Plasticity of Growth Rate in the High-Back Pygmy Swordtail, *Xiphophorus multilineatus*, in Response to Social Context and Maternal Effects

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This thesis titled

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by

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ABSTRACT

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Plasticity of Growth Rate in the High-Back Pygmy Swordtail, *Xiphophorus multilineatus*, in Response to Social Context and Maternal Effects

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Growth rate, which is influenced by genetics and environmental stimuli, is an important factor in determining when and at what body size animals reach sexual maturity. Examining mechanisms that produce variation in growth rate make it possible to determine the extent to which variation in growth rate may be adaptive. Offspring of *Xiphophorus multilineatus* females raised on high and low quality diets were measured at five points in ontogeny: exposure to large courter males and sexual maturity, to determine growth rate differences between treatment type and maternal diet. Before exposure, mother size, juvenile size at 14 days, and juvenile sex influenced growth rate. After exposure, the interactive effect of maternal diet, treatment type, and offspring sex was responsible for differences in growth rate. My results suggest that small mothers may invest more in male offspring, and that both maternal effects and social environment may influence growth rate to sexual maturity.

Approved: _____________________________________________________________

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BROAD INTRODUCTION

Growth rate, the amount of somatic tissue cultivated for a set period of time, is both genetically and environmentally determined and is crucial for survivorship and reproduction (Arendt and Reznick 2005). However, up to this point the importance of phenotypic variation in relation to growth rate has been underrepresented in the literature when compared with studies on genetic variation. A swelling body of evidence suggests that growth rate can be adaptively altered post-fertilization (Abrams et al. 1996; Blanckenhorn 1998). Altering growth rate may be adaptive to escape predation (Benard 2004), to escape intraspecific competition for food or mating resources (Madsen and Shine 2000), to reach sexual maturity at a larger size (Nylin and Gotthard 1998), to reach sexual maturity at an earlier age (Morey and Reznick 2000) or a combination of two or more traits in conjunction. In order for this to occur, an organism must divert more energy into growth processes.

According to Life-History Theory, organisms must partition the resources (energy) they obtain through maternal investment, photosynthesis, or consumption to functions of growth, development, repair, and reproduction (Gadgil and Bossert 1970; Stearns 1992). It follows that if an organism devotes more energy to growth, and the total energy available to the organism is fixed, then less energy can be allotted to other life functions (Williams 1966). It can be predicted then, that whenever an organism is not reproducing it should grow at a physiological maximum to obtain the benefits of large size. Strategically committing energy to growth at varying points in ontogeny may bear significant temporary or lifetime costs for reproduction, development, repair, and survival.
(Billerbeck et al. 2001; Fernandez and Bowser 2010; Madsen and Shine 1993; Reznick and Yang 1993). These costs provide an explanation as to why organisms don’t simply abandon other life functions solely to maximize growth. However, work by Royle et al. (2006) has shown that individuals that follow an alternative growth trajectory attain an equivalent body size as individuals on a “normal” growth trajectory with no significant fitness costs. In this study male Green Swordtails, *Xiphophorus helleri*, with the longest sexual ornaments (swords) displayed the greatest escape ability, which would be a good indicator of partner quality (Royle et al. 2006). In another study on Blue Wrasse, *Thalassoma bifasciatum*, fecundity or reproductive effort was positively correlated with growth rate contradictory to the predictions of Life-History Theory (Schultz and Warner 1989). More work must be done to elucidate causal relationships between environmental stimuli and accelerated or decelerated growth rates that likely differ based on the organism(s) in question.

Organisms capable of maintaining an internal core temperature independent of the ambient temperature (homeostasis), or homeotherms, differ in the degree to which environmental stimuli illicit changes in growth rate from organisms incapable of maintaining thermal homeostasis, or poikilotherms (Arendt 1997). Unlike their “warm-blooded” counterparts, poikilotherms must conform to temperature constraints on sex determination in some cases, foraging habits, reproduction, and growth rate (Angilletta et al. 2004; Rhen and Lang 1995). However, both poikilotherms and homeotherms share two common patterns for alternative growth rates: slow growth associated with resource limited environments and fast growth associated with high juvenile mortality as a result
of predation or competition (Arendt 1997). Temperature, along with resource stress (nutrient deprivation) and predation pressure, exert direct selection on juvenile growth rates (Dmitriew 2011).

Winemiller and Rose (1992) found that fishes employ three different growth strategies in response to variation in the aforementioned environmental stimuli: the “periodic”, “opportunistic”, and “equilibrium” strategies. Fish employ the “periodic” strategy when high periodic mortality occurs, typically in small size classes (Winemiller and Rose 1992). The “opportunistic” strategy is employed when individuals experience an environment prone to disturbance while the “equilibrium” strategy bears significant fruit when resource availability is diminished or zero (Winemiller and Rose 1992). These strategies alone can’t account for growth rate variation within species that associate in sometimes large, mixed sex social groups with opportunities for exposure of growing juveniles to mature adults. Species of the swordtail genus, *Xiphophorus*, represent an ideal system to study the influence of maternal investment (effects) and social context on growth rate because of their population structure.

Contemporary studies showing a relationship between age and size at maturity, which can be used to infer growth rate, and maternal effects as well as social context have helped generate a hypothesis of adaptive variation in growth rate across taxa (Jennings and Philipp 1992; Jorgenson et al. 2011; Reznick and Yang 1993). Phenotypic plasticity in growth rate has arisen in response to predation pressure, availability of potential mates, maternal investment in offspring, conspecific resource competition, etc. on multiple occasions asynchronously across taxa (MacDonald and Thompson 1985;
Newman 1992; Segers and Taborsky 2010; Sorci et al. 1996; Wiklund et al. 1991). The degree of plastic response varies within species as demonstrated by Bashey’s (2006) work regarding cross-generational environmental effects on guppy systems in Trinidad. Her results suggest that juvenile growth responses vary based on maternal resource environment and subsequent offspring investment, offspring diet regime, and selection for the heritability of offspring size. The evolution of alternative growth strategies represents an intersection of the diverse fields of behavior, developmental biology, genetics, and ecology.

The manners by which maternal effects impact juvenile growth rate are many and variable. A female of any species must partition a portion of her energy for investment in her offspring if she is to reproduce. Formation and maintenance of the egg typically represents the most obvious fraction of this investment, but other investment opportunities include yolk development, period of gestation if any, method of birth, and post-natal care (Byers and Moodie 1990; Kaplan 1992; Lee and Moss 1986; Rasanen et al. 2005). Maternal investment in offspring comes at a cost to her somatic growth and survival in most instances (Berube et al. 1996; Gomendio 1991; Hunt and Simmons 2004). Females in poor or highly variable environments (low nutrient or mate availability, high predation, temperature or moisture extremes, highly seasonal, etc.) may invest less in fecundity and favor self-preservation as a result (Arendt and Reznick 2005; Chong et. al 2004; Lyons 2011). Potential mothers in favorable environments generally utilize the opposite strategy: maximizing reproductive output sometimes at the cost of growth and survival (Bashey 2006; Kim 1999; Blount et al. 2002). This differential
investment in offspring often leads to variability in juvenile survival, initial size, and capability to sequester any available energy in the environment (Rutkowska and Cichon 2002; Sinervo 1990). Many organisms live in populations where juveniles may be exposed to individuals of varying age class, which introduces social context as an influence on adaptive growth rates.

The opportunities for social context to influence juvenile growth rate vary considerably in magnitude and direction. Mature individuals other than the offspring’s parents may cannibalize the young if resources are scarce or if male dominance has shifted in a hierarchically organized population (Claessen 2000; Grinnell and McComb 1996; Smith and Reay 1991). In these situations the juvenile represents new and undesirable competition for already scant food supplies while simultaneously being a store of energy themselves, or simply unnecessary investment in an individual that doesn’t possess the genes of the dominant male. In some species juveniles are able to take advantage of extra-familial care as other individuals offer food or protection (Dexter 1952; Gaston 1978; Hatchwell and Komdeur 2000). Sexually mature individuals in the population also represent potential mates when the juveniles themselves become capable of reproducing. The availability of mature sperm or mature eggs may exert pressure on juveniles to reach sexual maturity at an earlier age in some situations, while delaying maturation may be advantageous in species with determinant growth (Kruesi et al. 2011).

Timing and type of environmental stimuli exposure capable of inducing changes in juvenile growth rate may affect the size and strategy of observed growth response either directly or indirectly. In altricial birds, high growth rate is observed in species with
significant rates of predation during the vulnerable nesting stage (Case 1978; Lack 1968). The faster the juveniles can escape this stage-specific mortality, the better the chances for survival; thus the adaptive response would be to accelerate growth rate. A similar response is observed in species that inhabit highly seasonal environments, which can place significant time constraints on organismal life history events (Ludwig and Rowe 1990; Rowe and Ludwig 1991; Abrams and Rowe 1996). Organisms must be able to reach some set size or life stage by the end of the favorable season(s) or face drastically reduced chances for survival; so accelerated growth rate is frequently observed in circumpolar species (Houston and McNamara 1992; Abrams et al. 1996).

Natural resource deficiency is the most prevalent impetus for the development of alternative growth rates across taxa, since the amount of energy in a system is fixed and distribution will not always be equal; thus organisms in similar niches must compete. Under low resource conditions, both homeotherms and poikilotherms favor slow growth to maximize efficient utilization of all energy available while high resource conditions favor maximizing growth rate since somatic tissue cultivation can be sustained without threat (Niewiaroski and Roosenburg 1993; Riha and Berven 1991). It has been shown that juveniles of some species are capable of compensatory growth, a period of rapid growth rate following a period of reduced growth generally in response to nutrient deficiency (Yearsley et al. 2004; Mangel and Munch 2005). The point in ontogeny that the juvenile undergoes this compensation period as well as the duration of this compensation has impacts on lifetime survival and reproduction (Lindstrom et al. 2005; Sibly et al. 1985). Each of the aforementioned factors favoring alternative growth
strategies may confound the effects of one another (e.g. a sexually mature female experiencing a low resource environment slowing her growth rate and increasing her investment in fewer, larger offspring that are born into a large, mixed sex population), but experiments testing this kind of interaction are lacking. Thus multiple factors must be tested in conjunction, and not singly, to accurately determine the influence of each on altering juvenile growth rates.

To take a closer look at the potential adaptive alteration of growth rate due to maternal effects, I examined how changes in mother’s investment, due to manipulations in the mother’s resource environment, influenced the growth rates of their offspring in *Xiphophorus multilineatus*. Additionally, I determined if maternal investment impacted changes in their offspring growth rate in response to social context. Siblings were split between treatments, half being exposed to a large courter male during juvenile development and half controls with no exposure. Evidence from experiments on maternal diet manipulation in this species have shown that mothers alter investment in offspring (e.g. size at birth) based on the quality of their diets during juvenile development (Lyons 2011; Murphy unpublished data). In addition, a study on the related species *Xiphophorus helleri* found that juveniles altered the size at which they reached sexual maturity based on visual exposure to mature males with variable sword lengths: females reached sexual maturity sooner when exposed to the more preferred males with longer swords, while males took longer to reach sexual maturity, maturing at a larger size, when exposed to males with longer swords (Walling et al. 2007). Therefore, both maternal effect and social context have the potential to influence juvenile growth rates. Assuming that
plasticity in growth rate in response to the exposure to adult males is adaptive, I wanted to determine the extent to which the environment in which the mother was raised, in relation to the environment in which the offspring are raised, influence this adaptive response.

**Xiphophorus System**

*Xiphophorus* fishes are viviparous, or live-bearing, as well as lecithotrophic (Constantz 1989), which means that all maternal investment to the eggs is provided prior to fertilization. Lyons (unpublished data) found that in *Xiphophorus multilineatus*, the High-Back Pygmy Swordtail, females raised on the high quality diet produced more, smaller fry than females raised on the low quality diet. This result was similar to what had been detected in guppies *Poecilia reticulata* (Bashey 2006). Bashey (2006) placed half of the mothers on a low-food treatment and half on a high-food treatment then allowed full sibling sisters to drop fry. Mothers on the low-food treatment produced larger, leaner offspring in lower numbers than their high-food treatment sisters. This pattern supported the prediction that mothers in a non-competitive environment (high quality treatment) would increase fecundity rather than individual offspring investment (Bashey 2006).

Evidence that swordtails might adjust their growth rates based on social conditions comes from a study by Walling et al. (2007) on the green swordtail *Xiphophorus helleri*. Exposure to adult males bearing either “short” (up to 12 mm) or “long” swords (18mm or greater) influenced the age and size at sexual maturity. Age and size at sexual maturity differed not only between treatment groups, but also between
sexes, suggesting an interaction between offspring sex and growth strategy. Since growth rate defines the relationship between age and size, it can be inferred from these results, but testing for growth rate explicitly would eliminate potential confounding factors including initial offspring size.

In *X. multilineatus* the number of copies of Melanocortin-4 Receptor (MC4R) genes on the Y chromosome plays a large part in determining size at maturity for males; males exhibit determinate growth so the size they attain at maturity will be their maximum lifetime size (Lampert et al. 2010). This has implications for growth rate since differential growth strategies are observed among genotypes (Bono et al. 2009). Females have been shown to prefer larger males on average depending on female size, experience, and condition (Rios-Cardenas et al. 2007), larger males have higher fertilization success (Morris et al. 2010), larger males successfully deter rival males more often (Morris et al. 1995), and larger individuals could potentially evade predators more effectively (Basolo and Wagner 2004). Thus it would be advantageous for males to obtain maximum size at maturity, which might require altering growth rate.

It is hypothesized that altering growth rate would be advantageous for females more so than size, because females exhibit indeterminate growth and thus do not stop growing in standard length (SL, the distance from the tip of the mouth to the end of the caudal peduncle) and body depth (BD, the deepest linear measure from dorsal to ventral surfaces) at sexual maturity (Kallman 1989). However, once a female reaches sexual maturity, growth rates do decline. If juvenile females are not exposed to mature sperm, energy need not be devoted to reproductive tissue and females can maximize growth as
suggested according to Life-History Theory (Stearns 1989). However, if juvenile females are exposed to mature males, which they are consistently in wild mixed-sex populations, it may be adaptive to reach maturity at an earlier age by re-appropriating resources from somatic growth to reproductive effort.

Significant variation exists in the wild for growth rate to sexual maturity in males, which suggests differential plasticity for these traits among males of varying genotypes (Bono et al. 2011) and this may have implications for female mate preference and growth of juveniles sired by alternative genotypes. Female size and condition in turn influence preference for symmetry and size in males (Morris et al. 2006). This complex interacting web of genetic and environmental (social and resource) effects must be untangled to determine whether or not juvenile *X. multilineatus* alter growth rate to sexual maturity in response to a particular variable or set of variables.

This study investigated growth rates to sexual maturity of juvenile swordtails when broods were born to mothers raised on high and low quality diets, and fry were exposed to mature courter males throughout multiple stages of ontogeny. The changes in SL, BD, and growth rate before and after exposure to a large courter male were compared for differences in response by sex, by maternal diet, and by treatment group. Any observed differences in growth rate among males and females in treatment (exposure) groups would suggest that juvenile *X. multilineatus* alter growth strategy based on an interaction between sex and social context. Observed differences in the aforementioned traits among males and females born to mothers on different diets would suggest
alternative growth strategies based on an interaction between sex and maternal investment (effects).

It is hypothesized that there will be an interaction between sex and exposure when considering growth rate to sexual maturity, mirroring results found by Walling et al. (2007), such that females reach sexual maturity sooner in response to this treatment, while males increase growth and reach sexual maturity later at a larger size. It is also hypothesized that there will be a difference between observed growth rate before and after the first exposure based on the significant influence of maternal investment, which will be reflected in the initial size of the offspring. Finally, it is hypothesized that I will detect an interaction between maternal diet, sex, and exposure on growth rate after exposure. As all offspring will be raised in a low quality environment, I predict that offspring raised in the same environment as their mother (mother also raised in low quality diet) will be better able to respond adaptively to the social context of a large adult male. This study further enhances understanding of methods for plastically altering growth rate post-fertilization and reasons for doing so by determining how growth rate varies when maternal resource environment, social environment (exposure to a large male), and sex of the juvenile are considered together in the analysis.
CHAPTER 1: THE INFLUENCE OF MATERNAL DIET AND MALE EXPOSURE ON THE GROWTH RATE TO SEXUAL MATURITY OF JUVENILE *Xiphophorus multilineatus*

Abstract

Growth rate is an important factor in determining when and at what body size animals reach the onset of reproductive activity. While increasing growth rate can increase reproductive success by reaching sexual maturity sooner or at a larger size, it can also produce tradeoffs with other energetically demanding functions such as reproduction and development. Growth rate is influenced by genetics as well as environmental factors such as growing season, food availability, predation rate, and temperature fluctuations. Examining the mechanisms that produce variation in growth rate is an important first step towards determining the extent to which variation in growth rate is adaptive. Broods from *Xiphophorus multilineatus* females raised on high and low quality diets were split between exposure to an adult male and control treatments, and all raised on low quality diets. All fry were measured at 14 and 70 days, prior to treatment, at 100, 130, and 160 days after treatment, and at sexual maturity to determine how growth rates changed due to the exposure to an adult male. Mother’s size was significantly larger for females raised on the high quality diet. High quality mothers had larger fry than low quality mothers, but within each group larger females had smaller fry. In addition, fry were smaller from larger broods, and female fry were smaller than male fry. Growth rates prior to treatment were influenced by mother size, size of the juvenile at 14 days, and sex of the juvenile. These results suggest that mother’s investment can influence fry growth rate, and that this
investment is indirectly influenced by mother’s diet through mother’s size. The change in growth rate after treatment was explained by an interactive effect of maternal diet, exposure treatment, and sex of the offspring. Individuals from mothers on a diet that was high quality had a stronger growth response to the treatment, and the response to treatment was different between males and females. My results suggest that maternal effects can confound the ability of individuals to adjust their growth in response to social context.

Introduction

The influence of both maternal effects and social context on growth rate has been detected across a range of taxa (Dunn and Bale 2011; Blount et al. 2002; Rasanen et al. 2005; Sinervo 1990), and it has been suggested that the influence of these factors has led to the evolution of adaptive variation in growth rates (Arendt 1997; Conover 1990; Dmitriew 2011). Studies in butterflies and fishes have shown changes in age and size at sexual maturity in response to predation, maternal investment, and resource competition within species (Gotthard et al. 1994; Arendt and Reznick 2005). Experiments examining the impact of either social context or maternal effects on growth rate separately have been done in many species (Blanckenhorn 1998; Claessen et al. 2000; Benton et al. 2005); however, examining the influence of both variables simultaneously as an explanation of alternative growth trajectories is lacking (Segers and Taborsky 2010; Jorgensen et al. 2011). Analyzing phenotypic plasticity in growth rate from the lens of both maternal effects and social context makes intuitive sense for organisms that live in variable environments and associate in large, mixed sex groups where individuals of all life stages.
interact (Reznick and Yang 1993; Endler 1983). Offspring in these populations can experience intraspecific competition even before they emerge from the mother, as the competition for resources the mother experiences can impact how the mothers invest in their offspring, thereby creating disparity in size and mass at birth (maternal effects) (Chong et al. 2004). If mothers invest in offspring so as to increase the competitive abilities of their young, then it is possible that in highly variable environments, offspring that are born in to an environment that differs from the environment in which their mothers were raised could be at a disadvantage in their ability to respond to growth challenges.

The *Xiphophorus* system presents a unique opportunity to study the influence of maternal effects and social context on growth rate. Members of this live-bearing genus in the family *Poeciliidae* are native to freshwater and brackish drainages of Mexico, and associate in mixed sex schools of varying size creating opportunities for competition within and among species (Meyer et al. 2006; Simmons et al. 2008; Luo et al. 2005). It has been demonstrated that females of species including *X. helleri* and *X. multilineatus* differentially invest in fecundity based on resource environment and condition (Chong et al. 2004; Lyons 2011) and female *X. birchmanni* are capable of determining male mate quality based on pheromone cues as a signal of nutritional condition (Fisher and Rosenthal 2006). Juveniles of some species in the genus are capable of altering growth rates in response to sword length of the courter males they encounter (Walling et al. 2007), and altering the age and size at sexual maturity for males carries significant implications for success in intrasex competitions (Morris et al. 1992), evading predators
(Rosenthal et al. 2001; Royle et al. 2006), repairing oxidative damage from metabolism (Fernandez and Bowser 2010) and attracting mates (Morris 1998; Morris et al. 2006).

Based on a previous study of *X. helleri*, I predict that juvenile *Xiphophorus multilineatus* will be plastic in their growth rate response to social context. However, if mothers adjust their maternal investment based on their own juvenile environment, the ability of their offspring to have a plastic response will depend on whether or not the offspring are raised in an environment that is similar or not from the environment in which the mothers were raised. In order to test these hypotheses I measured the growth rates of offspring in a low quality environment born from mothers that had been raised on high or low quality diet (quality of diet based on crude protein percentage) and mated to a courter male from the largest size class, prior and subsequent to exposing the offspring to a large courter male. I first determined if mother’s diet during development influenced the size of their offspring at isolation and their growth rates prior to the exposure treatment. If there is a difference in fry size or growth depending on mother’s diet, this would suggest that maternal investment in initial offspring size may promote or inhibit accelerated growth in light of resources available to the juvenile. Second, I determined if the response of offspring raised on the same diet as their mothers (low quality diets) to exposure treatment was different from the response of offspring raised on a lower quality diet than their mothers, which would suggest that maternal effects can confound the ability of individuals to adjust their growth in response to social context.
Methods

Collecting and Raising Experimental Individuals

A previous study that examined condition-dependent female mate preferences (Lyons 2011) raised sisters on either a high or low quality diet, with diet quality determined by the proportion of crude protein to other nutrients. The low quality diet consisted of Nishikoi® wheatgerm pellets (Essex, England), which contained 20% crude protein in proportion to 62% carbohydrate, 7% moisture, 6% oil, 2.5% fiber and 2.5% ash. The high quality Tetramin® flakes (Melle, Germany), which contained 46% crude protein in proportion to 38% carbohydrate, 8% oil, 6% moisture, and 2% fiber (Lyons 2011). Twenty-five females from the previous study were selected to provide offspring for this experiment: 15 individuals from the low quality diet manipulation and 10 from the high quality. More low quality mothers were utilized because brood size was found to be small when compared with high quality mothers and for the current experiment, and I wanted the number of individuals from each diet manipulated mother type to be equivalent.

The females (mothers in the current study) were mated with courter males from the largest size class (Y-L). A virgin female was placed with two mature large courter males (mating block) within diet treatments for a period of one week, which generates the possibility of individuals from the same brood being paternal half-siblings and individuals from different mothers within diet treatments being paternal half-siblings. Therefore, in the analysis I controlled for mating block. Luo et al. (2005) has shown that multiple paternity is greatly reduced in *X. multilineatus* compared with other members of
the genus, with one male on average contributing more than 70% to the offspring, and as a result it is likely the juveniles from a single brood were all full siblings. Once mated, females were returned to the individual ten gallon aquaria from which they had been isolated since 15 days of age and allowed to drop fry. First broods of these mothers were counted and massed to determine offspring number and size (Lyons unpublished data). After mothers had dropped a second brood, they were removed from the juvenile tank on the day of birth to prevent cannibalism. Fry were then counted and maintained as a group until they reached two weeks of age. After they had passed this window of high mortality associated with excessive handling, at which time they could be isolated.

The timing of isolation, measurements, and exposure treatments has been summarized in Figure 1. At fourteen days, eight fry (or as many as could be collected) from each brood were isolated in separate ten gallon glass aquaria (41x21x22cm). When isolated, the fry were photographed using a Canon® Powershot SD 1200IS, with the images analyzed using ImageJ® (Rasband, W.S., NIH, Maryland, USA) to determine standard length and body depth. Half of each brood was assigned to a “no exposure” control treatment and half was assigned to an “exposure” treatment. All fry were maintained in two environmental chambers with a constant photoperiod and temperature of 13:11 h L: D, and 22°C respectively. Fry were fed Nishikoi® wheatgerm pellets ground finely into smaller particles ad libitum for five minutes once daily. After the allotted five minutes, any remaining food in the tank was netted and removed to prevent the growth of excess algae in the tanks that could supplement the juveniles’ diet. All tanks were scrubbed and half-drained accompanied by the addition of ten milliliters of
StressCoat®, ten milliliters of StressZyme®, and 1/8 of a cup of salt, and then refilled to inhibit the proliferation of algae as well as eliminate wastes once a month.

Courter Male Exposure Protocol

Twenty-eight courter males were isolated from two of the lines established in 538 liter cattle tanks (mesocosms) in the laboratory, measured for standard length and body depth, identified as symmetrical or asymmetrical with respect to vertical body bars, and separated into individual ten gallon tanks where they were fed Tetramin® flakes daily. Only males measuring 32-47 mm SL were used for exposure and considered “large” males; however, this included both Y-II and Y-L genotype courter males. Males were singly exposed to an individual fry via a plastic Penn Plax® Breed-n-show box (9.5x12.5x12.5cm) that was immersed at the front of the fry’s home tank. The Breed-n-show boxes were constructed with apertures at the top and bottom of the container so that juveniles could receive both visual and chemical (pheromone) cues from the exposure males.

For three days at a time, the fry were exposed to a mature male; during that time the exposure males were fed Tetramin® flakes ad libitum for five minutes once daily in much the same fashion as the fry were fed low quality food. Juvenile tanks were screened with opaque paperboard sheets so that only one male was visible to a fry at any one time to prevent fry or stimulus males from perceiving neighboring stimulus males. The stimulus males placed in their compartments were the only sexually mature fish that the juveniles in the exposure treatment saw from the day they were born in lab until they reached sexual maturity. Starting at seventy days of age, fry were exposed to a mature
male, and every thirty days subsequent to this first exposure they were exposed to a new male until every juvenile saw a total of four distinct males. Juveniles were photographed on the first day of each exposure using the Canon® Powershot SD 1200IS and measured for standard length and body depth using ImageJ® software similar to the procedure for measuring the juveniles throughout ontogeny. Fry in the control treatment were exposed to empty Breed-n-Show tanks during the same periods treatment individuals were exposed to mature males. Juveniles in the control treatment never saw an adult fish during the course of the experiment.

All juveniles were checked weekly for signs of sexual maturity: gonopodium formation by specialization of the anal fin in males and gravid spot appearance in females (Rosen 1960). Individuals that had reached sexual maturity prior to the last exposure (160 days) were photographed and measured with the same procedures as individuals that reached maturity after the fourth exposure. This experimental design yielded measurements of SL and BD for initial size, growth rate prior to exposure, and growth rate subsequent to exposure that were used to determine the influence of maternal diet and male exposure on the growth of juvenile *X. multilineatus*.

**Statistical Analysis**

All analyses were performed using R statistical software (R Development Core Team 2011). Data was analyzed utilizing a nested mixed effects model with REML. The nested structure was composed of mother’s identification within mating blocks. For all models the effect of mating block alone was removed because its estimate was very nearly zero. A normal probability plot of the residuals was used to check for normality of
the within group errors. Homogeneity of variance was checked using a plot of the
standardized within-group residuals versus the within-group fitted values for each model
that was analyzed. Normality of the random effects was similarly checked using a normal
probability plot for each of the levels (mother’s identification and mother’s identification
within mating blocks). Fitting the linear model was accomplished by plotting the
residuals against each of the explanatory variables. All models used met the assumptions
of linearity; only linear models were utilized.

Confidence intervals for the estimation of each explanatory variable were
obtained using a MCMC sampling of n=10,000. This sampled from the posterior
distribution of the parameters of a fitted model using Markov Chain Monte Carlo
methods. All variables hypothesized to have an impact were included in the initial model
with interactions of interest. All variables with a confidence interval including zero were
removed from the initial model. I determined the factors influencing fry size at time zero
(14 days), growth rate 1 (prior to treatment) and growth rate 2 (subsequent to treatment)
(Figure 1). GR2 is my estimate of plasticity in growth rate in response to treatment, with
differences in GR2 between the controls and individuals in the exposure treatment
indicating a growth response to the exposure.

Results

Maternal Diet and Sex Influences Fry Size

There was a significant difference in the mean size of mothers from the high
quality diet treatment and mothers from the low quality diet treatment; mothers raised on
high quality diets (hereafter “HQ mothers”) were larger than mothers raised on low
quality diets (hereafter “LQ mothers”) (HQ=42.45±3.55, LQ= 39.13±2.75, mean±SD mm, t=2.208, df=13.977, p-value=0.044; Figure 2). While HQ mothers produced larger fry than LQ mothers, within each group, larger females produced offspring that were smaller at 14 days (size 0) than smaller females, and offspring born into larger broods were smaller than offspring born into smaller broods (Table 1, Figure 3A, B). As this was the second brood for these females, it was hypothesized that some of the females cannibalized offspring from the first brood, adding a confounding resource to the study. In addition, within fry born to mothers on a common diet, male fry were larger than female fry at 14 days (HQ estimates: male=23.088, female=22.793; LQ estimates: male=21.948, female=21.653).

**Growth Rates**

Growth rate 1 (GR1, prior to exposure treatment) was influenced by an interaction between mother’s size, juvenile’s size at 14 days (size0), and sex of the offspring (Table 2). Growth prior to the first exposure was positive in relationship to mother’s size (estimate: SL_mom= 2.782, Figure 4), negative in relationship to offspring size at time 0 (estimate: size0=2.392), and male offspring grew faster than females (estimates: male=3.026, female=2.493).

The growth rate subsequent to exposure (GR2) was influenced by an interaction of maternal diet, exposure treatment, and sex of the juvenile (Table 3). Sons of LQ mothers grew faster than all other sex/diet combinations, with those in the treatment group growing faster than those in the control group (GR2 estimates: control=10.41, treatment=11.28, Figure 5). Similarly, sons of HQ mothers grew faster after exposure to a
large courtier male when compared to males born to HQ mothers that were exposed to empty tanks in the control group (GR2 estimates: control=9.55, treatment=10.74, Figure 5). The pattern for females was similar, however the response was in the opposite directions, depending on mother’s diet. Across females born to HQ mothers, those in the control treatment had a faster growth rate as compared with their sisters in the exposure treatment (GR2 estimates: control=8.75, treatment=7.63, Figure 5). Across females born to LQ mothers, those in the exposure treatment displayed faster growth rates as compared with their sisters in the control treatment (GR2 estimates: control=8.43, treatment=9.31, Figure 5).

**Discussion**

Growth rates of juvenile *X. multilineatus* were plastic, as full siblings had different growth rates depending on the social context in which they developed. The direction of the response to the presence of an adult male depended on the sex of the fry, and the magnitude of the response for males, as well as the direction of the response for females depended on whether or not their mothers were raised on the same low quality diet. Male offspring increased their growth rate if exposed to a larger adult male, a response that was stronger if mothers were raised on a high quality diet. Females from mothers on low quality diets also increased their growth rates after being exposed to an adult male, while females from mothers on high quality diets grew slower if exposed to a large adult male, presumably investing in reproduction rather than growth. I discuss the evidence that there are differences in maternal investment depending on mother’s diet as well as the potentially adaptive consequences of these maternal investments below. I also
discuss the adaptive consequences of the differences in plasticity in growth detected between males and females, and of maternal investment influencing plasticity.

The difference in size of the fry between offspring of low quality and high quality mothers at age 14 days suggests that maternal investment influenced juvenile size at birth, and that this investment was influenced by both the mother’s diet and the sex of the fry. Condition-dependent maternal investment has been detected in other poeciliid fishes (the guppy *Poecilia reticulata*, Reznick and Yang 1993), such that females on low quality diets or diets that switched from low to high quality produced heavier, less numerous offspring, and females on high quality diets produced lighter, more numerous offspring. Mothers may enhance the survivorship of their offspring in a nutrient poor or highly variable system by distributing their energy reserves across fewer offspring; making them larger (Bashey 2006; Segers and Taborsky 2010). In high resource or non-competitive environments mothers may instead increase their fecundity, investing less in each offspring; making them smaller (Smith and Fretwell 1974). This fits with life history theory predictions (Stearns and Koella 1986). First, given a certain gonadal mass a mother must allocate resources to her offspring and each increase in investment per offspring may result in decreased offspring number (Jorgensen et al. 2011). But second, investing more per offspring is important if the environment is poor, as the offspring will have fewer resources available to them after they are born. The pattern of investment I detected across the HQ and LQ mothers in *X. multilineatus*, however, was somewhat different. At 14 days old (size 0), both male and female offspring from HQ mothers were larger than their peers from LQ mothers, which suggests that HQ mothers used their
additional resources to invest more in each offspring; HQ mothers were on average larger than LQ mothers. However, within each diet treatment, fry were smaller from larger broods. This suggests that the investment strategy of the mothers from the different diet treatments was the same, but HQ mothers had more resources to invest, and put those additional resources into producing larger fry. The maternal effect of differential investment in offspring size as seen here may be considered adaptive if mothers are able to accurately predict the post-natal environment of their offspring.

Within maternal diet groups, male offspring were larger than female offspring, suggesting that mothers may invest more in male offspring. The mechanism for how females would accomplish this differential investment is not clear. Fishes of the genus *Xiphophorus* are lecithotrophic (Constantz 1989), which means that all maternal investment is provided to the eggs in the form of yolk prior to fertilization. An alternative hypothesis is that the larger size of male fry is due to genetic influences that differ between males and females. Evidence from prior studies suggests that there is variation in male growth rate associated with the MC4R genes on the Y chromosome. Males from the smallest size class carry the same alleles at this locus as females, and these smaller males grow slower than the larger courting males in the wild (Bono et al. 2011). Therefore, the difference between the sexes could reflect differences in genetically influenced growth rates that allowed males to reach a larger size at a given age, rather than differences in maternal investment between males and females. Further studies will be necessary to determine if both genetic differences and maternal effects influence the differences detected in the size of male and female fry.
The overall different growth rates between males and females, as well as the different responses of males and females to social condition for the fry from high quality mothers, suggests different optimal growth strategies for the sexes. As with other species of swordtail in the genus *Xiphophorus*, males exhibit determinate growth and cease increasing in size once they have reached sexual maturity (Kallman 1989). Females on the other hand, exhibit indeterminate growth and continue increasing in size after reaching sexual maturity, although growth rate is reduced (Kallman 1989). Since females continue growth past sexual maturity, increased initial size by growing faster will be less influential on female’s size throughout her reproductive life and thus selection to grow fast should be stronger on male offspring. Therefore, even though larger adult size increases reproductive success for both males (Bono 2009; Luo et al. 2005; Ryan et al. 1992) and females (Chong et al. 2004; Tudor and Morris 2009), it might be more important for males to have a faster growth rate prior to sexual maturity than for females.

The difference in growth rate prior to exposure (GR1) suggests that maternal investment influenced growth rate, and that maternal diet and sex of the offspring influenced this investment similar to juvenile size at age 14. There was a positive relationship between mother’s standard length and GR1, which suggests that maternal diet had an indirect effect on growth rate of their offspring. There was a negative relationship between size at age 14 and GR1, which suggests that larger offspring experienced slower growth than their smaller peers. Since larger mothers produced more numerous, smaller fry at size 0, larger mothers typically produced faster growing offspring. There was also a relationship between sex of the offspring and GR1, with male
juveniles growing faster than female juveniles. With male juveniles both being larger, and growing faster than female juveniles, this lends support to the hypothesis that a difference in growth rate between sexes is genetically influenced. These results suggest that it may be adaptive for juveniles that were born at a smaller size into an environment different from their mother’s environment to plastically alter their growth rate; potentially compensating for shortcomings in maternal investment and natal resource availability.

The response to social context depended on a fry’s sex and the maternal resource environment. Across male offspring of both LQ and HQ mothers, treatment individuals grew faster than control individuals, suggesting that males accelerated growth rate in response to exposure, regardless of the maternal investment. Male offspring accelerating growth rate in the presence of an adult male competitor may be adaptive if male size is important in deterring rival males and siring more offspring, which has been shown by previous studies (Luo et al. 2005; Rios-Cardenas et al. 2007), and growth is limited to the juvenile phase. If age at sexual maturity is strongly influenced by the number of Mc4R genes a male carries, then the one way to be a larger adult in response to an environment with large competitors is to grow faster. In addition, the fact that the control males did not grow as fast the treatment males also suggests a tradeoff between growth and some other trait. It would be interesting to determine if males in the exposure treatment show signs of developmental tradeoffs due to the faster growth as compared to their siblings in the control treatment. The interaction between maternal effects and exposure was due to the growth response of the females. Females of LQ mothers increased growth, while females of HQ mothers decreased growth rate in response to exposure to a large courter male.
Female offspring decelerating growth rate in the presence of a potential mate may be adaptive if the female is diverting energy from somatic growth to reproductive effort and investing in gonadal tissue to reach sexual maturity sooner. However, being able to do both (grow faster and invest in reproduction) would be the most adaptive, as females would mature sooner and at a larger size. If both females from HQ and LQ moms were investing more in reproduction when exposed to a male, these results could suggest that females that experienced a different resource environment (diet) than their mothers paid a cost of slower growth when they responded to social context by investing in gonadal growth. If only the HQ females were making this tradeoff, I would predict that females of HQ mothers would reach sexual maturity later but at the same size as females of LQ mothers. However, if the females of both LQ and HQ mothers trade off growth and reproduction, then I would predict that the LQ daughters would use the faster growth rate to mature at a larger size, but later than the HQ daughters. In either case, the differences in the responses between the LQ and HQ daughters suggests that the ability to have a plastic growth rate in response to social context would be adaptive in highly variable environments where natal resource availability is highly unpredictable for the mother. Further work analyzing the impact of growth rate on age and size at maturity for both sexes, as well as the potential costs (i.e. developmental stability) of accelerating or decelerating growth rate, may elucidate a potential trade-offs of plastically altering growth rate (Moller and Manning 2003).

Finally, these results have significant implications for any species that is commercially harvested for human purposes. Most species of fish are harvested for food,
but some, including *Xiphophorus multilineatus*, are coveted by aquaculturists and pet collectors for their brilliant colors, elaborate fins and swords, thrilling mating displays, and overall social affability in a community tank. Aquaculturists especially should take heed of the differences in growth rate between the sexes based on social context and maternal effects; raising juveniles within the appropriate population structure and nurturing mothers with the ideal amount and type of food can maximize growth and offspring number, and in turn profit. These results may also have implications for pet collectors seeking aesthetically pleasing male phenotypes free of fluctuating asymmetry; accelerating the growth rate of juvenile males may carry the associated cost of increased developmental instability leading to differences in vertical body bar number and coloration. Ecologists interested in maintaining operational sex ratios or enhancing the prevalence of one sex may find these results applicable to such a task; the age at which females reach sexual maturity could be influenced by both mother’s diet and exposure to a mature male resulting in earlier maturation. Inducing females to mature earlier could then increase the ratio of reproducing females to males in the population with impacts on mate availability and subsequent population structure.
REFERENCES


Table 1. Linear mixed-effects model of the factors influencing size at time 0 (14 days) with 95% confidence interval (errors shown in parentheses).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Estimate</th>
<th>CI 95% Lower</th>
<th>CI 95% Upper</th>
<th>pMCMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>22.79(3.29)</td>
<td>18.94</td>
<td>26.94</td>
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<tr>
<td>Standard Length(dam)</td>
<td>-0.22(0.07)</td>
<td>-0.30</td>
<td>-0.12</td>
<td>0.0001</td>
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<tr>
<td>Brood size</td>
<td>-0.11(0.03)</td>
<td>-0.15</td>
<td>-0.07</td>
<td>0.0001</td>
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<tr>
<td>Male Offspring</td>
<td>0.30(0.11)</td>
<td>0.07</td>
<td>0.59</td>
<td>0.0162</td>
</tr>
<tr>
<td>LQ Maternal Diet</td>
<td>-1.14(0.52)</td>
<td>-1.80</td>
<td>-0.54</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Table 2. Linear mixed-effects model of the factors influencing growth rate prior to exposure with 95% confidence interval (errors shown in parentheses).

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>CI 95% Lower</th>
<th>CI 95% Upper</th>
<th>pMCMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.68(1.37)</td>
<td>0.38</td>
<td>5.14</td>
<td>0.0254</td>
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<tr>
<td>Standard Length(dam)</td>
<td>0.10(0.03)</td>
<td>0.05</td>
<td>0.15</td>
<td>0.0001</td>
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<tr>
<td>Male Offspring</td>
<td>0.53(0.11)</td>
<td>0.32</td>
<td>0.75</td>
<td>0.0001</td>
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<td>Initial Size</td>
<td>-0.29(0.05)</td>
<td>-0.39</td>
<td>-0.20</td>
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Table 3. Linear mixed-effects model of the factors influencing growth rate subsequent to exposure with 95% confidence interval (errors shown in parentheses).

<table>
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<tr>
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<th>CI 95% Upper</th>
<th>pMCMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.38(0.22)</td>
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<td>0.03</td>
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<td>Male Offspring</td>
<td>0.36(0.28)</td>
<td>-0.19</td>
<td>0.92</td>
<td>0.1958</td>
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<tr>
<td>Treatment(Male Exposure)</td>
<td>-0.50(0.28)</td>
<td>-1.07</td>
<td>0.05</td>
<td>0.0784</td>
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<tr>
<td>LQ Maternal Diet</td>
<td>-0.15(0.29)</td>
<td>-0.68</td>
<td>0.41</td>
<td>0.6204</td>
</tr>
<tr>
<td>Male Offspring:Treatment(Male Exposure)</td>
<td>1.04(0.41)</td>
<td>0.23</td>
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<td>Male Offspring:LQ Maternal Diet</td>
<td>0.54(0.36)</td>
<td>-0.19</td>
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<td>Treatment(Male Exposure):LQ Maternal Diet</td>
<td>0.91(0.36)</td>
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<tr>
<td>Male Offspring:Treatment(Male Exposure):LQ Maternal Diet</td>
<td>-1.05(0.53)</td>
<td>-2.10</td>
<td>-0.01</td>
<td>0.0498</td>
</tr>
</tbody>
</table>
Figure 1. Age at which fry were measured, and a hypothetical relationship between the growth rate prior to exposure (GR1) and the growth rate subsequent to exposure (GR2). Growth rate prior to exposure = , growth rate subsequent to exposure = .
Figure 2. Comparison of the standard length (mm) of mothers raised on two different diets, measured post-partum their second brood.
Figure 3. Influence of (A) maternal size (SL, mm) and (B) brood size on juvenile size at time 0 (14 days). Female offspring of HQ mothers=○ and —, female offspring of LQ mothers=● and ——, male offspring of HQ mothers=△ and ——, male offspring of LQ mothers=▲ and ——.
Figure 4. Influence of maternal size (SL, mm) on juvenile growth rate prior to exposure (GR1). Female offspring=■ and –, male offspring=△ and –.
Figure 5. Estimates (with standard errors) of the interactive influence of maternal diet, treatment type (exposure to adult males), and offspring sex on growth rate. Female offspring of HQ mothers=○ and –, female offspring of LQ mothers=● and ..., male offspring of HQ mothers=△ and –, male offspring of LQ mothers=▲ and ...
Figure 6. The average growth rate of juveniles from isolation (14 days) through 3rd exposure (130 days) as influenced by the interaction of maternal diet, offspring sex, and treatment type (exposure to adult male). Female offspring of HQ mothers in control=○ and —, female offspring of HQ mothers in treatment=○ and …, female offspring of LQ mothers in control=●and —, female offspring of LQ mothers in treatment=●and …, male offspring of HQ mothers in control=△ and —, male offspring of HQ mothers in treatment=△ and …, male offspring of LQ mothers in control=△ and —, male offspring of LQ mothers in treatment=△ and ….