

THE RELATIONSHIP BETWEEN 11-KETOTESTOSTERONE, CORTISOL, AND TESTOSTERONE AND
CONDITION INDEX FOR *Poecilia reticulata*

Shelly Gupta & Brandy G. Kalbach
California Polytechnic State University, San Luis Obispo
Date Submitted: March 2011

Abstract

The guppy, *Poecilia reticulata*, is a common study organism for behavioral and other ecological experiments (Houde, 1997). As far as we know, no previous data have been compiled regarding hormones and condition index of guppies. In this experiment we examined the relationship between circulating levels of three hormones (testosterone, 11-ketotestosterone and cortisol) and condition index in males and also made comparisons among the hormone levels, for three guppy sites from the northern range of Trinidad – Aripo River, Marianne River and Small Crayfish River. Hormone samples were obtained using non-invasive water borne techniques and quantified by our collaborator. The condition index of guppies was calculated as the ratio of mass per length³. We predicted the following relationships: positive relationships between testosterone (T) and 11-ketotestosterone (11KT), condition index (CI) and 11KT, and CI and T; negative relationships between 11KT and cortisol (C), T and C, and CI and C. We found: a positive relationship between 11KT and C, 11KT and T, and C and T for the Small Crayfish River population. Aripo River and Marianne River populations displayed no significant relationship between 11KT and C, and 11KT and T. The Aripo River population showed a significant positive relationship between C and T, but the Marianne River did not show a significant relationship. Overall, there were no relationships between any of the hormones and CI for any site. Our findings indicate that there may be genetic differences among the populations indicated by the hormone differences. Further experimentation is necessary for more comprehensive understanding of the functions of these hormones in the guppies.

Introduction

Hormonal effects on morphological and behavioral characteristics have been widely investigated in several taxa (e.g. cichlids, Antunes & Oliveira, 2009; Guppies, Clemens et al., 1966; Eastern fence lizards, Cox et al., 2005; cichlids, Early et al., 2006; male bluegills, Kindler et al., 2006; Japanese eels, Ozaki et al., 2006). Androgens, such as testosterone (T), have been linked to the production of traits such as body coloration, which can either be intensified or reduced with increasing levels of androgens, depending on the organism (Sköld et al., 2008). Body size, in particular, seems to be variable depending on the species, as seen in gorillas and

chimpanzees (Leigh & Shea, 1996) and the individual's life history stage (Calder III, 1984). Androgens, namely testosterone, affect size, growth, aggression, and the production of other hormones in a variety of organisms (humans, Harrison et al, 2000; *Sceloporus* spp., Cox & John-Alder, 2005; rats, Huag & Guatvik, 2011). 11-ketotestosterone (11KT) is a potent androgen in fish along with testosterone (Cavaco et al., 2001), that induces spermatogenesis (African catfish, Cavaco et al., 2001; Japanese eel, Ozaki et al., 2006), whereas testosterone has been shown to influence pituitary gonadotroph development in African catfish (Cavaco et al., 2001). Cortisol, a stress hormone (Marieb & Hoehn, 2010) can be examined as a measure of response to stress (Cohen et al., 1997), as well as being considered as a causative or inhibiting agent to other hormones (Marieb & Hoehn, 2010). In general, hormones regulate the metabolic function of other cells in the body (Marieb & Hoehn, 2010) and so may have important influences on body size.

In sexually dimorphic species in which the males are larger than the females, males sometimes use physical combat for securing access to mates (Marieb & Hoehn, 2010; Andersson, 1994); larger size is sometimes advantageous under sexual selection (Kraak & Bakker 1998). The victors of aggressive competitions for mates are those that are more aggressive, a trait correlated with high levels of testosterone (Antunes and Oliveira, 2009). In *Poecilia reticulata*, large males generally compete to court females, while smaller males perform sneak copulations (Bisazza et al., 2001). Females show a preference for larger males, larger ornaments and high courtship activity (Bisazza et al., 2001; Houde, 1997).

T is a primary male sex hormone that influences physical appearance and behavior in several ways. T levels have been linked with body length and mass in eastern fence lizards (*Sceloporus undulates*) inhibiting growth in juvenile males (Cox et al., 2005), and T in brown

anoles increases growth rate (Cox et al., 2009). T may affect growth rate by directly affecting growth hormone (GH) synthesis, as it does in the pituitary glands in goldfish (Holloway and Leatherland, 1998). In humans, it is known that T production and release are controlled by the luteinizing hormone (LH) of the anterior pituitary gland (Marieb & Hoehn, 2010). During puberty, an increased amount of LH is released, which increases the amount of T. T influences growth hormone (GH) release, which increases bone length, bone width and muscle mass. Thus, testosterone has an indirect positive effect on body size in humans (Marieb & Hoehn, 2010). Androgens also cause aggression either by reducing “activation of the neural pathway of impulse control and self-regulation” (humans, Mehta & Beer, 2009) or by preparing the male to fight (e.g. eastern fence lizards, *Sceloporus undulatus* Cox et al., 2005; brown anoles, *Anolis sagrei*, Cox et al., 2009; cichlid fish, *Oreochromis mossambicus*, Antunes & Oliveira, 2009)., Increased levels of T also positively correlate with aggressive behaviors in cichlid fish (Antunes & Oliveira, 2009). Clemens et al. (1966) found that immature guppies fed fish food mixed with T had a larger anal fin-to-snout length compared to control guppies prior to maturity; however, after reaching maturity, the T-enhanced guppies exhibited a smaller anal fin-to-snout length than the control guppies. Thus the amount of T found in the guppies must vary throughout its life span.

The fish-specific androgen, 11-ketotestosterone (11KT), is also an important hormonal mediator of spermatogenesis (African catfish, Cavaco et al., 2001; Japanese eel, Ozaki et al., 2006), and has been studied in sticklebacks and bluegill sunfish (stickelback, Oliveira et al., 2001a; bluegill sunfish, Magee et al., 2006). T is a precursor of 11KT (Idler et al., 1963), and in some cases, such as during pre-spawning stages in male bluegill sunfish, both T and 11KT positively correlate with each other (Magee et al., 2006).

Cortisol (C) is a steroid hormone (Marieb & Hoehn, 2010) primarily released under stressful conditions (Marieb & Hoehn, 2010) and has not been found to influence physical attributes. For example, coloration had no significant correlation with C levels in frigatebirds (Osorno et al, 2010). This cannot be generalized to other species. Body size may influence cortisol levels, rather than the other way around; however, in rainbow trout, plasma cortisol levels were not correlated with body size (Yue et al., 2006).

The guppy has been an ideal organism of study across several disciplines, ranging from ecological genetics to behavior (Houde, 1997). Environmental effects on behavior and life history evolution have been studied in the past; however, hormones have not been widely investigated in this species. Though males have not been selected to be larger than females, size may be an important factor among males during male-male competition (Rodd & Sokolowski, 1993). Guppies are native to the freshwater rivers of Trinidad and Tobago (Houde, 1997), which is where the guppies in our study originated from.

In the present study, we investigated fish sites originating from three sites in northern Trinidad: Aripo, Marianne, and Small Crayfish rivers. We used water-borne technique to collect circulating levels of the hormones, T, 11KT, and C, and measured the condition index (mass to length-cubed ratio). We determined if there was a correlation between the condition index of the fish and C, 11KT and T levels. Finally, we investigated relationships among the hormones – C and 11KT levels, 11KT and T levels, and C and T levels.

Because T is a precursor of 11KT (Idler et al., 1963), and T levels are proportionally related to growth hormone, we predicted that T and 11KT would be positively correlated, and will also be positively correlated with condition index. Glucocorticoids, such as cortisol, have been shown to directly inhibit the levels of androgen hormones, such as testosterone (Welsh et

al., 1982). Therefore, we predict that there will be a negative correlation between C and 11KT, and between C and T. Furthermore, since C inhibits T (Welsh et al., 1982), we predict a negative relationship between C and condition index.

Materials and Methods

Maintenance of Poecilia reticulata

Guppies were obtained from the Aripo, Marianne and Small Crayfish Rivers of Trinidad and Tobago and initially kept in 10-gallon stock tanks at California Polytechnic State University, San Luis Obispo. There were three stock tanks for each site, from which guppies were selected for experimentation. There was no interbreeding among the sites before or throughout the experiment. Initially, adult females and a male were removed from stock tanks and placed in 2-gallon tanks to breed. The adults were fed twice a day on weekdays and once a day on the weekend with a small feeding of either *Tetra*® Tetracolor Tropical Flakes, brine shrimp, spirulina, or bloodworms. First feeding was between 9 a.m. and 12 p.m., and the second feeding was between 3 p.m. and 9 p.m. After a period of approximately two weeks the babies were removed from the adult tanks and put into another 2 gallon tank where they were fed HBH® Baby Bites, until they became big enough to eat the adult food.

The guppy lab room was maintained at a constant 78°F temperature to simulate the tropical environments of Trinidad and Tobago. Water was regularly made and contains deionized water, pH down, pH stable, RO Right, and Start Right. The 10-gallon tanks were cleaned out biweekly, and the 2-gallon tanks were cleaned weekly.

Breeding of Poecilia reticulata

Females from a given site were randomly selected and isolated into individual 2-gallon tanks to breed with a male from the same site. Post birth, the babies were kept in the mother's tank for approximately two weeks before being placed in their own individual 2-gallon tank at densities of 2-6 fish per tank; if a litter exceeded this amount, the babies were split into separate tanks. The tanks were labeled with the site tag, mother's identification, litter number, and approximate date of birth. Once sex of the babies could be distinguished they were separated by sex into new 2-gallon tanks until further selection into home tanks. Sex was determined using the visual cues of coloration and gonopodium development.

Home Tank Isolation and Focals

Home tanks contained males from the same site but potentially from different litters at densities of one to four fish per tank. Each tank was designated a number and within each tank the fish were labeled W, X, Y, or Z. For the remainder of the experiment each fish was identified by its tank number and corresponding letter. Each guppy's color patterns were sketched in order to identify males in subsequent observations.

Home Tank Focal Observations

After the guppies were identified, home tank observations were conducted. The observer stood about five feet from the tank and the guppies were given a two-minute acclimation period where the observer watched the fish. Each focal male was observed for 5 minutes. The behavioral observations were recorded on data sheets. Behavior included sequence of behavior events as well as tallying the number of each event. Events recorded included displays, chases, bites, lateral displays, and face-offs. This was done at three times of day: between 9 am and 12

pm, between 2pm and 4pm and between 6pm and 9pm. These data were collected as part of a larger experiment.

Hormone Collection

These methods follow Earley, et al 2006. Following the third observation session, hormone samples were collected from each male, using a non-invasive water borne technique (Earley, et al., 2006). Two beakers were filled with water, one meant for a male guppy, the other as a control. The water was the same as the water used in all the tanks, and was treated to achieve appropriate pH, temperature and hardness. The control water was used to obtain a background hormone reading for hormones that may have been present in the water. One male guppy was selected from the home tank and placed in its designated beaker, and hormones were allowed to exude into the water for 60 minutes. The samples were stored in 200mL aliquots at -80°C immediately following collection, until extraction.

Obtaining Guppy Measurements

After hormone collection each guppy was sedated using MS 222, Tricaine Methane Sulfonate (Sigma®) to obtain length and weight measurements. The standard length was recorded using digital calipers to three significant figures, measuring from the tip of the snout to the caudal peduncle line. Mass was obtained using a digital balance to three significant figures. After measurement, the fish was returned to a general site-specific stock tank. Condition index for guppies is the ratio of the mass to length-cubed (g/mm^3).

Hormone Extraction

The samples were allowed to thaw at room temperature and filtered through Watman filter paper to remove particulate contaminants. 11KT, C, and T were extracted with C18 solid phase extraction columns using a 24-port vacuum manifold. The hormones were eluted through the columns with methanol and deionized water, leaving a pellet of hormone. These samples were labeled, frozen and sent to Dr. Ryan Earley's lab at the University of Alabama for hormone quantification using enzyme immunoassays.

Data Analysis

Levels of 11KT, C, and T were obtained from Dr. Ryan Earley in units of pg/mL. Correlation statistical analysis was performed in order to determine if there was any relationship between the condition indices and each hormone, as well as among hormones. The data was analyzed according to differing river sites (Aripo River, Marianne River and Small Crayfish River). Log transformed values of the hormone levels were used for analysis using JMP Version 7.0.1 statistical software. Results differed from predictions made (Table 1).

Results

Multiple pairwise Pearson's correlations were performed in order to determine if there was any relationship between condition index and each hormone, as well as among hormones. There were no significant relationships found when the guppy sites were grouped and compared to the hormones; therefore, data was also analyzed by individual site (Aripo River, Marianne River and Small Crayfish River). The corrected values of hormone levels were used for all analysis (log-transformed values). Results differed from predictions made (Table. 1).

Table 1: Predicted and actual results for condition index and hormone data and among hormones. Left side indicates predictions, and right side (italics) indicates results. (“+” indicates a significant relationship; “-” indicates a negative relationship; Site abbreviations are: Aripo River (AR), Marianne River (MR) and Small Crayfish River (SCR). “NS” indicates non-significant correlation.)

	11-Ketotestosterone	Cortisol	Testosterone	Condition Index
11-Ketotestosterone		+: SCR, NS: AR, MR	+: SCR, NS: AR, MR	NS
Cortisol	-		+: AR, SCR, NS: MR	NS
Testosterone	+	-		NS
Condition Index	+	-	+	

The Relationships Between Hormones and Condition Index

11-Ketotestosterone: There was no significant relationship between the condition index and 11KT levels for any guppy site (Fig. 1).

Cortisol: There was no significant relationship between the condition index and C levels for any site (Fig. 2).

Testosterone: There was no significant relationship between the condition index and T levels for any site (Fig. 3).

The Relationship Among the Hormones

Cortisol and 11-Ketotestosterone: The Aripo and Marianne River sites had no significant relationship between C levels and 11KT levels. On the other hand, Small Crayfish River showed a significant relationship between the levels of C and 11KT (Fig. 4).

11-Ketotestosterone and Testosterone: The Marianne River site had no significant relationship between 11KT and T levels. On the other hand, there was a significant relationship between 11KT and T for the Aripo and Small Crayfish River sites (Fig. 5).

Cortisol and Testosterone: The Aripo and Marianne River sites had no significant relationship between C levels and T levels. On the other hand, Small Crayfish River showed a significant relationship between the levels of C and T (Fig. 6).

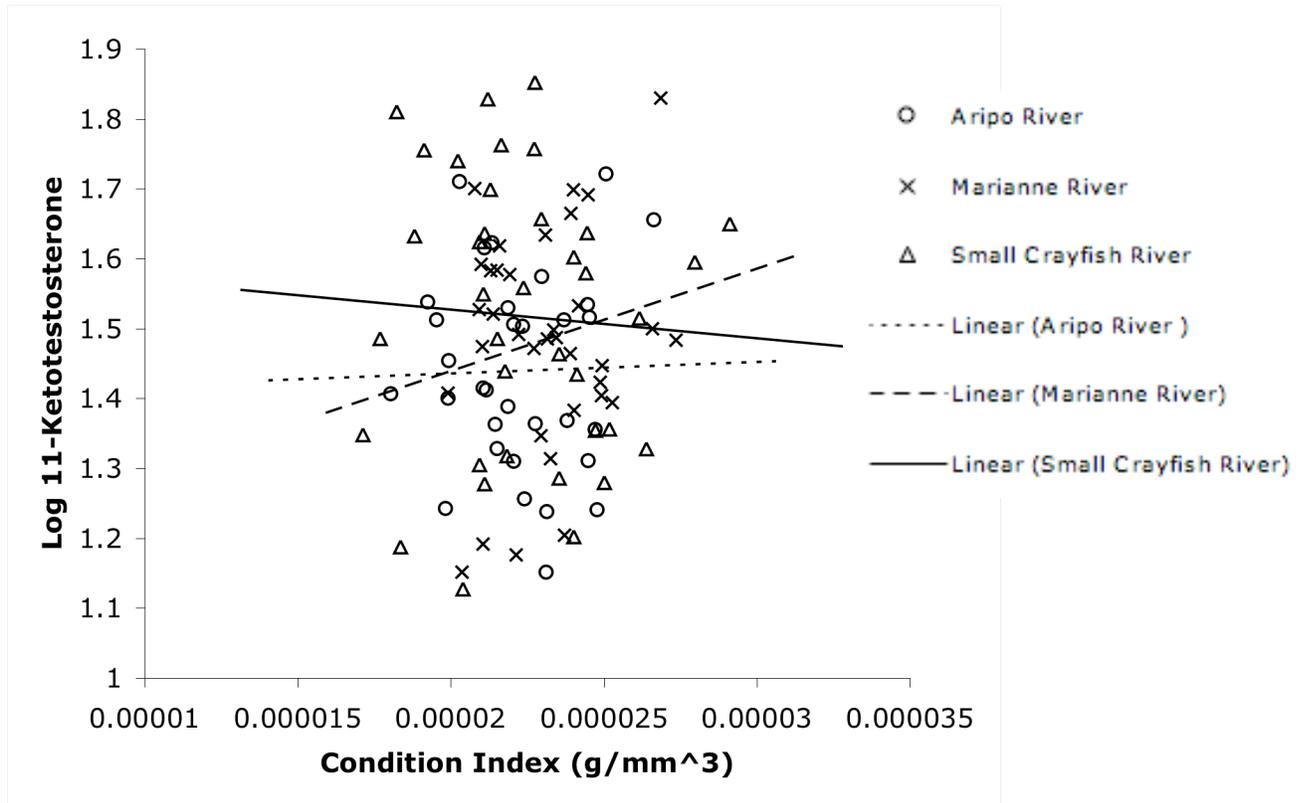


Figure 1: The relationship between condition index and log 11-ketotestosterone levels for the three sites. There was no significant relationship between condition index and 11KT levels for any site. (Aripo River – $r^2 = -0.0328$, $F_{stat} = 0.0156$, $df = 31$, $p\text{-value} = 0.901$; Marianne River – $r^2 = 0.0019$, $F_{stat} = .064$, $df = 34$, $p\text{-value} = 0.310$; Small Crayfish River – $r^2 = -0.0252$, $F_{stat} = 0.116$, $df = 36$, $p\text{-value} = 0.734$).

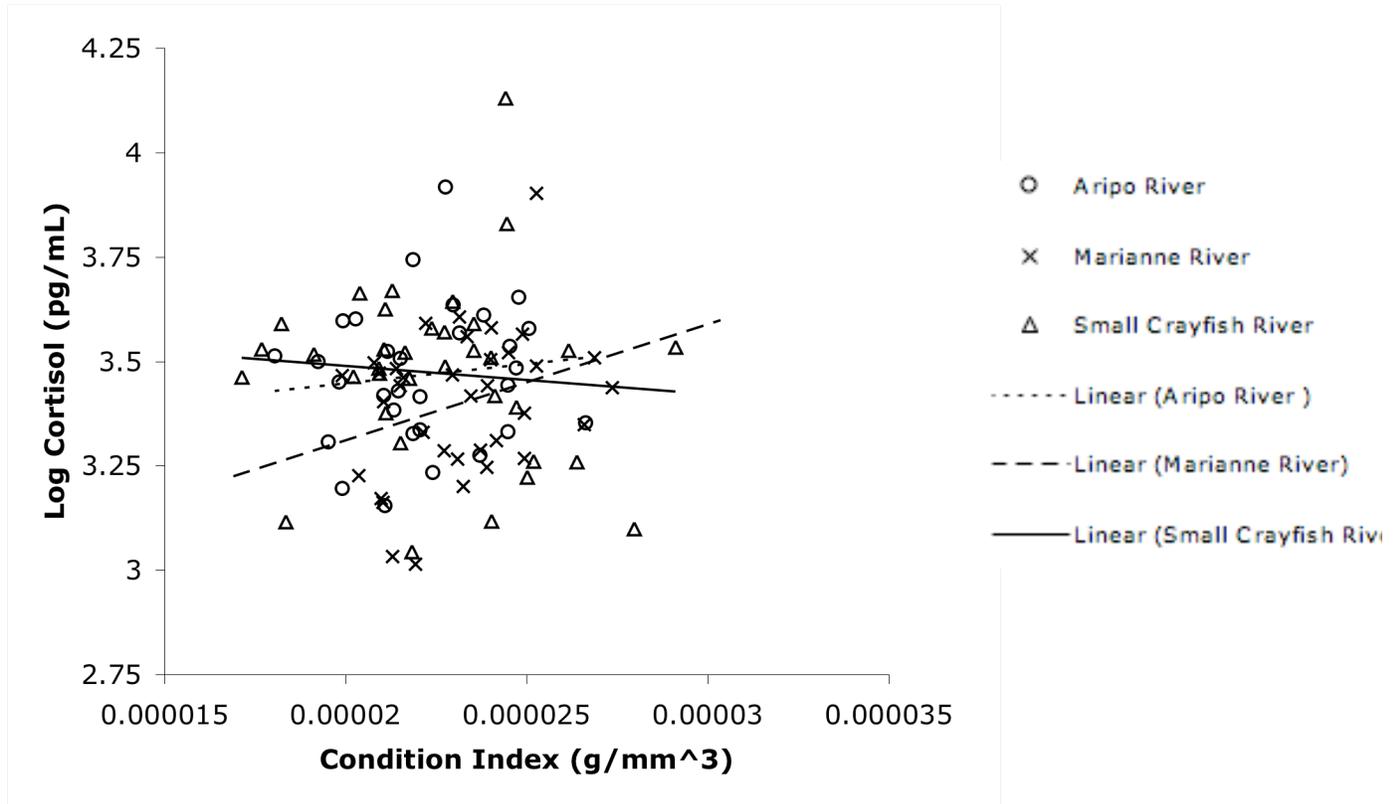


Figure 2: The relationship between condition index and log cortisol levels for the three sites. There was no significant relationship between condition index and C levels for any site. (Aripo River – $r^2 = -0.0218$, $F_{\text{stat}} = 0.381$, $df = 29$, $p\text{-value} = 0.542$; Marianne River – $r^2 = 0.0639$, $F_{\text{stat}} = 3.389$, $df = 35$, $p\text{-value} = 0.0744$; Small Crayfish River – $r^2 = -0.0223$, $F_{\text{stat}} = 0.258$, $df = 35$, $p\text{-value} = 0.615$).

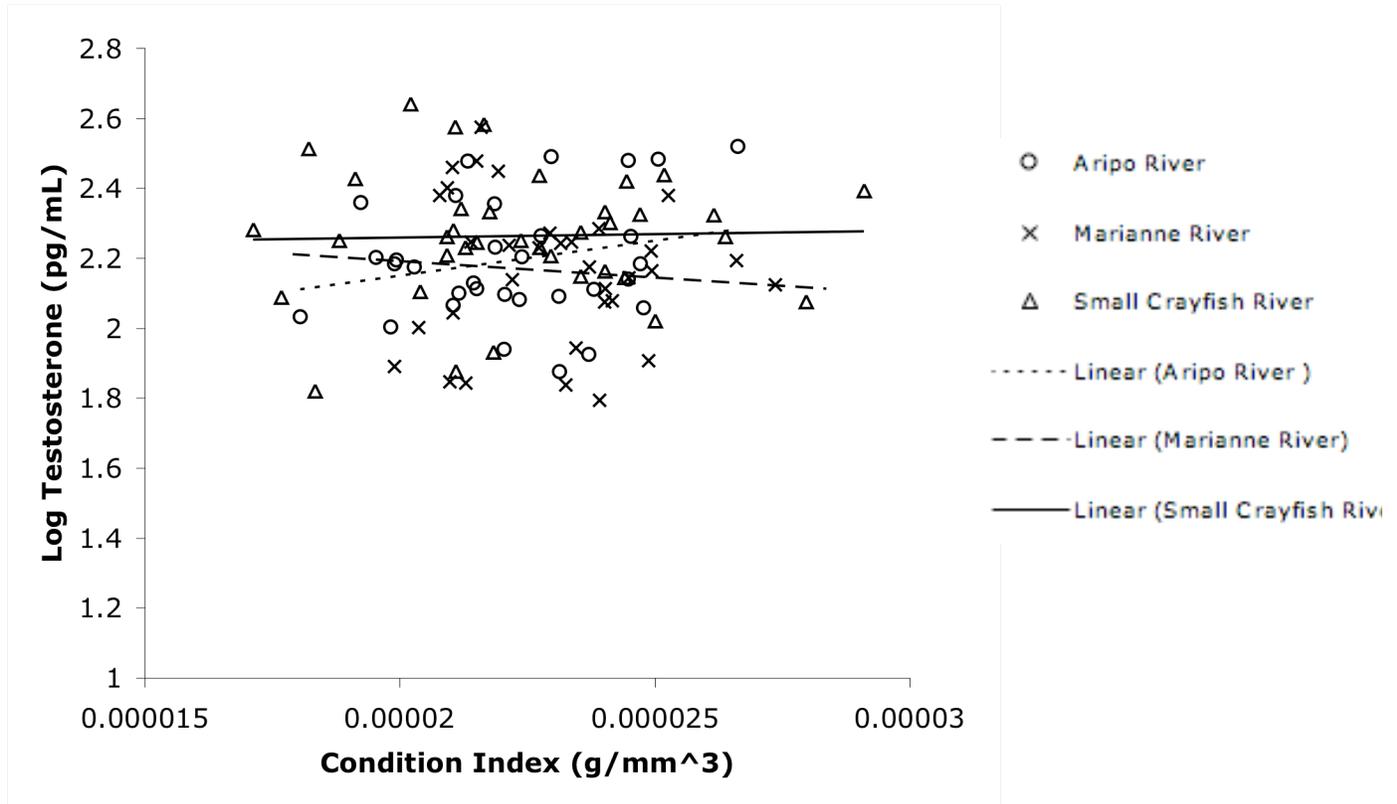


Figure 3: The relationship between condition index and log testosterone levels for the three sites. There was no significant relationship between condition index and T levels for any site. (Aripo River – $r^2 = 0.0209$, Fstat = 1.66, df = 31, p-value = 0.207; Marianne River – $r^2 = -0.0251$, Fstat = 0.216, df = 32, p-value = 0.645; Small Crayfish River – $r^2 = -0.0277$, Fstat = 0.0297, df = 36, p-value = 0.864).

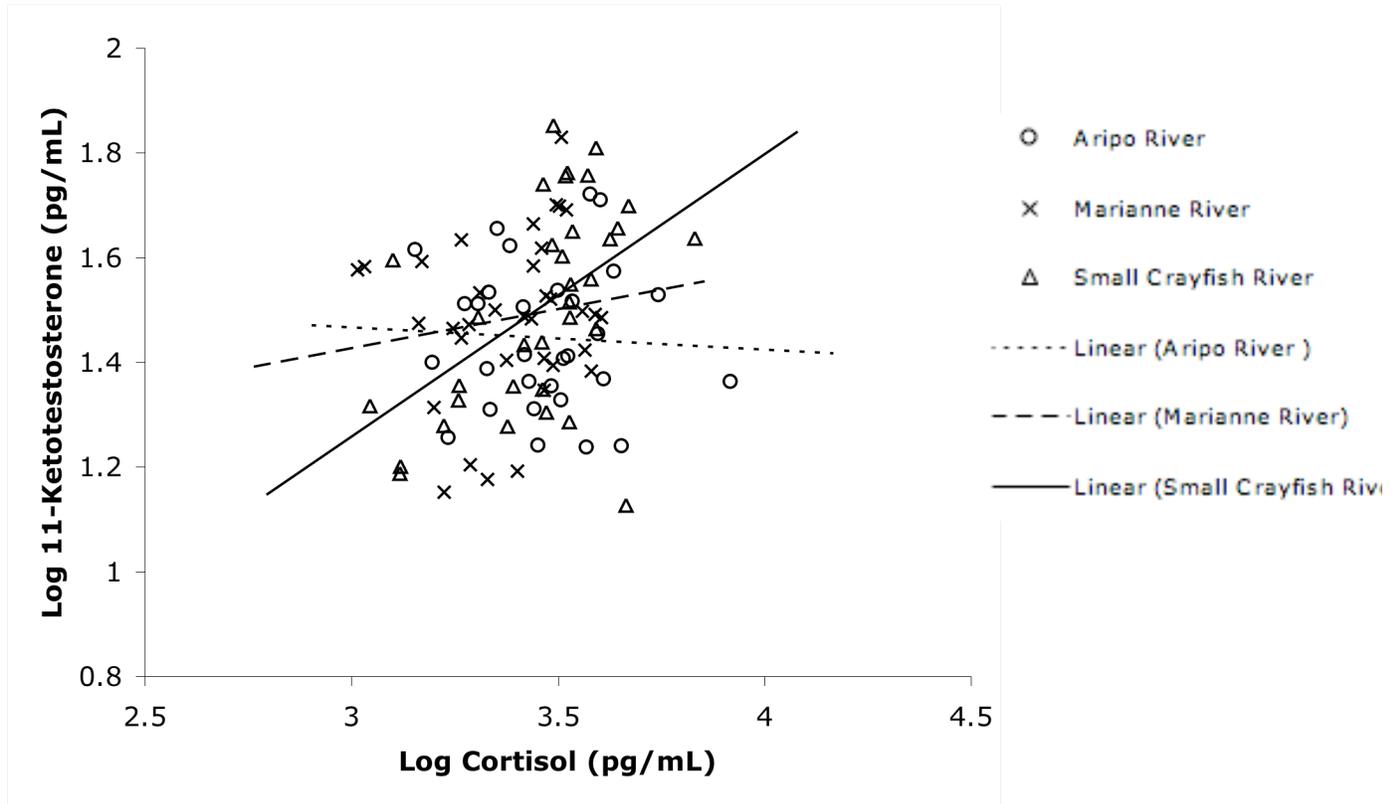


Figure 4: The relationship between cortisol and 11-ketotestosterone levels for the three sites. There was no significant relationship found between the hormones for the Aripo and Marianne River sites (Aripo River – $r^2 = -0.033$, $F_{stat} = 0.0732$, $df = 29$, $p\text{-value} = 0.0732$; Marianne River – $r^2 = -0.0077$, $F_{stat} = 0.739$, $df = 34$, $p\text{-value} = 0.396$). There was a significant relationship found between the hormone levels of the Small Crayfish River site (Small Crayfish River – $r^2 = 0.0223$, $F_{stat} = 10.5$, $df = 33$, $p\text{-value} = 0.003$).

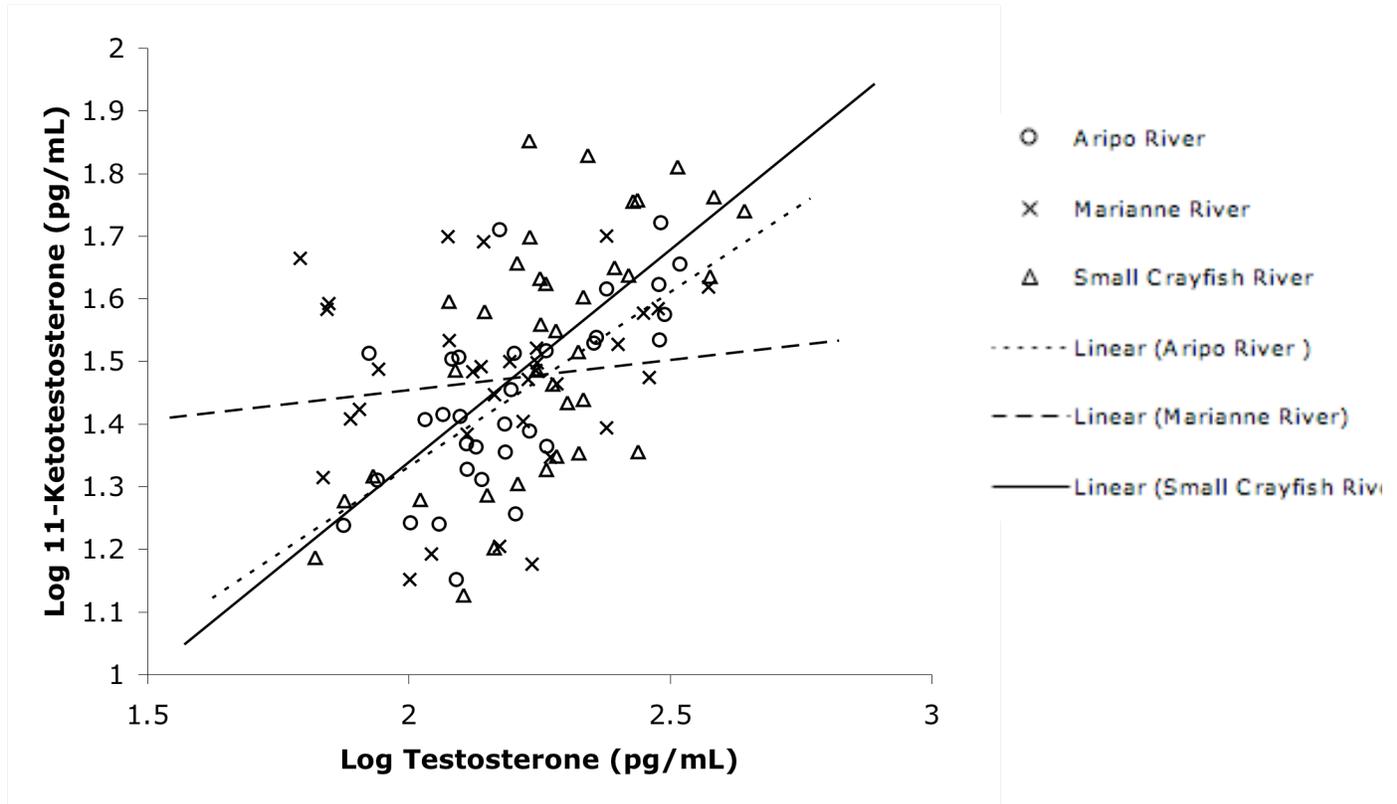


Figure 5: The relationship between 11-ketotestosterone levels and testosterone among the three sites. There was no significant relationship found between the hormone levels of the Marianne River site (Marianne River – $r^2 = -0.0134$, $F_{stat} = 0.576$, $df = 32$, $p\text{-value} = 0.434$). There was a significant relationship found between the hormone levels of the Aripo and Small Crayfish River sites (Aripo River – $r^2 = 0.433$, $F_{stat} = 24.66$, $df = 31$, $p\text{-value} < 0.0001$; Small Crayfish River – $r^2 = 0.378$, $F_{stat} = 22.9$, $df = 36$, $p\text{-value} < 0.0001$).

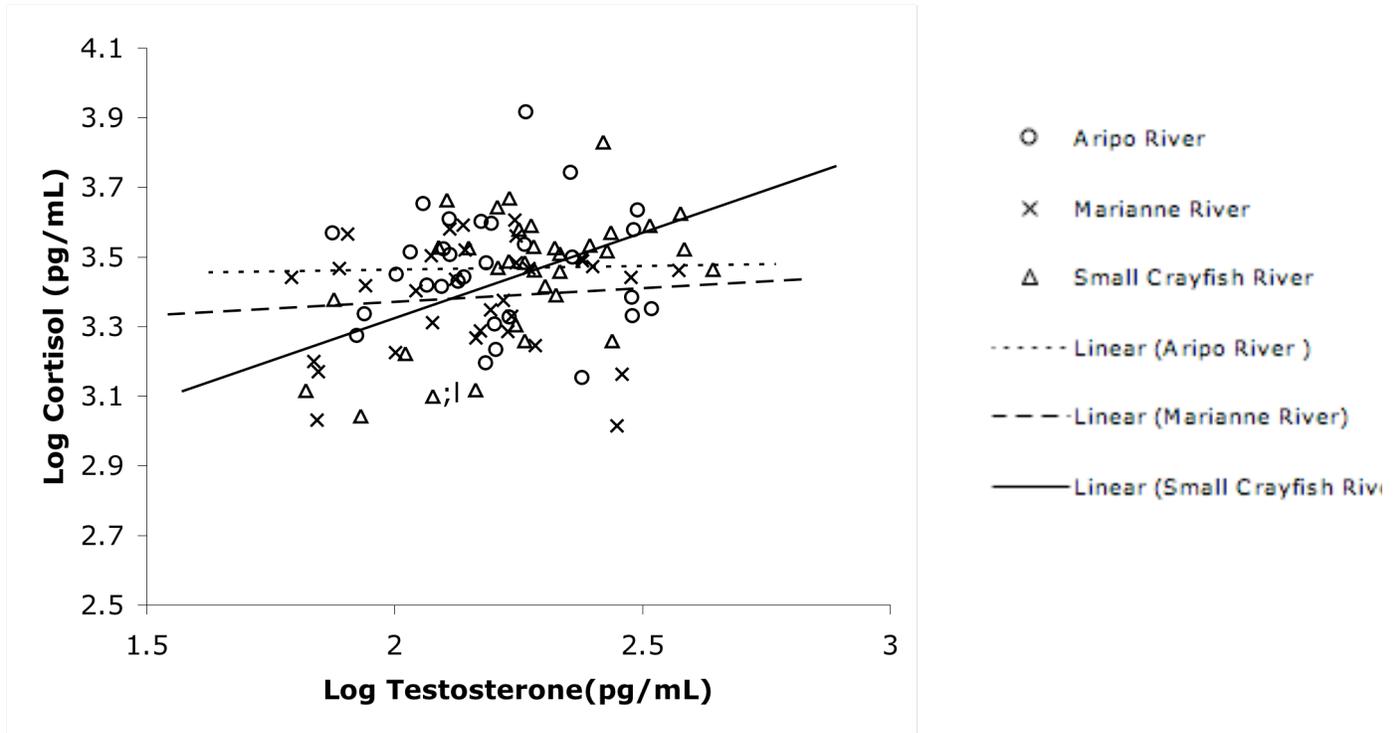


Figure 6: The relationship between cortisol levels and testosterone among the three sites. There was no significant relationship found between the hormone levels of the Aripo and Marianne River sites (Aripo River – $r^2 = -0.0352$, $F_{stat} = 0.0137$, $df = 29$, $p\text{-value} = 0.908$; Marianne River – $r^2 = -0.021$, $F_{stat} = 0.342$, $df = 32$, $p\text{-value} = 0.563$). There was a significant relationship found between the hormone levels of the Small Crayfish River site (Small Crayfish River – $r^2 = 0.240$, $F_{stat} = 11.40$, $df = 33$, $p\text{-value} < 0.0019$).

Discussion

Poecilia reticulata is a model species for behavioral and ecological research (Houde, 1997); however, previous studies of endocrine relationships in guppies have not been studied thoroughly. In the present study we were interested in seeing if there was a relationship between condition index and hormones, and among the different hormones. There was no relationship found between the hormones and condition index. On the other hand, one river, the Small Crayfish, showed the expected positive relationship with 11-ketotestosterone (11KT) and testosterone (T) and an unexpected positive relationship between 11KT and cortisol (C). However, interestingly, the Small Crayfish River and Aripo River sites showed an unexpected positive relationship between C and T, while there was no relationship seen for C and T of the Marianne site. Furthermore, Aripo River and Marianne River showed no relationship between C and 11KT, T and 11KT. This variation among sites in hormone levels could be attributed to genetic variation (Shrey, et al., 2011).

The Relationship between Hormones and Condition Index

11-Ketotestosterone, Testosterone

T has been shown to influence both bone and skeletal muscle growth in humans and other organisms (Marieb & Hoehn, 2010). Specifically, in humans, a three-fold increase in growth hormone (GH) secretion was found with an increase in gonadal steroid hormones (including testosterone) during puberty (Meinhardt & Ho, 2006). When it comes to the concentrations and localization of T, Barannikova et al. (2002) found that T was higher in both plasma and gonadal tissues in sturgeons. However, in most bony fish, 11KT has been found at higher plasma concentrations than T (Barannikova et al., 2002). In fish, 11KT is an important androgen

associated with spermatogenesis (Cavaco et al., 2001), gonadal masculinization and sperm production (Nakamura, 1981). Because there is variability in the importance and roles that T and 11KT play in different fish species (e.g. guppies, Takahashi, 1975; Mozambique tilapia, Nakamura, 1981), it was important to measure both hormones in this study. According to Bouyer et al. (2008), gonadal steroids stimulate GH secretion more in females than in males in sexually dimorphic transgenic mice. Similar to the transgenic mice, guppies are a sexually dimorphic species, in which the females are larger than the males (Houde, 1997). In the transgenic mice, females had an increased expression of receptors on GHRH neurons compared to males, allowing more release of GH into the system (Bouyer et al., 2008). Therefore, we expected to find a positive relationship between CI and T or 11KT. However, in the three sites of guppies we observed, no relationship was found between condition index and any of the hormones. We speculate that the male guppies, like transgenic mice (Bouyer et al., 2008), have fewer receptors on their GHRH neurons, and therefore are not as affected by GH via T stimulation as females may be. The effect of T on female guppy CI should be investigated in future studies.

Another explanation for the lack of relationship between androgens and CI in male guppies is that male guppies do not necessarily need to be selected for and therefore invest their T for other purposes like gonopodium development, especially given that it has been found that females prefer males with larger gonopodium (Langerhans et al., 2005). Specifically for 11KT, an important androgen that is associated with spermatogenesis in fish (Cavaco et al., 2001), CI and 11KT may have shown no significant relationship because the guppies we used were already mature adults at the time of hormone collection, and therefore 11KT may no longer play a significant role. In order to more thoroughly understand the relationship between these two

androgens and condition index in guppies, future research should examine the correlations during development.

Cortisol:

C and CI were found to have no relationship in the guppies. Cortisol is a stress response hormone that has been negatively correlated with size in some species, including some fish (juvenile rainbow trout; Barton et al., 1987). Juvenile rainbow trout were continuously fed cortisol for 10 weeks while another group was chronically stressed via direct handling; both groups had reduced growth (Barton et al., 1987). However, Yue et al., (2006) found that different-sized rainbow trout did not display differing plasma cortisol levels. The experiment involved handling and movement of the fish from one location to another, so the initial stress of being displaced may have been masking any potential effect of body size (Yue et al., 2006). Displacement of fish was also involved in our study. Moving the guppies from their home tanks into temporary hormone collecting beakers may have caused a strong spike in cortisol that is not representative of baseline levels in the absence of direct handling. Future experiments should look to minimize handling stress of the guppies.

*The Relationship Among the Hormones***Cortisol vs. 11-Ketotestosterone**

There was a positive relationship between C and 11KT. Our prediction was based on the fact that T is the precursor for 11KT (Idler et al., 1963) and if the relationship between C and T is expected to be negative (Welsh et al., 1982) then the relationship between C and 11KT will probably be negative. However, we speculate the positive relationship between the hormones is indicative of variation of the function of T and 11KT as discussed in more detail in later sections. There may be a genetic benefit to having 11KT and C correlate positively depending on its function. Ozaki et al. (2006), found that cortisol does not have a direct effect on spermatogenesis, but cortisol induced DNA replication in spermatogonia of Japanese eel, which potentiates 11KT levels and caused spermatogonial proliferation (Ozaki et al., 2006). Thus, C and 11KT could be seen as a positive correlation, since C may be indirectly assisting 11KT. Increased stress causes an increase in cortisol levels (Martinez-Porchas et al., 2009). We also speculate that when organisms are displaying aggressive behavior they could be under stressful conditions to maintain their territory or for gaining access to a female, which would indicate positive up-regulation of cortisol and androgens simultaneously. In another study conducted with sticklebacks (Siebre et al., 2007), no correlation was found between C and 11KT. Therefore, the function seems to be a determining factor in understanding if there is a relationship between 11KT and C.

11-Ketotestosterone vs. Testosterone

As expected, a positive relationship was seen between 11KT and T for the guppies in our study. We expected a positive correlation because T is the precursor for 11KT (Idler et al., 1963). According to the “challenge hypothesis” (Wingfield et al., 1990), androgens levels

increase in accordance with social interaction. In other studies, the exact function of each hormone has been determined, and different relationships between 11KT and T have been found (eg Cavaco et al., 2001; Kindler et al., 1989). A study of African catfish (Cavaco et al., 2001) showed the effects of T and 11KT on juveniles – T treatment stimulated gonadotroph activity, while 11KT treatment drove sperm formation. However, when 11KT and T were combined, the stimulatory effect of 11KT was inhibited (Cavaco et al., 2001). Kindler et al. (1989), analyzed T and 11KT concentrations in male bluegills, *Lepomis macrochirus*, and found that the concentration changes were due to seasonal variation. T was found to be associated with spermatogenesis periods and gonad development, while 11 KT was correlated to periods of behavior displayed by spawning parental males (Kindler et al., 1989). Thus, the roles of 11KT and T do not have concrete functions across all species. Therefore, since the effects of 11KT and T can be antagonistic in some situations and positively related in others, elucidating the nature of the relationship in *Poecilia reticulata* requires further testing.

Cortisol vs. Testosterone

Previous research has indicated that there is a negative correlation between C and T (Welsh et al., 1982), as we had also predicted. However, we found a positive relationship between C and T. Brownlee et al. (2005) looked at the relationship between T and C in humans at rest and post exercise. A negative relationship between T and C was found post exercise; however, there was no correlation between T and C for the subjects at rest (Brownlee et al., 2005). Therefore, it seems that the condition and environment may be indicative of the hormone levels. Moreover, Strier, et al. (1998), performed a study analyzing the fecal matter of marquis (*Brachyteles arachnoids*) in Brazil for T and C levels. Their results indicated differences among C and T when compared to the copulatory period, season and age; however, all males exhibited

high levels of cortisol following a slight elevation in T levels (Strier, et al., 1998). Strier, et al. (1998) attributed this to the low levels of aggression for mating choice seen for this species. Therefore, we can speculate that there are cases that indicate a positive correlation between C and T, but, as opposed to the marquis, our results cannot be attributed to low aggression since guppies have been shown to compete for mates through high levels of aggression (e.g. Houde 1997; Kolluru & Grether, 2005). Thus, there must be an alternative answer to the positive correlation that was seen.

A positive relationship could be indicative of endocrine up-regulation for the guppies due to handling techniques (Martinez-Porchas, et al., 2009). As stated in our methods, the guppies were removed from their home tanks where there were other males. An aggression behavior study was being conducted simultaneously to our present study, thus we know that within the home tanks, male guppies were competing and showing displays on a regular basis (Kolluru, G.R.; personal comment, 2011). Wingfield, et al. (1990), coined the term “the challenge hypothesis” to define the relationship between T and aggression – androgen production is stimulated by social interaction. Their findings indicated that T levels are most involved with aggression associated with reproduction (Wingfield et al., 1990). T levels are strongest in reproductive aggression in situations of social instability (Wingfield et al, 1990). Also, research with cichlid fish performed by Oliveira et al. (2001b), concluded that bystander fish also exhibit rises in androgen hormones; thus, it can be assumed that the fish that may not be physically aggressive but are still in the same tank would also have a rise in androgen hormones. The guppies in the home tanks that were behaving aggressively in their home tanks are speculated to have increasing T levels. The behavioral data study indicated that majority, if not all, of the

guppies showed aggressive behavior; thus, we can speculate that the increased T levels are due to the home tank conditions.

When the guppies were removed from the tanks, they were placed directly into beakers of fresh water and left for sixty minutes, to acclimate and exude hormones. This initial collection was our sample taken and analyzed; however, other samples were also measured following behavior research conducted for the other study, but it was not analyzed for our purposes. This unpublished data shows that the cortisol and testosterone levels decreased throughout the day (Kolluru, G.R.; personal comment, 2011). Therefore, it can be speculated that handling techniques and a new environment can attribute to stressful conditions for fish (Martinez-Porchas et al., 2009) and can lead to an increase in cortisol levels.

Individually in the two situations, T up-regulation due to aggression and C up-regulation due to stress, could have caused us to witness the unexpected positive correlation between cortisol and testosterone. However, this could also be due to different intrinsic or extrinsic factors that may upregulate hormones (Martinez-Porchas et al., 2009), which would require further analysis for the guppies.

Variation among sites

Close-related species often show a variety of similarities (Endler, 1995). Differences were witnessed among the sites between hormone relationships (Table 1). These differences can be attributed to genetic variation (eg Houde, 1997; Endler, 1995; Shrey et al., 2011; Hurt et al., 2008). Endler (1995) reviewed the variations found within the *Poecilia reticulata*. Endler (1995) described that traits of the guppies covary with one another, with predation and other extrinsic factors. There was also evidence that there was geographical variation in behavior and

morphology of the guppies. Some influences of differences within the species are the location of the rivers and the amount of human disturbance. Over time, this can influence the genetics of the site in the river system and alter the characteristics of the fish. This may have been witnessed within our study – specifically regarding the guppies of the Small Crayfish River site. This site showed positive correlations among all the hormones observed (between C and 11KT, 11KT and T, and C and T). We speculate that the Small Crayfish River site may be showing signs of speciation because the fish showed consistent endocrine up-regulatory activity that the other sites did not show. Aripo River also exhibited an indication of a positive relationship between C and T, which could be attributed to the geographical differences of the rivers. Small Crayfish and Aripo River are smaller and rivers with a slower current than the Marianne River, which is deeper and has a faster current (Kolluru, G.R.; personal comment, 2011). We speculate that the fish in the Marianne River may not interact with the other guppies or other competitors as often or regularly because they are in constant motion. Further investigation is necessary to measure the degree, if any, of genetic variation hormone upregulation trait among the sites. Shrey et al. (2011) showed that introducing a species of sparrows into an established site showed genetic variation. Therefore, a study of introducing or mixing sites may show if there is genetic variation among the sites and/or genetic isolation.

In conclusion, we found that there was no correlation between the three hormones, C, T, and 11 KT, and condition index for any of the guppy sites we studied. There were positive correlations between all the pairs of hormones – 11KT and C, T and 11KT, and T and C; however, this was not consistent across sites. The lack of relationship between the hormones and condition index could be due to the role of androgens playing a bigger role in male secondary

sex characteristics (Langerhans et al., 2005) and C being unrepresentative of normal base level concentration due to handling stress. There are some studies that have indicated a positive relationship between 11KT and C (Ozaki et al., 2006) as we have found in the present study, while others have found no correlation between C and 11KT (Siebre et al., 2007). The positive distribution of 11KT and C in the guppies can be attributed to stressful and aggressive behavior occurring simultaneously or potentiation of hormone activity (Ozaki et al., 2006). The positive distribution between 11KT and T can be attributed to T being the precursor for 11KT (Idler et al., 1963) as we had expected. However, further research has shown that 11KT and T usually display different functions in species that would require further research in the guppies. C and T levels were an unexpected positive relationship that is most likely attributed to the home tank environments and the aggressive behavior among the males (Wingfield et al., 1990) and to handling techniques that may have elevated stress levels of the guppies (Martinez-Porchas et al., 2009). The variation among the different sites (Aripo River, Marianne River and Small Crayfish River) shows that there could possibly be genetic variation within the species (eg Houde 1997; Endler 2005), which is a very intriguing and possible future study to conduct so other changes that can be seen among the sites. Further evidence of differentiation among sites for other traits and finding the functions of the hormones would be an interesting study and would help provide more evidence for one of the most well-known model organisms for ecology, the guppies (Houde, 1997).

Acknowledgements

We are extremely grateful for the guidance and critique from our advisor, Dr. Gita Kolluru. This research would not have been possible without Julia Walz, Samantha Alburn, Rebecca Nuffer, Kate Downey, and Alexandra Barbella. Also, our study and results would have been incomplete without the assistance of Dr. Ryan Earley and his undergraduate assistant, Amanda Hanninen.

Literature Cited

- Antunes, R. A., & Oliveira, R. F. (2009) Hormonal Anticipation of Territorial Challenges in Cichlid Fish. *Proceedings of the National Academy of Sciences*, 106 (37): 15985-5989.
- Barannikova, I. A., Dyubin, V. P., Bayunova, L. V., & Semenkova, T. B. (2002) Steroids in the Control of Reproductive Function in Fish. *Neuroscience and Behavioral Physiology*, 32(2): 141-148.
- Barton, B. A., Schreck, C. B., & Barton, L. D. (1987) Effects of Chronic Cortisol Administration and Daily Acute Stress on Growth, Physiological Conditions, and Stress Responses in Juvenile Rainbow Trout. *Diseases of Aquatic Organisms*, 2: 173-185.
- Bisazza, A., Vaccari, G., & Pilastro, A. (2001) Female Mate Choice in a Mating System Dominated by Male Sexual Coercion. *Behavioral Ecology*, 12(1): 59-64.
- Bouyer, K., Faivre-Bauman, A., Robinson, I. C. A. F., Epelbaum, J., & Loudes, C. (2008) Sexually Dimorphic Distribution of sst2A Receptors on Growth Releasing Hormone Neurones in Mice: Modulation by Gonadal Steroids. *Journal of Neuroendocrinology*, 20: 1278-1287.
- Brownlee, K. K., Moore, A. W., & Hackney, A. C. (2005) The Relationship Between Circulating Cortisol and Testosterone: Influence of Physical Exercise. *Journal of Sports Science and Medicine*, 4: 76-83.
- Cavaco, J. E. B., Bogerd, J., Goos, H., & Schulz, R. (2001) Testosterone Inhibits 11-Ketotestosterone-Induced Spermatogenesis in African Catfish (*Clarias gariepinus*). *Biology of Reproduction*, 65: 1807-1812.
- Clemens, H.P., McDermitt, C., & Inslee, T. (1966) The Effects of Feeding Methyl Testosterone to Guppies for Sixty Days after Birth. *Copeia*, 1966 (2): 280-84.
- Cox, R. M., Skelly, S. L., & John-Adler, J. B. (2005) Testosterone Inhibits Growth in Juvenile Male Eastern Fence Lizards (*Sceloporus undulatus*): Implications for Energy Allocation and Sexual Size Dimorphism. *Physiological and Biochemical Zoology*, 78(4): 531-45.
- Cox, R. M., Stenquist, D. S., Henningsen, J. P., & Calsbeek, R. B. (2009) Manipulating Testosterone to Assess Links between Behavior, Morphology, and Performance in the Brown Anole *Anolis sagrei*. *Physiological and Biochemical Zoology*, 82(6): 686-98.
- Earley, R., Edwards, J. T., Aseem, O., Felton, K., Blumer, L. S., Karom, M., & Grober, M. S. (2006) Social Interactions Tune Aggression and Stress Responsiveness in a Territorial Cichlid Fish (*Archocentrus nigrofasciatus*). *Physiology & Behavior*, 88: 353-63.
- Endler, J. A. (1995) Multiple Trait Coevolution and Environmental Gradients in Guppies. *Tree*, 10(1): 22-29

- Holloway, A. C. & Leatherland, J. F. (1998) Neuroendocrine Regulation of Growth Hormone Secretion in Teleost Fishes with Emphasis on the Involvement of Gonadal Sex Steroids. *Reviews in Fish Biology and Fisheries*, 8: 409-429.
- Houde, A. E. (1997) *Sex, Color, and Mate Choice in Guppies*. Princeton, NJ: Princeton UP.
- Hurt, C., Anker, A., Knowlton, N. (2008) A Multilocus test of simultaneous divergence across the isthmus of the panama using snapping shrimp in the genus *Alpheus*. *Evolution*, 63(2): 514-530.
- Idler, D. R., Truscott, B., Freeman, H. C., Chang, V., Schmidt, P. J., & Ronald, A. P. (1963) In vivo metabolism of steroid hormones by sockeye salmon: (a) impaired hormone clearance in mature and spawned pacific salmon (*O. nerka*) (b) precursors of 11-ketotestosterone. (abstract) *Canadian Journal of Physiology and Pharmacology*, 41(4): 875-87.
- Kindler, P. M., Phillip, D. P., Gross, M. R., Bahr, J. M. (1989) Serum 11-Ketotestosterone and Testosterone concentrations associated with reproduction in male bluegill (*Lepomis macrochirus*: Centrarchidae (abstract). *General and Comparative Endocrinology*, 75(3): 446-453.
- Kolluru, G. R., and Grether, G. F. (2004) The effects of resource availability on alternative mating tactics in guppies (*Poecilia reticulata*) *Behavior Ecology*, 16(1): 294-300.
- Kraak, S. B. M. & Bakker, T. C. M. (1998) Mutual Mate Choice in Sticklebacks: attractive males choose big females, which lay big eggs. *Animal Behaviour*, 56: 859-866.
- Langerhans, R. B., Layman, C. A., & DeWitt, T. J. (2005) Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species. *PNAS*, 102(21): 7618-7623.
- Magee, S., Neff, B. D., & Knapp, R. (2006) Plasma Levels of Androgens and Cortisol in Relation to Breeding Behavior in Parental Male Bluegill Sunfish, *Lepomis macrochirus*. *Hormones and Behavior*, 49(5): 598-609.
- Marieb, E., & Hoehn, K. (2010) *Human Anatomy and Physiology* (8th Ed.) San Francisco: Pearson Education.
- Martinez-Porchas, M., Martinez-Cordova, L. R., & Ramos-Enriquez, R. (2009) Cortisol and Glucose: Reliable indicators of fish stress? *Pan American Journal of Aquatic Sciences*, 4(2): 158-178.
- Mehta, P., & Beer, J. (2009) Neural Mechanisms of the Testosterone-Aggression Relation: The Role of Orbitofrontal Cortex. *Journal of Cognitive Neuroscience*, 22(10): 2357-2368.

- Meinhardt, U. J., Ho, K. K. Y. (2006) Modulation of Growth Hormone Action by Sex Steroids. *Clinical Endocrinology*, 65: 413-422.
- Nakamura, M. (1981) Effects of 11-Ketotestosterone on Gonadal Sex Differentiation in *Tilapia mossambica*. *Bulletin of the Japanese Society of Scientific Fisheries*, 47(10): 1323-1327.
- Oliveira, R. F., Carneiro, L. A., Goncalves, D. M., Adelino V. M., & Grober, M. S. (2001a) 11-Ketotestosterone Inhibits the Alternative Mating Tactic in Sneaker Males of the PeacockBlenny, *Salaria pavo*. *Brain, Behavior and Evolution*, 58: 29-37.
- Oliveira, R. F., Lopes, M., Carneiro, L. A., & Canario, A. V. M. (2001b) Watching fights raises fish hormone levels. *Nature*, 409: 475.
- Osorno, J. L., Nunez-de la-Mora, A., D'Alba, L., & Wingfield, J. C. (2010) Hormonal Correlates of Breeding Behavior and Pouch Color in the Magnificent Frigatebird, *Fregata magnificens*. *General and Comparative Endocrinology*, 169(1): 18-22.
- Ozaki, Y., Higuchi, M., Miura, C., Yamaguchi, S., Tozawa, Y., & Miura, T. (2006) Roles of 11 β -Hydroxysteroid Dehydrogenase in Fish Spermatogenesis. *Endocrinology*, 147: 5139-5146
- Skold, H. N., Amundsen, T., Svensson, P. A., Mayer, I., Bjelvenmark, I., & Forsgren, E. (2008) Hormonal Regulation of Female Nuptial Coloration in a Fish. *Hormones and Behavior*, 54(4): 549-56.
- Shrey, A. W., Grispo, M., Awad, M., Cook, M. B., McCoy, E. D., Mushinsky, H. R., Albayrak, T., Bensch, S., Burke, T., Butler, L. K., Dor, R., Fokidis, H. B., Jensen, H., Imboma, T., Kessler-Rios, M. M., Marzal, A., Stewart, I. R. K., Westerdahl, H., Westneat, D. F., Zehindj Iev, P., & Martin, L. B. (2011) Broad-scale latitudinal patterns of genetic diversity among native European and introduced house sparrow (*Passer domesticus*) populations. *Molecular Ecology*, 20: 1133-1143.
- Siebre, M., Katsiadaki, I., & Scott, A. P. (2007) Non-invasive measurement of 11-ketotestosterone, cortisol, and androstenedione in male three-spined stickleback (*Gasterosteus aculeatus*). *General and Comparative Endocrinology*, 152(1): 30-38
- Strier, K. B., Ziegler, T. E., and Wittwer, D. J. (1998) Seasonal and Social Correlates of Fecal Testosterone and Cortisol Levels in Male Marquis (*Brachyteles arachnoids*) (abstract). *Hormones and Behavior*, 34(2) 125-134.
- Takahashi, H. (1975) Masculinization of the Gonad of Juvenile Guppy, *Poecilia reticulata*, Induced by 11-ketotestosterone. *Bull. Fac. Fish. Hokkaido Univ.*, 26(1);11-22.
- Welsh, T. H., Bambino, T. H. & Hsueh, A. J. (1982) Mechanism of Glucocorticoid-Induced Suppression of Testicular Androgen Biosynthesis In Vitro. *Biology of Reproduction*, 27: 1138-1146.

Wingfield, J. C., Hegner, R. E., Dufty, A. M., Ball, G. F. (1990) "The Challenge Hypothesis": Theoretical Implications for Patterns of Testosterone Secretion, Mating Systems and Breeding Strategies. *The American Society of Naturalists*, 136(6): 829-846.

Yue, S., Duncan, I. J. H., & Moccacia, R. (2006) Do Difference in Body Conspecific Body Size Induce Social Stress in Domestic Rainbow Trout? *Environmental Biology Fish*, 76: 425-431.