrected for male size differences were calculated and compared by Pearson product-moment correlations and the significance level was adjusted using a sequential Bonferroni correction method (Rice 1989). I further examined the influence of male SL on courtship display rates with receptive females, post-predator activity level, and post-predator inspection time by Pearson product-moment correlations. All statistical analyses were performed with JMP Version 5.1 software (Cary, North Carolina, USA).

RESULTS

Behavioral Variation between Situations within a Context

Male size (SL) significantly influenced behaviors in mating and inspection contexts but had no significant effect on activity levels (Table 3.1). There were no significant differences among the three male populations tested in rates of courtship, levels of boldness, or overall activity levels (Table 3.1). Males from all three populations performed significantly more courtship displays to receptive females than to non-receptive females and spent significantly more time inspecting the social group than the predator (Table 3.1, Figure 3.2A and C).

Activity levels were similar between situations (post social group or post predator stimulus; Table 3.1, Figure 3.2B).

Pearson product-moment correlations showed positive associations between a male's responses to different situations within all three behavioral contexts (Table 3.2). I found a significant positive association between the level of courtship displays performed by a male with the two types of females (receptive and non-receptive, Figure 3.3A). Males that were more active following inspection of the social group were also more active following inspection of predators (Figure 3.3B), regardless of whether they viewed the predator stimulus first ($r = 0.530$, $P < 0.001$) or the social group stimulus first ($r = 0.579$, $P < 0.001$). There was a positive association between the level of boldness with a social group and boldness with a predator, which was marginally non-significant after Bonferroni correction ($r = 0.249$, $P = 0.015$, $\alpha = 0.0125$; Figure 3.3C). These results suggest that individual males vary in their expression of different behaviors but a given male shows consistency across different situations within behavioral contexts.

Table 3.1. Results of ANCOVA (male size (SL) as covariate) for each behavioral context for males of *Poecilia latipinna* from three different populations. Significant effects ($P < 0.05$) are shown in bold.

Context	Measure	Source	df	F	P
Mating	Display	Size	1	24.166	**<0.001**
		Population	2	0.645	0.526
		Situation	1	12.788	**<0.001**
		Error	181		
Activity	Sum of 5 Minutes	Size	1	0.066	0.798
		Population	2	2.897	0.058
		Situation	1	2.406	0.123
		Error	172		
Inspection	Approach Time	Size	1	18.945	**<0.001**
		Population	2	0.876	0.418
		Situation	1	19.884	**<0.001**
		Error	173		

Table 3.2. Results of correlation analyses. For each pairwise comparison, the Pearson product-moment correlation value (r) is followed by the P value. Values above the diagonal are full correlation values, and values below the diagonal are partial correlation values, where the effect of male size (SL) has been removed. Significant correlations (P < 0.05) are shown in bold.

		Mating		Activity		Inspection	
		Receptive	Non-Receptive	Post Social	Post Predator	Social Group	Predator
Mating	Receptive	- -	0.250 **0.011***	-0.085 0.410	-0.055 0.600	0.007 0.943	0.250 **0.014***
	Non-Receptive	0.215 **0.041**	- -	-0.062 0.547	-0.211 **0.039**	0.054 0.602	0.063 0.542
Activity	Post Social	-0.057 0.595	0.002 0.989	- -	0.570 **<0.001***	0.183 0.074	0.115 0.267
	Post Predator	-0.064 0.554	-0.203 0.056	0.554 **<0.001***	- -	0.143 0.166	0.159 0.122
Inspection	Social Group	-0.112 0.298	0.037 0.727	0.172 0.106	0.107 0.320	- -	0.249 **0.015**
	Predator	0.154 0.152	0.066 0.541	0.109 0.311	0.144 0.179	0.131 0.220	- -

*Significant correlations at P < 0.05 by sequential Bonferroni correction (Rice 1989).

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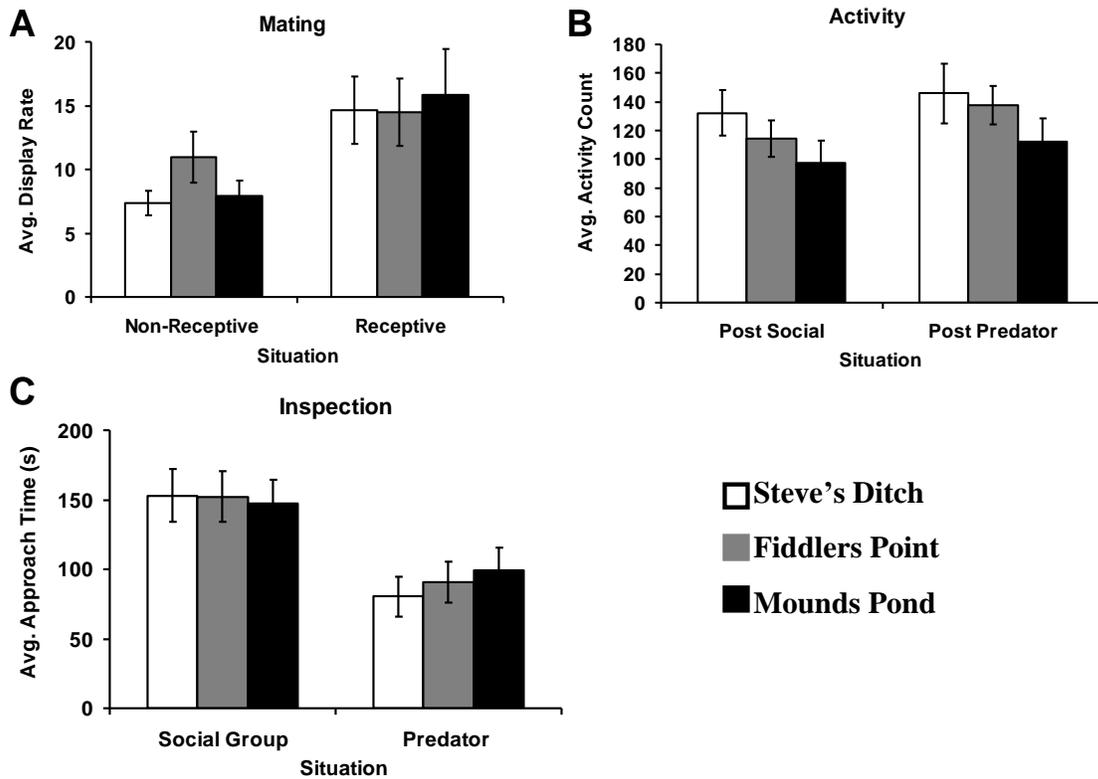
Figure 3.2: Mean (± SE) behavioral differences between situations in three distinct contexts for three different populations of males of *P. latipinna*. (A) Mating: number of courtship displays in response to receptive versus non-receptive females. (B) Activity: number of squares crossed during post social period (following presentation of social group) and post predator period (following presentation of predator). (C) Inspection: time spent in association with social group versus predator.
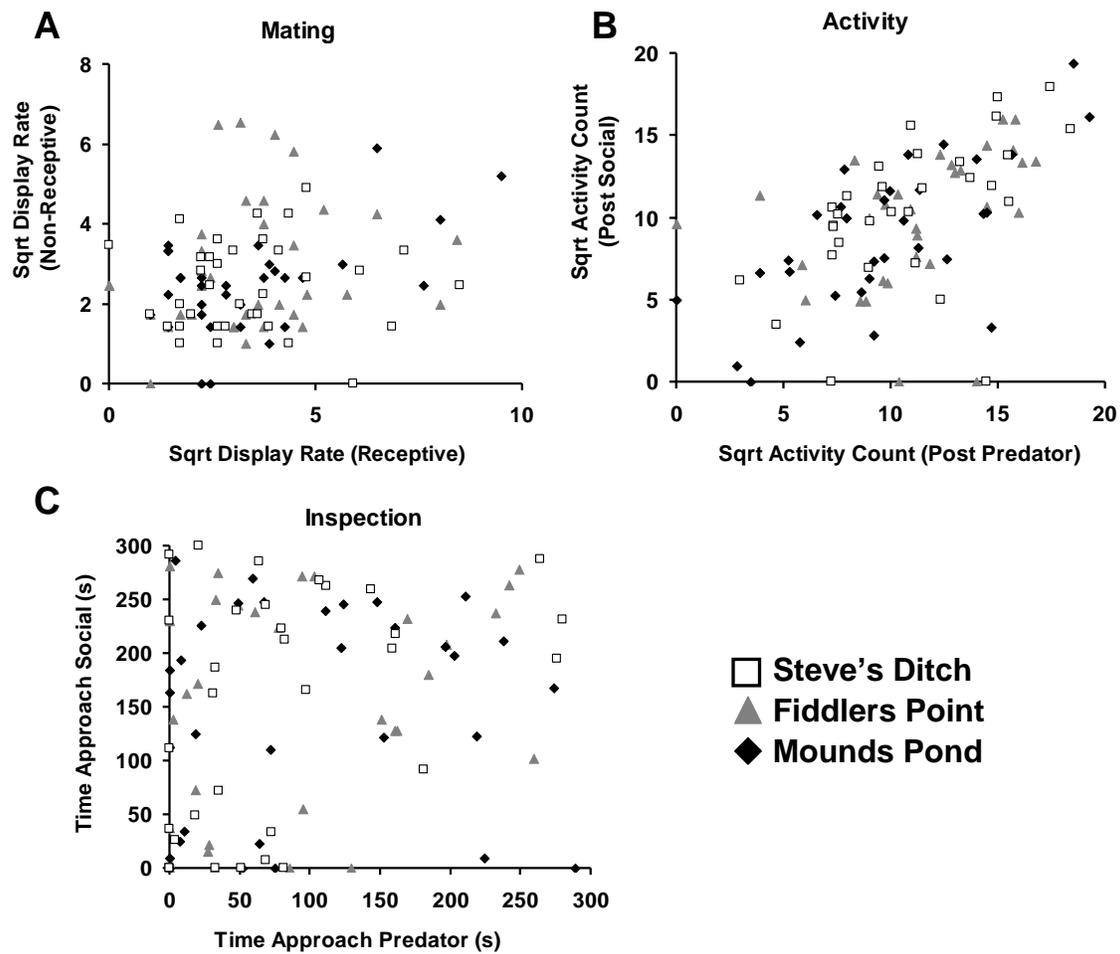
Figure 3.3: The relationship between behaviors in different situations within each of three distinct behavioral contexts for three different populations of males of *P. latipinna*. (A) Mating Context: number of courtship displays in response to receptive versus non-receptive females. (B) Activity Context: number of squares crossed during post social period (following presentation of social group) and post predator period (following presentation of predator). (C) Inspection Context: time spent in association with social group versus predator.

Pearson product-moment correlations showed a significant positive association between mating and inspection behaviors (Table 3.2; Figure 3.4); males with higher courtship display rates also were bolder (spent more time) when inspecting a predator. No other between-context comparisons were significant (Table 3.2).

Figure 3.4: The relationship between behaviors in the mating context (number of courtship displays in response to receptive females) versus inspection context (time spent in association with predator) for three different populations of males of *P. latipinna*.
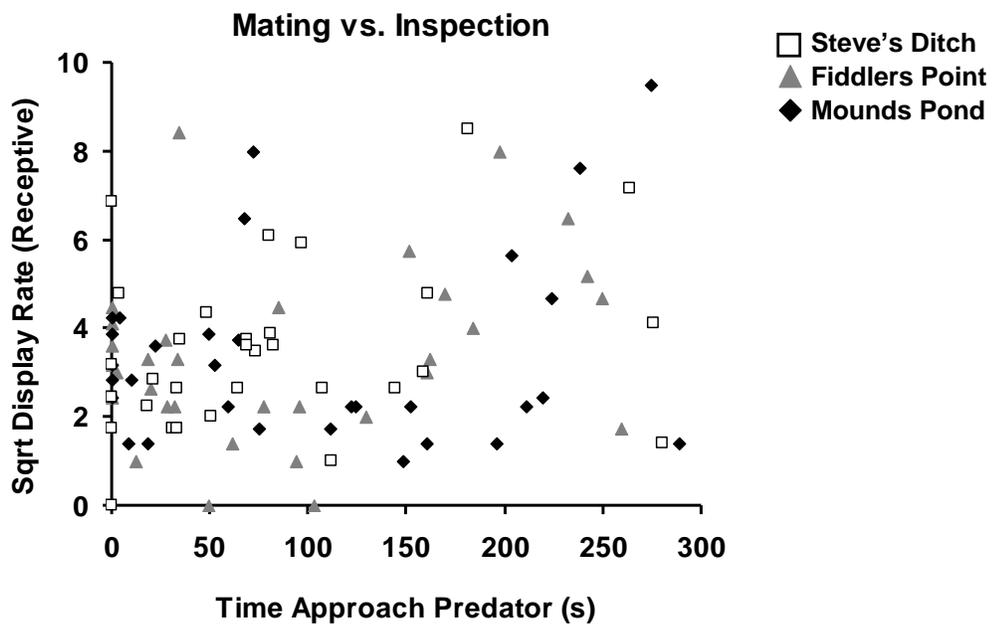
Influence of Male Size on Behavioral Variation

Results of the ANCOVA showed a significant effect of male size on levels of mating and inspection behaviors but not activity levels (Table 3.1). Pearson product-moment correlations showed that male SL was positively associated with behavior levels in mating and inspection but not activity contexts (Figure 3.5). The effect of SL was greatest ($r = 0.461$, $P < 0.001$) on courtship display rates (Figure 3.5A). Male SL did not influence activity levels ($r = 0.051$, $P = 0.634$; Figure 3.5B) but was significantly positively associated with time spent approaching predators ($r = 0.317$, $P = 0.003$; Figure 3.5C). Thus, body size appears to be positively associated with boldness as well as courtship display rates in sailfin molly males.

Partial correlations adjusting for differences in male size in pairwise comparisons within and between behavioral contexts showed only a significant positive association between post-social and post-predator activity levels (Table 3.2). Thus, some males are more active than others, regardless of their SL. No other pairwise partial correlations were significant (Table 3.2) suggesting that male size has a larger effect on the expression of mating and inspection behaviors in sailfin mollies than it does on activity level.
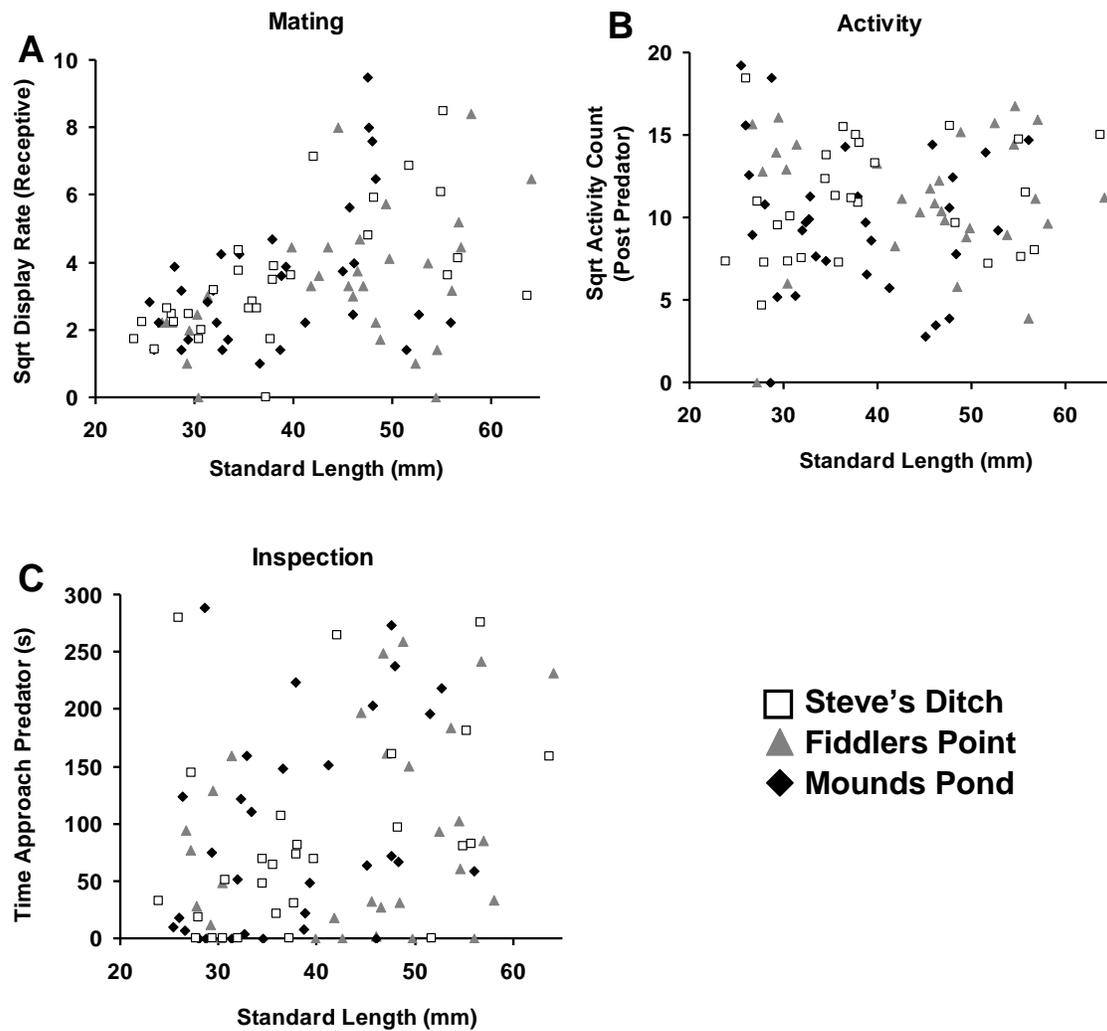
Figure 3.5: The relationship between male size (SL) and behaviors in three distinct behavioral contexts for three different populations of males of *P. latipinna*. (A) Mating Context: number of courtship displays in response to receptive females versus male SL. (B) Activity Context: number of squares crossed during post predator period (following presentation of the predator) versus male SL. (C) Inspection Context: time spent in association with predator versus male SL.

DISCUSSION

Evidence of Behavioral Syndromes in Sailfin Mollies

Behavioral syndromes are most often defined as suites of correlated behaviors across contexts (Sih et al. 2004a). In my study, the best evidence for a true behavioral syndrome in mollies was demonstrated by the significant positive association between courtship display rate with receptive females and boldness as measured by time spent inspecting a predator. While a number of studies have examined boldness associations across inspection, activity and foraging contexts (reviewed by Sih et al. 2004b), fewer studies have compared behavioral associations between male mating behaviors and behaviors in other contexts (e.g., Sih and Watters 2005; Stapley and Keogh 2005; Wilson et al. 2010). For example, in guppies, bolder males have a higher willingness to approach and inspect predators and novel food sources, and resume normal foraging behavior more quickly after a disturbance as compared to shier males (Godin and Dugatkin 1996; Piyapong et al. 2010). Females prefer to mate with bolder males (Godin and Dugatkin 1996) but whether or not bolder males exhibit higher rates of courtship displays has not been investigated. Boldness in male mollies, as in male guppies, may be an indicator of increased viability (bolder males put themselves at risk of predation, but may be better able to escape piscivorous predators or deter predator attack (e.g., Godin and Davis 1995)). A positive

association between levels of courtship (preferred by females (Ptacek and Travis 1997)) and bold behavior towards predators in mollies may suggest that these traits serve as indicators of high viability. Variation among males in courtship display rates and boldness level may provide females with multiple proximate cues for mate choice (Johnstone 1996).

Influence of Male Size on Behavioral Associations

Male size at maturity (as measured by SL) had its strongest influence on mating and inspection behaviors. Larger males were more likely to be "courters" and more bold in inspecting predators. Interestingly, male size did not appear to influence activity levels in males, but individual variation among males did exist in activity level with some males being more active in both post social and post predator periods than others.

In mollies, the influence of male size on courtship display rates appears to be greatest for *P. latipinna*, as other sailfin species show little influence of male size among displaying males (Ptacek et al. 2005; Hankison and Ptacek 2007). Male size at maturity in *P. latipinna* has a known genetic basis (Travis 1994a, b) controlled in a similar manner to the Y-linked *P* locus with multiple alleles for male size in *Xiphophorus* (Kallman 1989). Courtship display rates have been found to show a pattern of Y-linked inheritance in several sailfin species as well, including *P. latipinna* (Ptacek 2002; Loveless et al. 2010), and the potential exists

for linkage disequilibrium to arise between Y-linked alleles for large male size and Y-linked alleles for high rates of courtship display. While the genetic basis for boldness in inspection behavior is unknown in sailfin mollies, my study suggests phenotypic associations between this behavior and male size at maturity as well.

Previous studies have found a positive association between male body mass and boldness score in a related tropical poeciliid, *Brachyraphis episcopi* (Brown and Braithwaite 2004; Brown et al. 2005, 2007a, b). This effect was strongest for populations from high predation sites (Brown et al. 2007a, b). These studies suggest that variation in the natural selection regime (e.g., predation risk) among populations may contribute to variation among individuals in associations between morphology and behavior. Sailfin molly populations are known to vary considerably in the types of predators that males are exposed to (Travis and Trexler 1987; Trexler et al. 1994) and other environmental features (e.g., temperature, salinity), which influence life history traits and survival rates of males of different body sizes (Trexler et al. 1992; McManus and Travis 1998). Thus, variation among populations in the strength and direction of natural selection on male size may contribute to individual variation in the associated behaviors of courtship display and boldness in *P. latipinna*.

Finally, the strong association between large male size, high courtship display rates and boldness in inspecting predators may also be maintained by female mating preferences for larger, bolder males (Ptacek and Travis 1997;

MacLaren et al. 2004; MacLaren 2006). In a similar manner, female guppies

prefer more brightly colored (Houde and Endler 1990; Endler and Houde 1995)

and bolder (Godin and Dugatkin 1996) males. Thus in sailfin mollies, due to its

Y-linked inheritance, male size may be a better predictor of mating success than

other phenotypic traits (Ptacek and Travis 1997; MacLaren et al. 2004; MacLaren

2006; Kozak et al. 2008) and larger males that are bolder may more easily draw

the attention of females.

   Does Individual Variation Constrain Population Divergence in Sailfin Mollies?

Unlike previously reported studies on interpopulation variation in mating

behaviors in *P. latipinna* (Farr et al. 1986; Ptacek and Travis 1996), this study

found no differences among populations in behaviors in any context, mating,

activity or inspection (Table 3.1). Interestingly, variation among male size

distributions in my three populations (all three with males ranging between 25

and 60 mm SL) was considerably less than that reported in a previous study (few

to no males > 40 mm SL previously in Fiddlers Point or Mounds Pond

populations; Ptacek and Travis 1996) and likely contributed to the lack of

population differentiation in mating behavior found in this study. High levels of

gene flow have been demonstrated among natural populations of *P. latipinna*

(Trexler 1988; Trexler et al. 1990) and stochastic environmental perturbations

such as hurricane events followed by wide-spread flooding (e.g., Hurricane

Dennis in 2005), potentially contribute to high levels of mixing between sailfin molly populations. Fluctuations among populations in the strength and direction of natural selection may also contribute to variation among years in the level of population divergence (Travis 1994b; Langerhans and DeWitt 2004).

Population variation in the presence or absence of behavioral syndromes or the strength of associations within behavioral syndromes has been observed in several fish species (threespined stickleback (*Gasterosteus aculeatus*), Bell and Stamps 2004; Bell 2005; *B. episcopi*, Brown and Braithwaite 2004; Brown et al. 2007a), usually associated with differences among populations in levels of predation. While direct measures of predation risk have not been made in different *P. latipinna* populations, variation does exist in the suite of predators (e.g., wading birds vs. piscivorous predators) present, but all populations likely experience strong predation pressures (Travis and Trexler 1987). This is unlike the situation in many poeciliids that live in low or high predation populations (e.g., guppies, swordtails, *Gambusia affinis*, Endler 1983; Basolo and Wagner 2004; Langerhans et al. 2004). Thus, population differentiation among males of *P. latipinna* may reflect differences in the relative balance of natural selection favoring small male size (earlier maturity and greater potential lifetime reproductive success) and sexual selection favoring large male size (females preference for larger males) (Travis 1994b; Ptacek and Travis 1997) among different populations.

Results of my study suggest that individual differences in behavioral associations among males may also contribute to decreasing the degree of population divergence in male behaviors. While male size explains some of the variation among males in rates of courtship displays and level of boldness, activity levels were independent of male size, and size alone does not explain all of the variation among individual males in mating or inspection behaviors (Figure 3.5). Individual males may be more or less active, independent of male size, and variability in activity may draw the attention of both females and predators. Future studies should focus on quantifying predation pressure as well as mating advantages of different male behavioral types in different populations of *P. latipinna* in order to better understand the evolution of behavioral flexibility in sailfin mollies.

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APPENDICES

Appendix A. Non-size adjusted morphological trait means (mm) and variances for all north Florida *Poecilia latipinna* populations collected in 2005 and 2007.

Population	Collection Year	Sample Size	SL Avg	SL Var	LDF Avg	LDF Var	LFFR Avg	LFFR Var
Wacissa River	2005	9	27.182	26.197	7.530	6.563	3.048	2.222
Mounds Pond	2005	12	27.594	91.770	6.746	19.432	2.817	5.100
Lighthouse	2005	11	28.587	10.971	6.637	2.693	2.562	0.265
Bald Point	2005	12	31.963	26.634	8.058	4.446	3.499	0.968
Fiddlers Point	2005	12	34.143	17.970	9.140	4.375	3.362	0.845
Live Oak	2005	19	34.713	34.981	9.318	7.744	3.521	2.392
Marine Lab	2005	17	60.370	93.844	21.557	31.128	12.563	22.791
Pinhook	2007	22	31.113	36.765	8.619	10.792	2.892	2.648
Mounds Pond	2007	87	32.600	37.123	9.388	9.247	3.301	3.840
Steve's Ditch	2007	74	34.547	90.045	10.362	27.692	3.409	5.778
Fiddlers Point	2007	41	44.866	122.671	16.112	41.042	6.632	18.065

Population	LLFR Avg	LLFR Var	DFR# Avg	DFR# Var	DFA Avg	DFA Var	PDD Avg	PDD Var
Wacissa River	3.140	4.070	7.667	4.500	21.712	1116.111	12.695	2.437
Mounds Pond	2.794	4.447	8.083	2.447	21.031	1157.026	12.582	11.009
Lighthouse	2.950	0.354	8.091	1.691	11.088	24.249	13.916	2.532
Bald Point	3.898	1.988	8.833	1.788	22.936	239.531	14.368	4.492
Fiddlers Point	4.046	1.276	10.083	1.720	36.006	508.418	15.519	1.484
Live Oak	4.420	3.857	9.316	4.006	43.368	1584.591	15.479	5.217
Marine Lab	12.727	14.705	12.765	3.066	375.275	38454.167	22.419	6.586
Pinhook	4.447	9.767	11.136	1.552	55.366	10151.499	12.953	1.161
Mounds Pond	4.837	8.208	11.782	2.731	67.191	6416.346	13.242	2.348
Steve's Ditch	5.133	15.756	11.000	3.836	88.766	25353.408	13.605	3.524
Fiddlers Point	9.953	25.635	12.049	2.948	218.770	44201.626	14.874	3.481

Appendix A. cont.

Population	DMB		PAD		LG		DCP	
	Avg	Var	Avg	Var	Avg	Var	Avg	Var
Wacissa River	7.487	3.375	13.685	17.764	6.023	10.722	4.336	1.709
Mounds Pond	8.776	14.014	14.746	25.001	4.799	1.181	4.874	5.168
Lighthouse	8.880	1.895	16.727	7.399	4.699	0.550	4.250	0.398
Bald Point	10.102	4.393	15.782	4.252	5.150	0.869	5.952	1.911
Fiddlers Point	11.441	3.086	18.546	8.890	5.696	1.002	6.149	1.085
Live Oak	11.321	4.751	18.782	11.329	6.032	1.205	6.429	2.013
Marine Lab	21.096	12.109	30.877	22.536	8.529	1.073	12.908	5.828
Pinhook	9.350	4.426	15.053	8.788	5.662	0.474	5.865	2.787
Mounds Pond	9.806	4.488	15.853	10.690	5.528	1.272	6.319	2.381
Steve's Ditch	10.407	11.763	16.228	21.189	5.897	1.750	6.996	6.697
Fiddlers Point	13.882	12.798	19.437	24.046	6.596	2.468	10.174	9.022

Population	LCF		HCF		CFA		BA	
	Avg	Var	Avg	Var	Avg	Var	Avg	Var
Wacissa River	4.805	2.144	4.442	8.023	21.808	447.473	155.513	4917.154
Mounds Pond	4.904	2.857	5.862	10.707	28.908	874.853	192.101	27787.526
Lighthouse	4.795	0.084	3.829	1.452	17.338	40.569	170.841	2120.438
Bald Point	5.190	1.294	7.426	3.466	37.626	247.814	228.498	7220.091
Fiddlers Point	6.695	1.377	7.692	3.791	46.793	325.682	260.640	5019.681
Live Oak	6.322	1.570	8.040	4.508	47.982	379.877	271.938	9826.445
Marine Lab	12.101	5.339	17.478	8.532	195.920	3513.355	869.953	62197.684
Pinhook	5.986	4.213	8.017	15.399	52.634	2321.574	205.176	10433.895
Mounds Pond	6.045	2.135	10.129	9.545	61.527	1042.468	226.923	8079.162
Steve's Ditch	7.602	8.367	11.050	30.294	84.287	6233.153	275.756	36642.522
Fiddlers Point	9.865	7.372	16.720	25.323	160.212	8021.020	483.519	53550.866