Pair Bond Dynamics: Evaluating Longitudinal Changes in Behavior and 11-Ketotestosterone in Parental Convict Cichlids (Amatitlania Siquia)

PAIR BOND DYNAMICS: EVALUATING LONGITUDINAL CHANGES IN BEHAVIOR AND 11-KETOTESTOSTERONE IN PARENTAL CONVICT CICHLIDS (AMATITLANIA S*IQUIA*)

By

CELINE RICHARDS

Under the Direction of Edmund Rodgers, PhD

ABSTRACT

Bi-parental care and pair bonding often coincide in nature. The reproductive success of the organisms that apply this strategy is dependent upon defensive behaviors and territorial aggression. Some of these organisms also display affiliative behavior within the pair pond during the time of parental care. The behavioral dynamics that occur over the course of the pair bond and their relationship to the reproductive success of the organism is not well understood. Convict cichlids (Amatitlania siquia) form pair bonds during the breeding season and provide bi-parental care; their behavioral repertoire is ideal for studying pair bonding. The androgen profile of organisms that provide parental care through aggressive means is also not fully understood. We provide a context based comparative view of the role of behavior and androgens in the pair bonds of these organisms. Observations were conducted in both the lab and the field; samples of 11-ketotestosterone (KT) were collected from the males. We found that there is an effect of fry stage on the behavior of the parent. Data suggests that there is some effect of fry stage on androgen levels in males.

INDEX WORDS: Convict cichlids, Pair Bond, Behavior, Affiliation, Aggression, Bi-Parental Care, 11-ketotestosterone

PAIR BOND DYNAMICS: EVALUATING LONGITUDINAL CHANGES IN BEHAVIOR AND 11-KETOTESTOSTERONE IN PARENTAL CONVICT CICHLIDS (AMATITLANIA S*IQUIA*)

By

CELINE RICHARDS

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

in the College of Arts and Sciences

Georgia State University

2019

PAIR BOND DYNAMICS: EVALUATING LONGITUDINAL CHANGES IN BEHAVIOR AND 11-KETOTESTOSTERONE IN PARENTAL CONVICT CICHLIDS (AMATITLANIA S*IQUIA*)

By

CELINE RICHARDS

Committee Chair: Edmund Rodgers

Committee: Matthew Grober

Jonathan Sylvester

Edmund Rodgers

Electronic Version Approved:

Office of Graduate Studies

College of Arts and Sciences

Georgia State University

May 2019

DEDICATION

This is dedicated to my family.

ACKNOWLEDGEMENTS

I would like to acknowledge all of the people that have helped and supported me throughout my time at Georgia State University. All of the students that assisted me as well the Biology department especially my advisors.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

KT	ll-ketotestosterone
11-KT	ll-ketotestosterone

1 INTRODUCTION

Selective and exclusive affiliation towards mate and selective aggression toward all others is a condition that defines a pair bond (Aragona, 2005) (Kleiman 1977). For pair bonded animals their social relationship and coordination is critical to long term success. Bi-parental care occurs when a mated pair work together to care for their offspring (Barlow, 1992). Some bi-parental animals form pair bonds and mate monogamously (Wisenden, 1995). Convict cichlids are a species of freshwater fish native to Central America (Wisenden, 1994). Males and females of this species are territorial, and they participate in bi-parental care. Convict cichlids are also seasonally monogamous, and they pair bond during the breeding season in order to care for their offspring (Snekser, 2019). In addition to providing parental care, convict cichlids display behaviors such as aggression and affiliation (Sowersby, 2019) (Tittaferrante, 2015).

Convict cichlids are sexually dimorphic; males are larger than females (Estrela, 2016). Females have a darker appearance with visible orange coloration when reproductive. There are behavioral sex differences as well such that females are more aggressive than males overall. In a study from Wisenden in 1994, he observed that parental roles also differed between the sexes. Males would typically defend offspring from larger predators that were further from the nest and females would typically defend the offspring from smaller predators that were closer to the nest. There is also a disparity between the sexes in terms of reproductive potential within a breeding season; most females breed once within a season (1-2), while males have the possibility of having multiple broods (1-4) (Wisenden 1995). Parental investment is a critical aspect of parental care, and it is directly related to the fitness of the individual. In their natural environment, convict cichlids and their fry face extreme predation pressure and so their fitness is directly related to how well they care for their fry. Males and females of this species both undergo sexual selection to find mates, and evidence suggests that both males and females should be very invested in each clutch (Trivers, 1972). However, individuals that have the opportunity to breed only once will place more value in their offspring and generally provide extensive parental care. There is a tradeoff in terms of offspring number and parental care as providing extensive parental care may delay the organism from reproducing again (Ratikainen, 2018). So, the interaction between parenting and reproducing and its effect on reproductive success are determinants of parental investment. Because most female convict cichlids typically only breed once during a breeding season their reproductive success is completely dependent on their mate and the set of offspring that they produce. Males however, could at least double their reproductive success within any given season. This means that the females are more invested, and therefore provide more parental care, for any given group of her offspring to maximize her reproductive success. Loss of offspring would have a much greater effect on the female's reproductive success than the males. We hypothesize that the pair bond dynamics between the male and female of the pair will change over the course of fry development. We also predict that females will have higher rates of all behaviors than males within pairs because females have a higher investment in the offspring than the males.

Studies evaluating pair bonding in this fish have not yet evaluated the changes in behavior over the course of fry development. We hypothesize that the dynamics of the pair bond will change as the fry get older and there is more opportunity for the male to abandon the clutch and reproduce again within the season. The goal of this study is to observe and record behavior and hormone in both the lab and the field environments and provide a comparative view of the role of these behaviors in the pair bonds of these organisms.

Parental care and androgens have and inverse and often context dependent relationship. Male cichlids have the highest androgen levels during courtship and have levels significantly lower while parenting (van Breukelen, 2015). Androgens and specifically 11-Ketotestosterone, which is the primary androgen in fishes, is also related to territoriality and aggression (Oliveira, 2002). A 2015 study has also shown that brain levels of 11-KT may regulate the expression of parenting behavior in a territorial fish (Pradhan, 2014). Investigating androgen concentrations in the context of an organism that provides parental care and actively defends a territory may provide insight toward this relationship (Rodgers, 2006). We hypothesize that the male androgen levels should increase near the age of independence for fry as the chance to reproduce increases and the parental role changes. No previous study has focused of the same fish longitudinally as the fry get larger. We hope to evaluate the changes in 11-KT over the course of fry development for more insight into how this hormone is linked to pair bonding and parental care behaviors.

1.1 Purpose of the Study

Determine whether there is an effect of fry stage on the behavior of the parent, longitudinally. Determine whether there is a difference between the male in female in rates and types of behavior due to the difference in investment in a given clutch. Determine whether there is an effect of fry stage on levels of 11-ketotestosterone in male convict cichlids.

1.2 Expected Results

Behavioral dynamics of the pair should be different at different stages of fry development. We hypothesize that females should have higher rates of behavior than males in all stages of fry development; also, female affiliation should be highest for pairs with late stage fry and male parental behaviors should be lower for males with late stage fry. We hypothesize that the male androgen levels should increase near the late stage of fry development.

2 EXPERIMENTS

Experiments include all methods for data collection in both the field and the laboratory environments. The lab experiment and the hormone sampling procedure were conducted in coordination with one another. The data was collected as a part of a research project so the number of people that conducted behavioral observations differs between the lab and field experiments. All behavioral data was collected with the standardized ethogram for the behavioral observations.

2.1 Field evaluation of behavior within pairs of convict cichlids with fry at different stages of development

2.1.1 Hypothesis

Behavioral dynamics of the pair should be different at different stages of fry development. We hypothesize that females should have higher rates of behavior than males in all stages of fry development; also, female affiliation should be highest during the late stage of fry development and all male behaviors should be lower for males with late stage fry. Female affiliation should be related to higher rates of male investment.

2.1.2 Methods

Identify pairs based on shared defense of fry. Conduct underwater paired 10-minute observations of pairs with fry in the natural environment of the streams/pools of Costa Rica via snorkel and underwater notebooks. Allowed for 2-3-minute wait prior to observations. Observations take place from 8am-4pm during the dry/breeding season in the streams/ pools of the Rio Cabuyo at the Lomas de Barbuda Biological reserve as well as the Llanos de Cortez falls both in the Guanacaste region of Liberia, Costa Rica in March of 2018 and 2019. Each observation consists of tallies for all affiliative (approach (w/o returning), touch, nip, body wave, change orientation toward), aggressive (displacement, bite, chase, displays), and parental behaviors (leaf flipping, approach, herding, picking up) for the male and the female. Observe each pair only once at a single point in fry development. Overview of behaviors recorded is as follows: Affiliative behaviors are behaviors directed towards the mate, aggressive behaviors are behaviors directed towards the predators/ any other fish and parental behaviors are behaviors directed towards the fry. After observation immediately collect and stage fry based on length. A single random fry was collected from each observed clutch using either a slurp gun or a net. Fry were then photographed for later staging and returned to nest site or collected and anesthetized in ethanol and placed in an Eppendorf tube for staging. Fry staging details are as follows: fry 0-7mm in length categorized as early, fry 7-10mm in length are categorized as middle stage, and fry that are 10+mm in length are categorized as late stage.

2.2 Lab evaluation of behavior within pairs of convict cichlids with fry over time

2.2.1 Hypothesis

Behavioral dynamics of the pair should be different at different stages of fry development. We hypothesize that females should have higher rates of behavior than males in all stages of fry development; also, female affiliation should be highest during the late stage of fry development and all male behaviors should decrease as the fry age.

2.2.2 Methods

Data shall be collected in the form of paired 10-minute behavioral observations of the pairs with fry. Each observation consists of tallies for all affiliative, aggressive, and parental behaviors for the male and the female. The tanks are set up with 1 pair in a 20 gallon (see Figure 2.1) and a small school of 2-6 tetra fish which function as predators. A nest site is provided in each tank. Pairs are created by visually sexing the papilla of male and female fish with a dissecting microscope and adding a random male and female to each tank ensuring that the females are smaller than the males. Or pairs are created by moving established pairs from the group tank. For the pairs of fish with fry, at least 2 observations per developmental stage; before the pair have free swimming fry, when the brood is 1 week old or less, when fry are 3-4 weeks old, and when fry are 5-7 weeks old. Take 11-kt hormone samples from male for each stage. Remove a single fry from tank and record standard length. All observations and hormone sampling must take place before 6pm and not within 1 hour of feeding. The day 1 is noted as when we first see wrigglers.

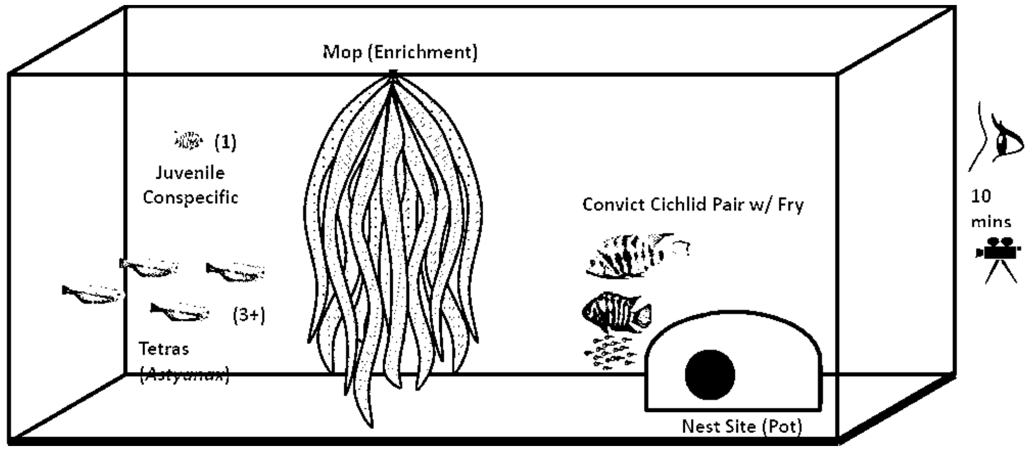

Figure 2.1 Diagram of the Experimental Tank Layout in the Lab

2.3 Sampling 11-ketotestosterone from parental male convict cichlids

2.3.1 Hypothesis

We hypothesize that the male androgen levels should be elevated near the late stage of fry development.

2.3.2 Methods

Lab hormone collection and extraction follows the procedures outlined in Wong et al. 2008. After one of the observations at each fry stage the male of the pair was placed in a beaker containing 200mL of heated water. The male will remain in the beaker for 1 hour and after that time has passed, the water will be poured through the net into a fresh beaker and set aside for extraction and the male will be returned to the tank. All beakers and nets will be rinsed in ethanol to prevent contamination of hormones. For the field sampling procedure, following observation of a random pair. Males were caught and placed into a cup of 200-300mL of prefiltered midstream water and they were left there for 30mins. Steroids were extracted using Lichrolut C18 columns (Carlisle, 2000). Elution with 4ml of methanol into a test tube, the methanol was evaporated with concentrated air currents in a warm water bath (35°C). Samples were re-suspended in 210µl of assay buffer. An 11-Ketotestosterone EIA Kit from Cayman Chemicals

Inc. was used to process samples which were run in duplicate. Samples with concentrations that were not within the range of the values of the standard curve were excluded from the analysis.

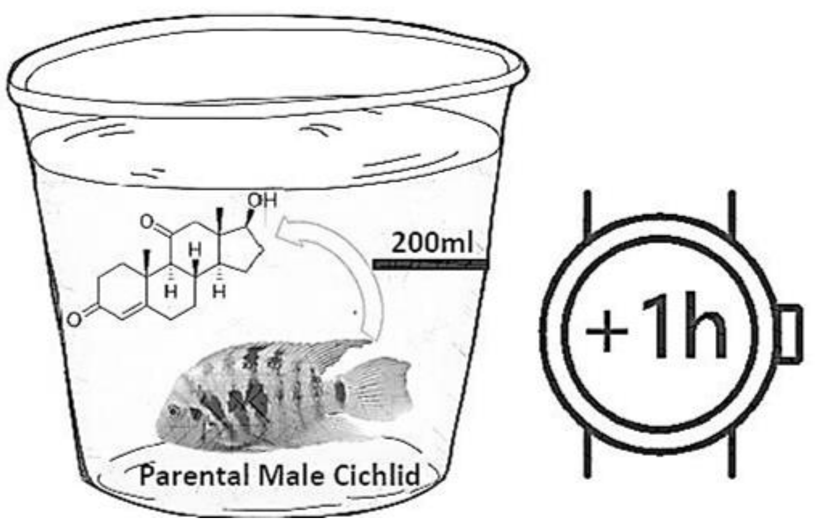

Figure 2.2 Diagram of Hormone Sampling from Water

3 RESULTS

All of the results were analyzed using the Microsoft excel and GraphPad Prism statistical software. There were fish that were not used in the analysis due to premature fry loss. There were several instances of loss of fry before the end of the experiment, most likely due to cannibalism. Also, observed were pairs that did not reproduce at all during the experiment. Also observed were pairs that broke (or showed high levels of aggression and no affiliation towards each other). The samples that included those factors were excluded from the analysis. Only 1 instance of illness/ death or infection was observed in the experimental animals. To determine whether there is an effect of fry stage on the behaviors of the parents, an analysis on the variance/ANOVA was conducted. Linear regressions were used to assess the relationship between the behaviors of the male and the behaviors of the female in the context of the pair bond. Paired t tests were also used in order to compare the hormone concentrations of the males with early and late stage fry.

3.1 Results of field evaluation of behavior within pairs of convict cichlids with fry at different of development

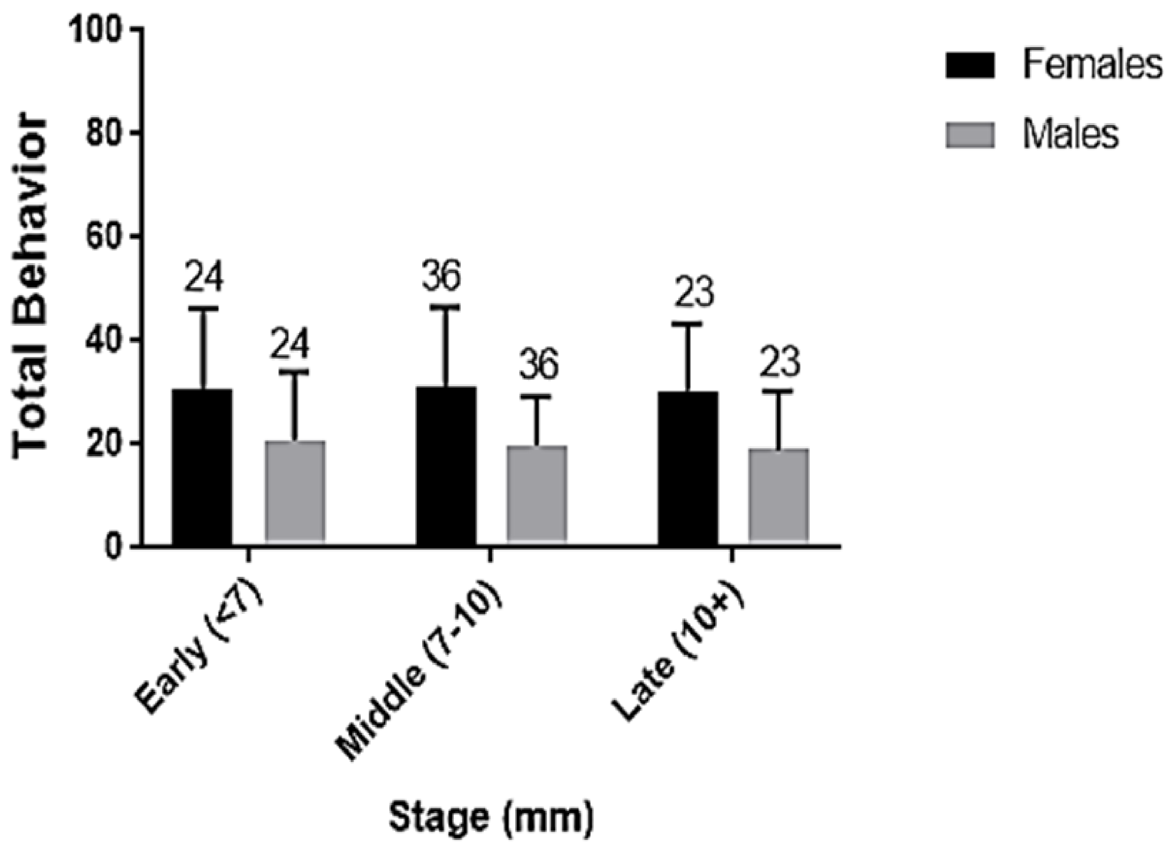

Figure 3.1 Effect of Fry Stage on Total Behavior in Males and Females

Data for total behavior includes all aggressive, affiliative, and parental behaviors. Data was analyzed with a two-way ANOVA statistical test. There is a significant effect of sex ($p<0.05$) on fry stage. Females show higher rates of behaviors overall. Numbers above error bars correspond to sample size. Error bars represent the standard deviation.

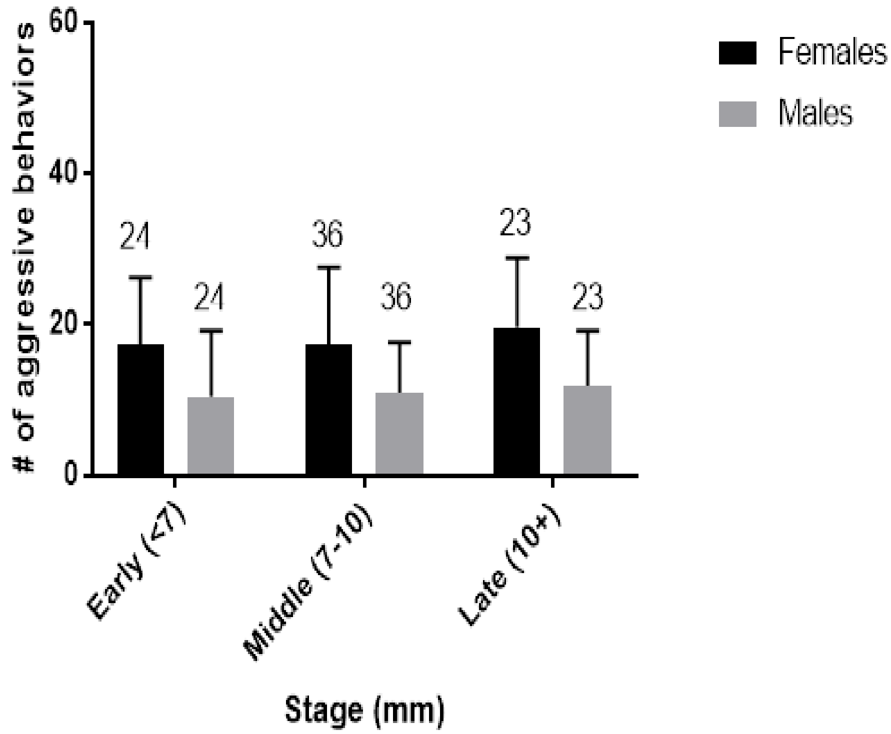

Figure 3.2 Effect of Fry Stage on Aggressive Behavior in Males and Females

Data was analyzed with a two-way ANOVA statistical test. There is a significant effect of sex (p<0.05). Females are significantly more aggressive. Fry stage (p>0.05) is not significant. Females are move aggressive overall. Numbers above error bars correspond to sample size. Error bars represent the standard deviation.

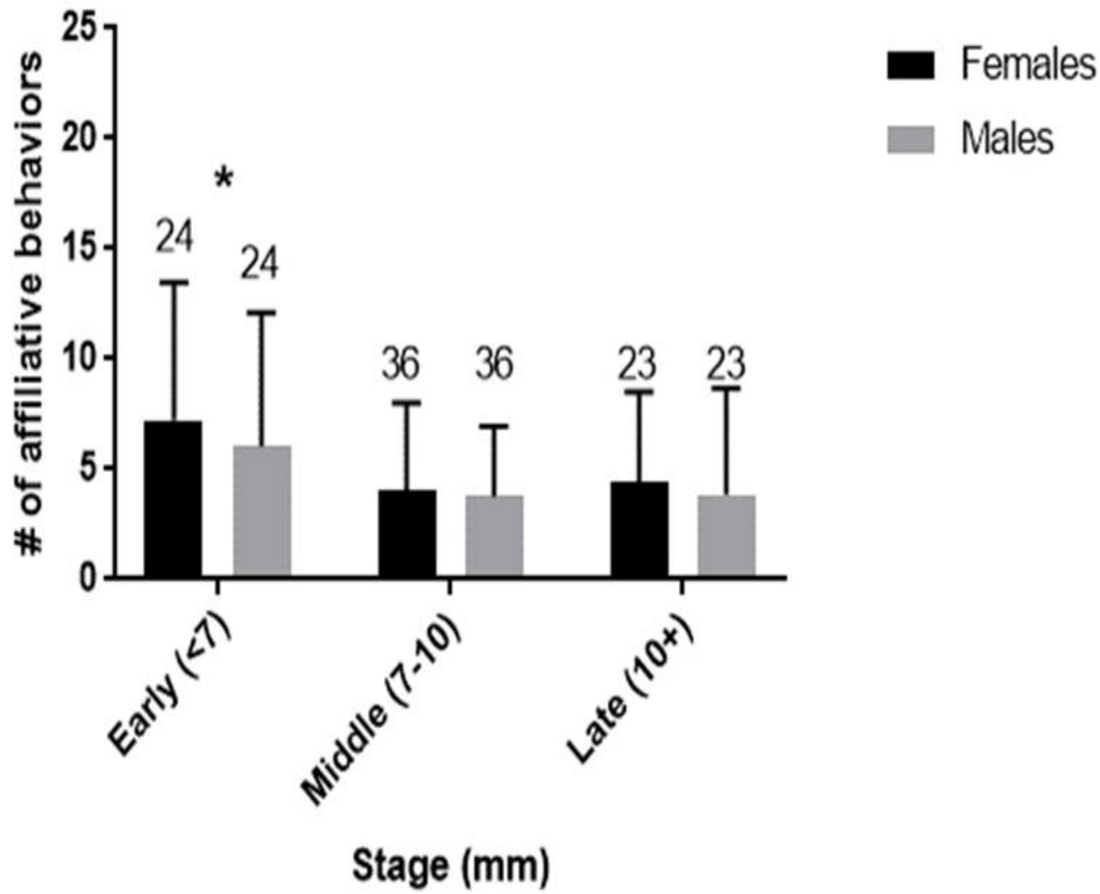

Figure 3.3 Effect of Fry Stage on Affiliative Behavior in Males and Females

Data was analyzed with a two-way ANOVA statistical test. There is a significant effect of fry stage ($p<0.05$) on affiliative behavior. Early stage affiliation is significantly higher than middle and late stage affiliation. Numbers above error bars correspond to sample size. Asterisk denotes significant stage. Error bars represent the standard deviation.

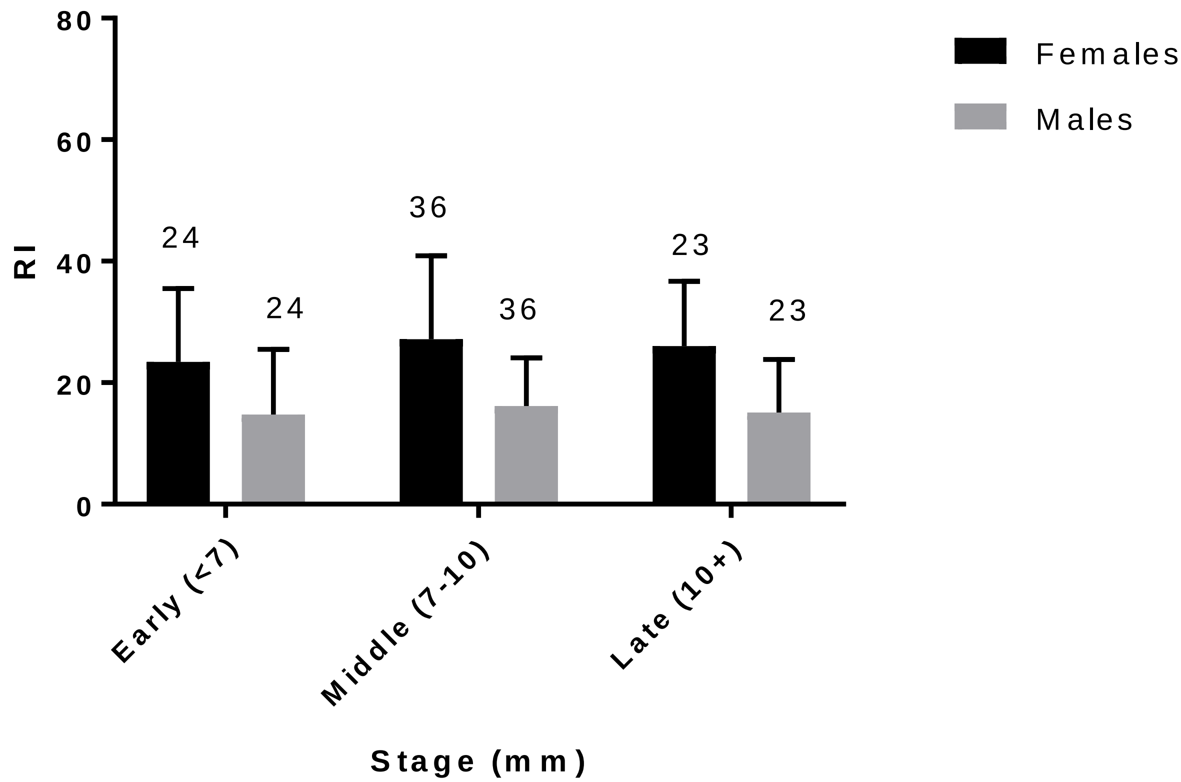

Figure 3.4 Effect of Fry Stage on (Parental) Reproductive Investment in Males and Females

RI (Reproductive investment) includes all parental and aggressive behaviors. Data was analyzed with a two-way ANOVA statistical test. There is a significant effect of sex ($p<0.05$) Reproductive investment behavior. Females have significantly more behaviors than males during all stages. Males show the same amount off reproductive investment behaviors throughout parenting in the field. Numbers above error bars correspond to sample size. Error bars represent the standard deviation.

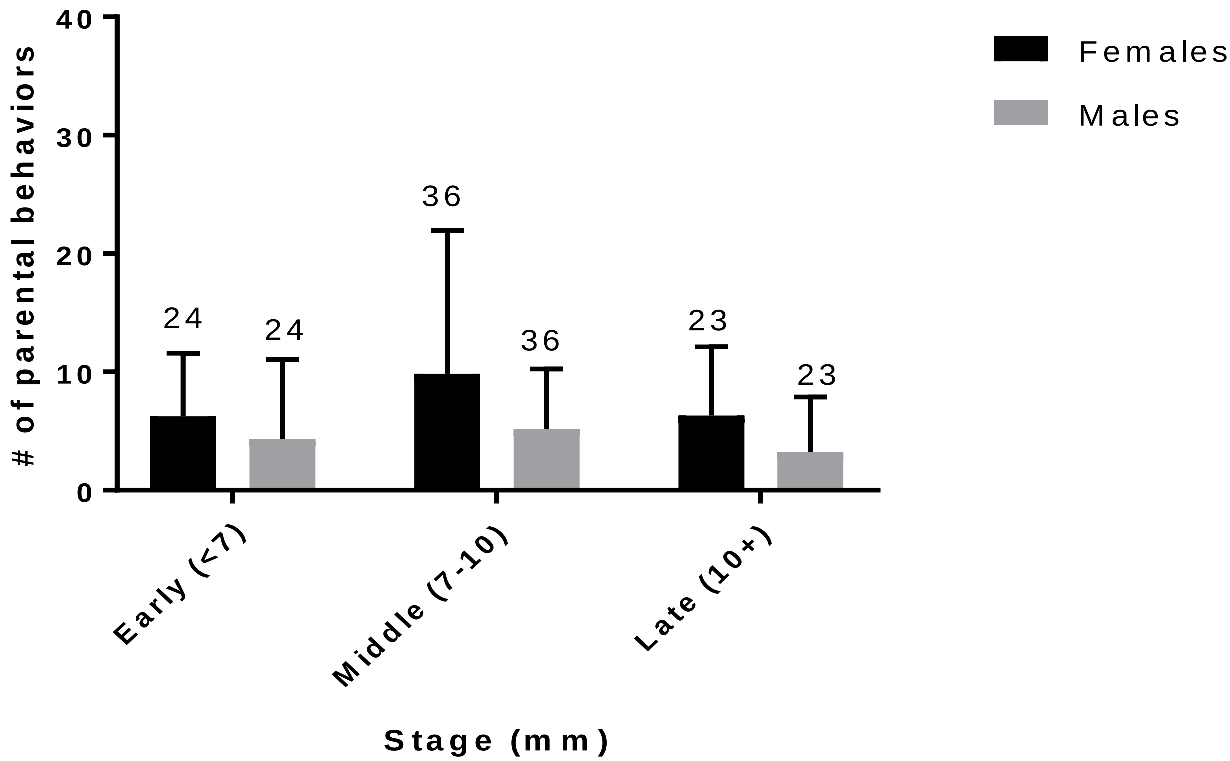

Figure 3.5 Effect of Fry Stage on the Parental Behaviors of the Males and Females

Data was analyzed with a two-way ANOVA statistical test. There is a significant effect of sex ($p<0.05$) parental behavior. Middle stage has the highest total behavior for females. Females have significantly more behaviors than males during all stages. Males show the same amount off parental behaviors throughout parenting. Error bars represent the standard deviation.

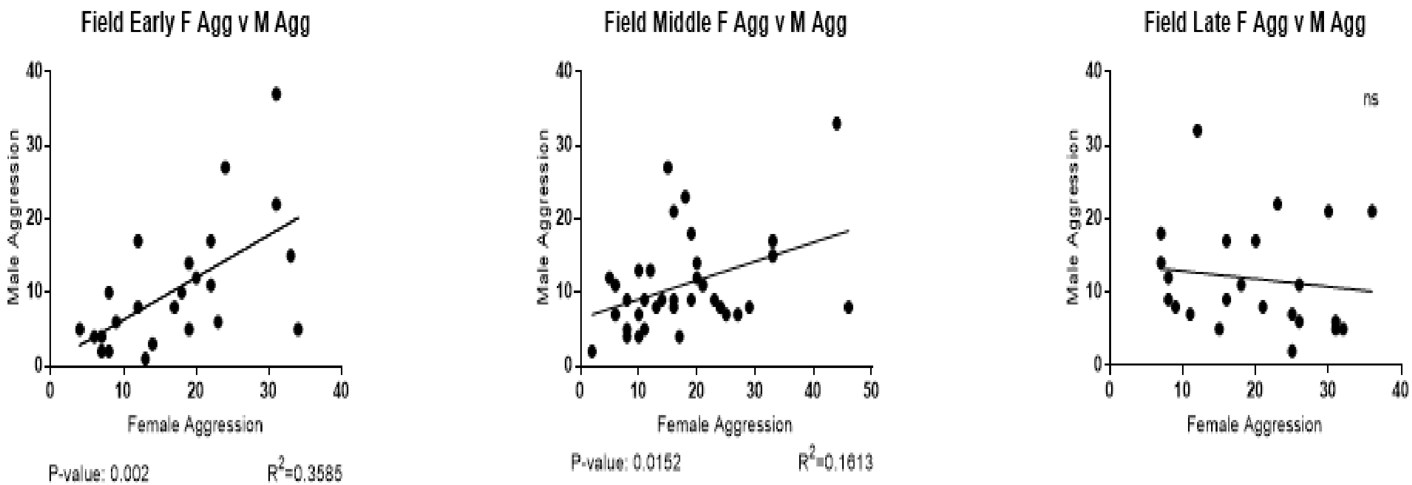

Figure 3.6 Correlations between Aggressive Behaviors Produced By the Males and Females

Data includes linear regression of the aggressive behavior produced by the male and the female in pairs at different stages of fry development. There is a correlation between the aggressive behaviors produced by members of pairs with early and middle stage fry. This value is significant p-value>0.05. There is no correlation between aggressive behaviors produced by pairs with late stage fry.

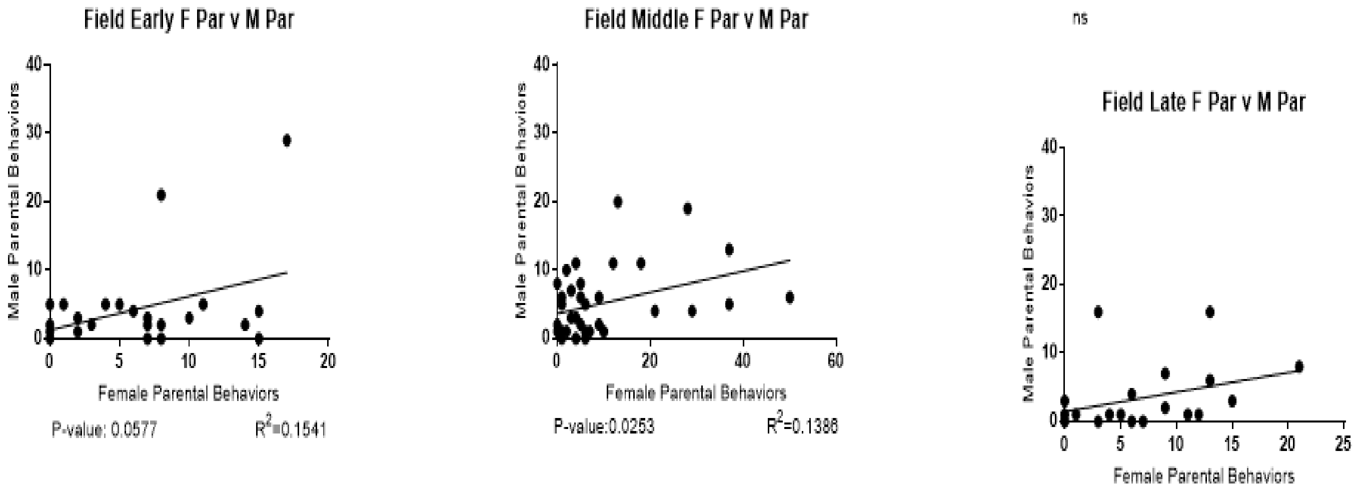

Figure 3.7 Correlations between Parental Behaviors Produced By the Male and Female

Data includes linear regression of the parental behavior produced by the male and the female in pairs at different stages of fry development. There is a correlation between the parental behaviors produced by members of pairs with early and middle stage fry. This value is significant p-value>0.05. There is no correlation between parental behaviors produced by pairs with late stage fry.

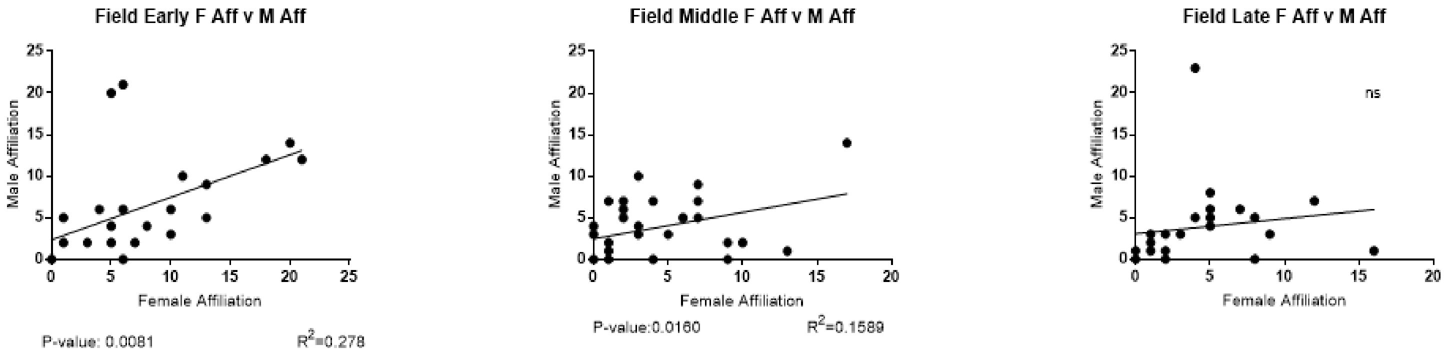

Figure 3.8 Correlation between Affiliative Behaviors Produced By the Males and Females

Data includes linear regression of the aggressive behavior produced by the male and the female in pairs at different stages of fry development. There is a correlation between the affiliative behaviors produced by members of pairs with early and middle stage fry. This value is significant p-value>0.05. There is no correlation between affiliative behaviors produced by pairs with late stage fry.

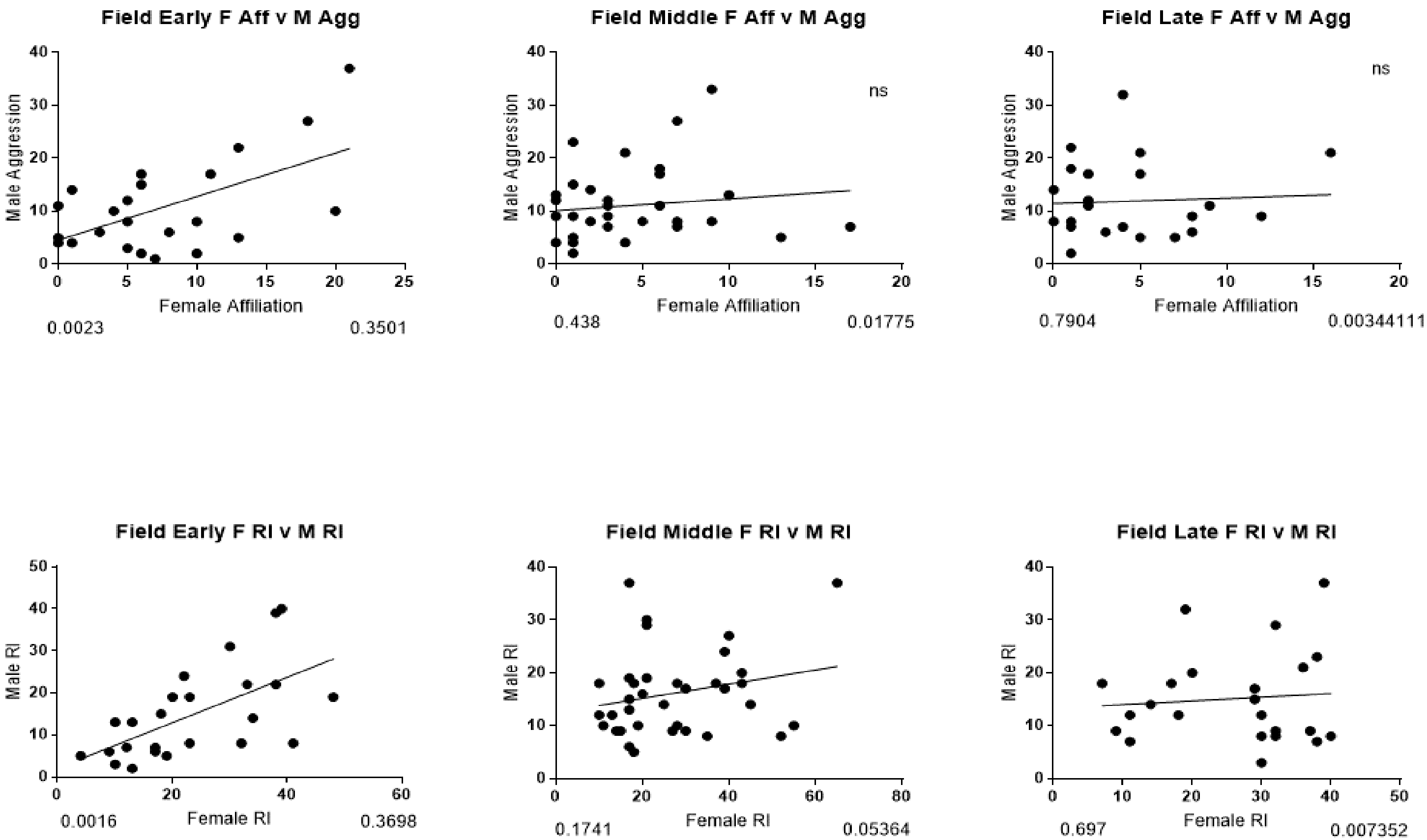

Figure 3.9 Correlations between (Parental) Reproductive Investment of the Males and Females and Male Aggression and Female Affiliation at All Stages

Interesting relationship between male parental behavior and female aggressive behavior for pairs with middle stage fry; data includes linear regressions of the aggressive behavior produced by the male and affiliative behaviors produced by the female in pairs at different stages of fry development (upper). Data also includes linear regressions of the reproductive investment behavior produced by the male and reproductive investment behaviors produced by the female in pairs at different stages of fry development (lower). There is a correlation between male and female behaviors produced by members of pairs with early stage fry. This value is significant p-value>0.05. There is no correlation between aggressive behaviors produced by pairs with middle and late stage fry. Bottom left of each graph indicates p-value and bottom right indicated the R^2 value.

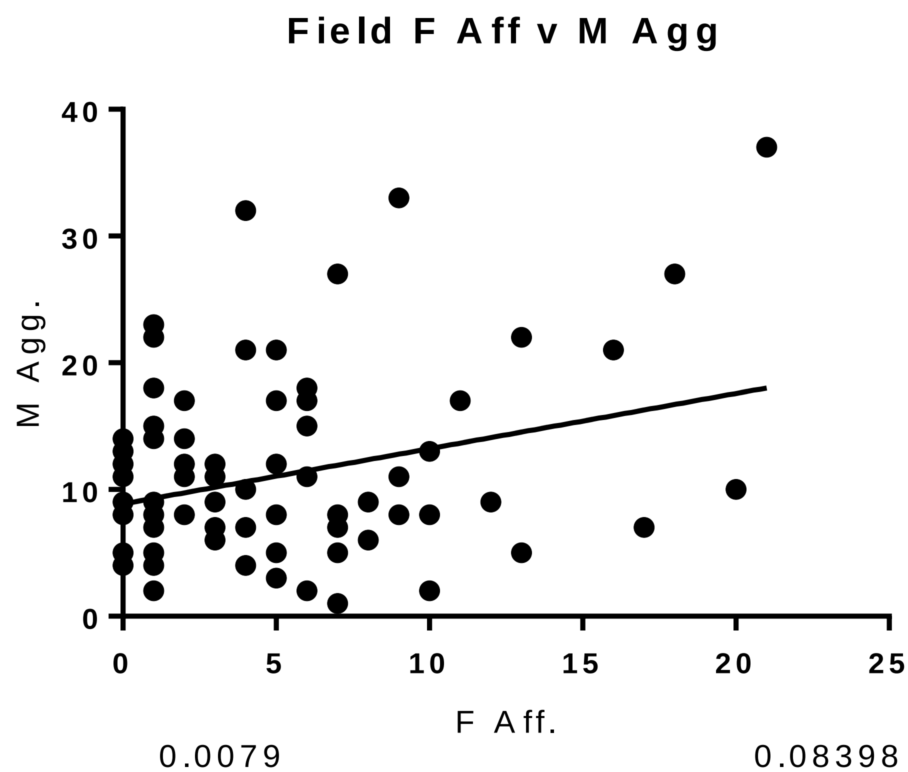

Figure 3.10 Correlations between Female Affiliation and Male Aggression Overall

Data includes linear regression of the aggressive behavior produced by the male and affiliative behaviors produced by the female in pairs overall (includes early, middle, and late). There is a correlation between male and female behaviors. This value is significant p-value>0.05. The same relationship is present in the lab animals (figure not shown).

3.2 Results of lab evaluation of affiliation within pairs of convict cichlids over the course of fry development

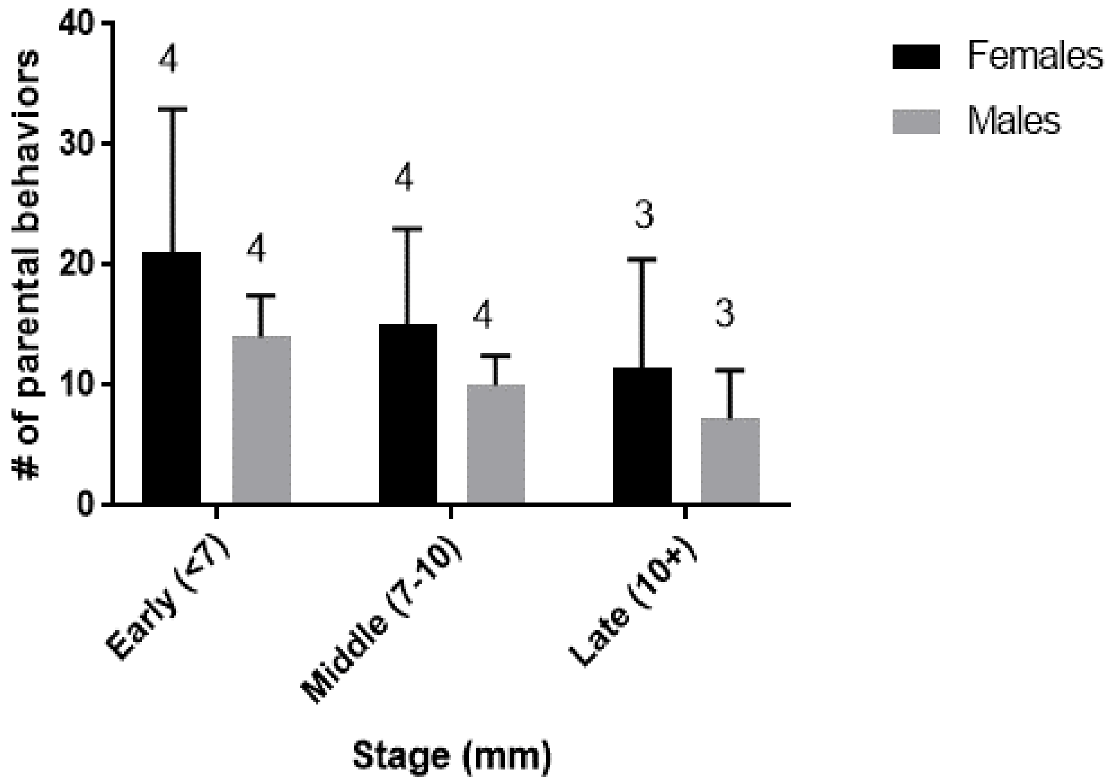

Figure 3.11 The Effect of Fry Stage on Parental Behaviors in the Lab

Data was analyzed with a two-way ANOVA statistical test. Numbers above error bars correspond to sample size. Females show more parental behaviors than males. There is a decrease in parental behaviors from early to late stage. Stage is significant ($p<0.05$). Sex is significant ($p<0.05$). Late stage males show significantly less parental behaviors than early stage males. Error bars represent the standard deviation.

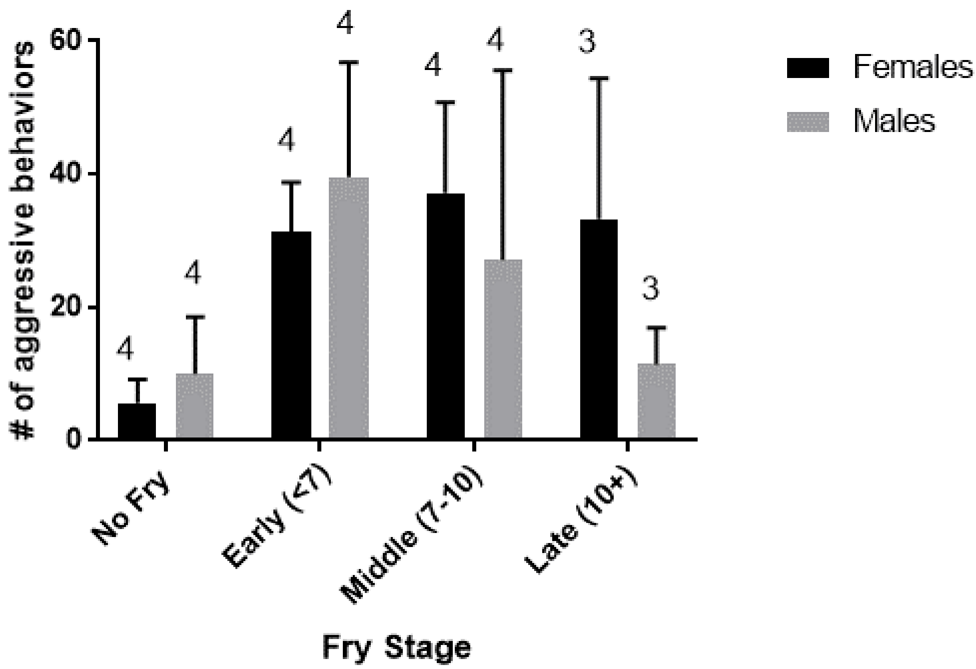

Figure 3.12 The Effect of Fry Stage on Aggressive Behavior of the Parents in the Lab Environment

Data was analyzed with a two-way ANOVA statistical test. Numbers above error bars correspond to sample size. Stage is significant ($p<0.05$). Late stage males show significantly less aggressive behaviors than early stage males. Late stage males have levels of aggression similar to males with no fry. Error bars represent the standard deviation.

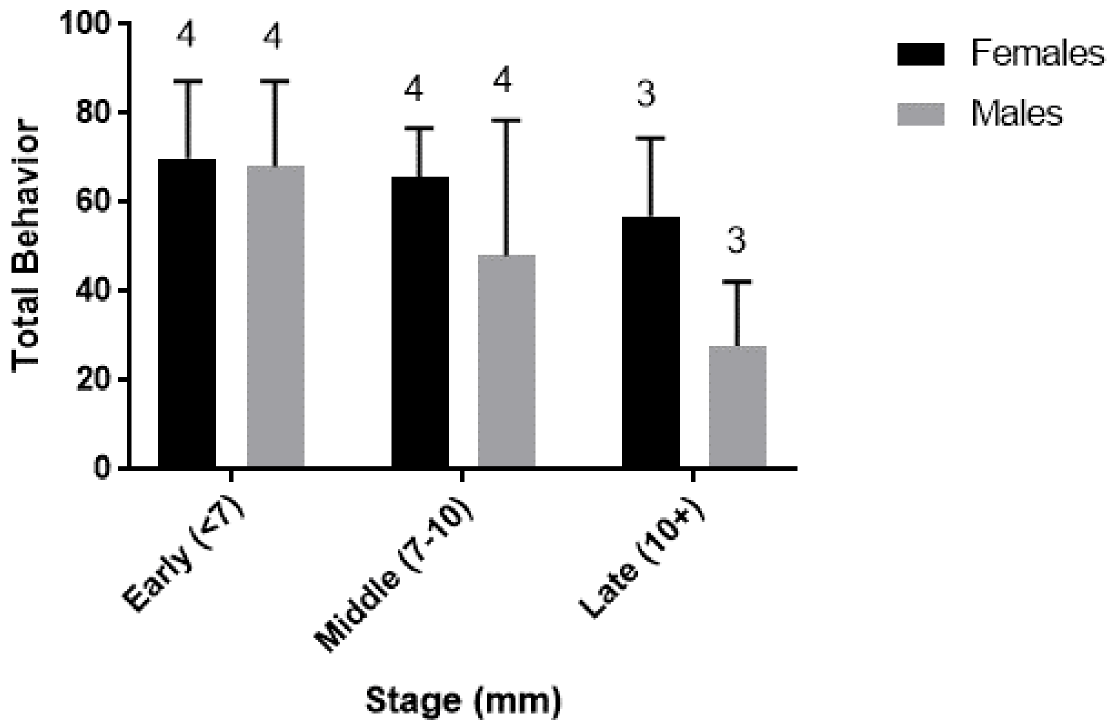

Figure 3.13 The Effect of Fry Stage on the Total Behaviors Produced By the Parents in the Lab Environment

Data for total behavior includes all aggressive, affiliative, and parental behaviors. Data was analyzed with a two-way ANOVA statistical test. There is a significant effect of sex ($p<0.05$) on fry stage. Females show higher rates of behaviors in late stage. Numbers above error bars correspond to sample size. Late stage males show significantly less behaviors than early stage males. Error bars represent the standard deviation.

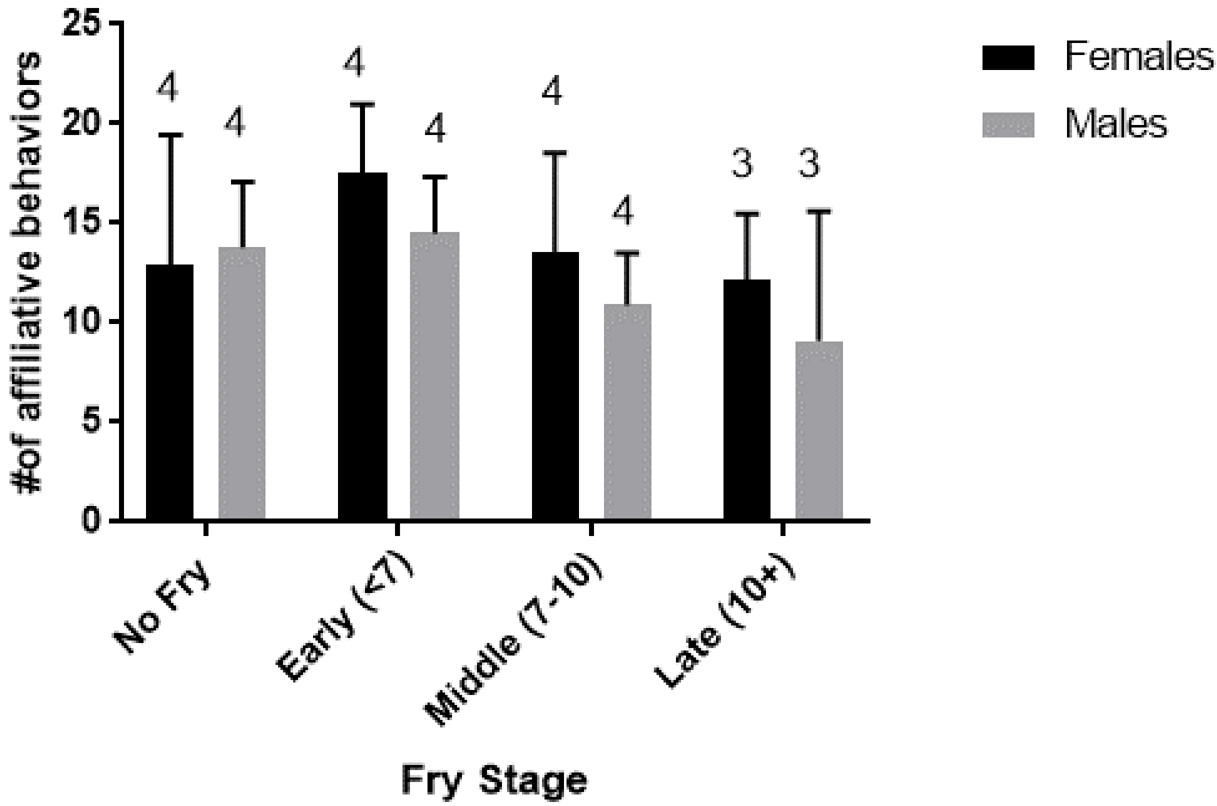

Figure 3.14 The Effect of Fry Stage on Affiliative Behaviors of the Parents in the Lab Environment

Data was analyzed with a two-way ANOVA statistical test. There is no significant effect ($p>0.05$) of fry stage or sex. Numbers above error bars correspond to sample size. Trend toward decrease in behavior as fry get larger but (nonsignificant). Error bars represent the standard deviation.

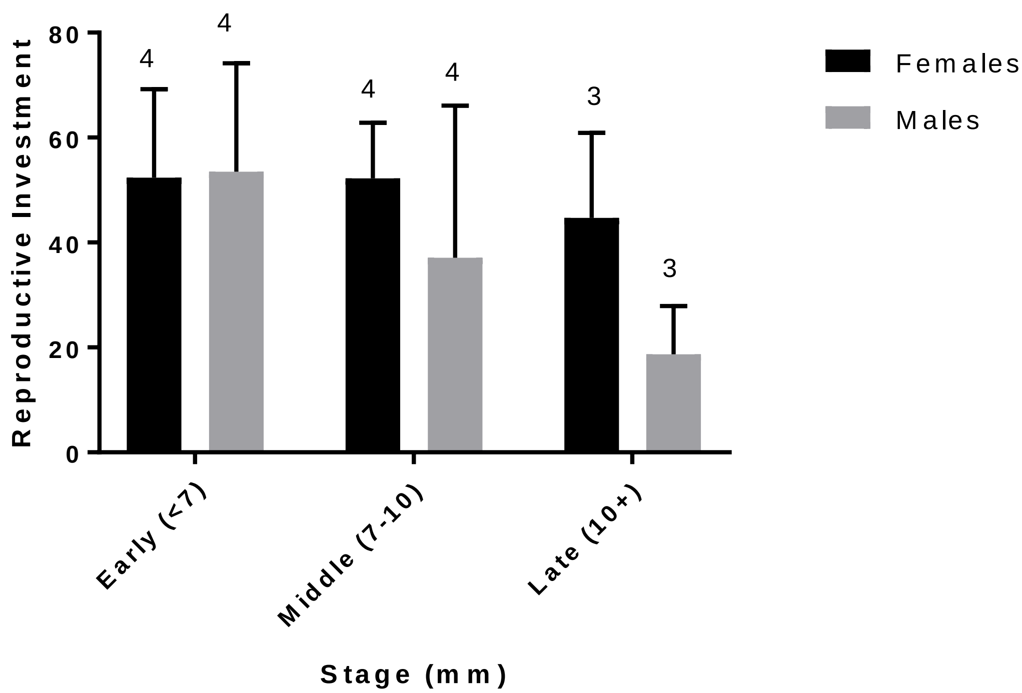

Figure 3.15 Effect of Fry Stage on the (Parental) Reproductive Investment of Males and Females (Lab)

Data was analyzed with a two-way ANOVA statistical test. There is a significant effect of stage ($p<0.05$) on fry stage. Females show higher rates of behaviors in late stage. Numbers above error bars correspond to sample size. Late stage males show significantly less behaviors than early stage males. Male reproductive investment decreases significantly as the fry get larger. Females have similar reproductive investment throughout development. Error bars represent the standard deviation.

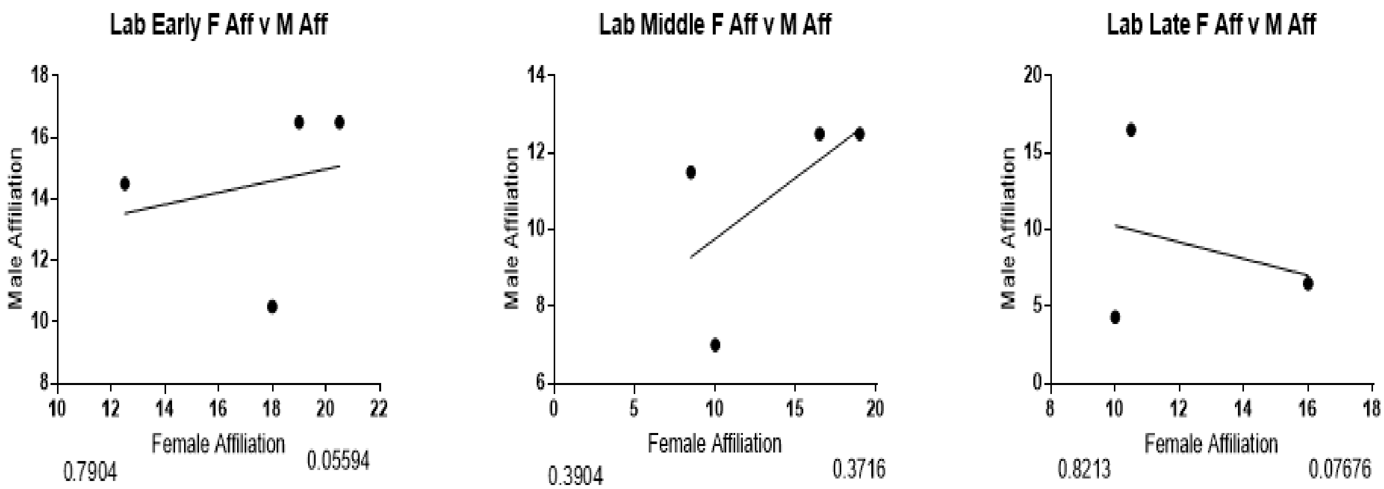

Figure 3.16 Correlations between the Affiliative Behaviors Produced By Males and Females in a Pair during Early Middle and Late Stages of Fry Development

Data includes linear regression of the affiliative behavior produced by the male and the female in pairs at different stages of fry development. There is no correlation between affiliative behaviors produced by pairs with late stage fry. Bottom left of each graph indicates p-value and bottom right indicated the R^2 value.

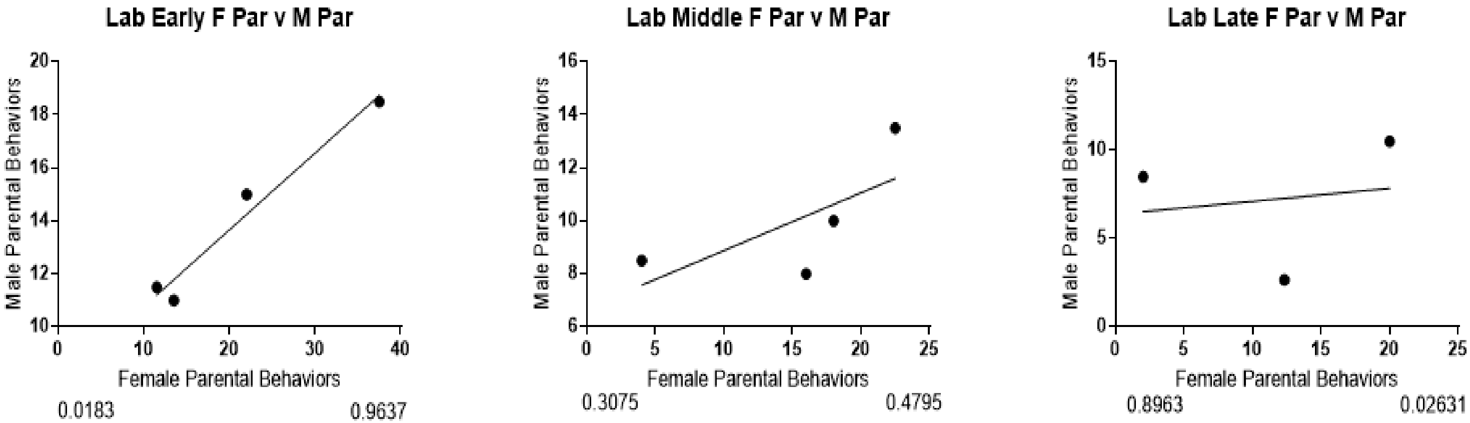

Figure 3.17 Correlations between the Parental Behaviors Produced Between the Pair during Early, Middle, And Late Stages of Fry Development

Data includes linear regression of the parental behavior produced by the male and the female in pairs at different stages of fry development. There is no correlation between parental behaviors within the pairs at middle and late stages. Early stage shows a significant correlation $p<0.05$. Bottom left of each graph indicates p-value and bottom right indicated the R^2 value.

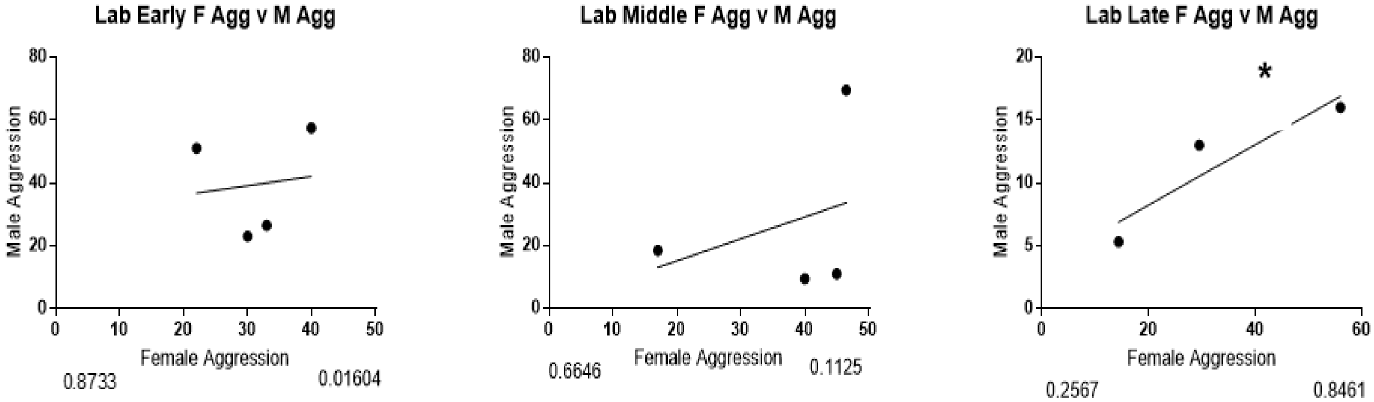

Figure 3.18 Correlations between Aggressive Behaviors Produced Within the Pairs during Early, Middle, And Late Stages of Fry Development

Data includes linear regression of the aggression behavior produced by the male and the female in pairs at different stages of fry development. There is no correlation between aggressive behaviors with in the pairs during any stage. Bottom left of each graph indicates p-value and bottom right indicated the R^2 value.

3.3 Results of sampling 11-ketotestosterone from parental male convict cichlids in the lab

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S1	S1	S1 9-27-18 NO FRY	T2 9-5-18 NO FRY	G4 11-13-18 EARLY	T2 10-1-18 POST EARLY FRY DIED	G1 9-13-18 MIDDLE	G4 11-29-18 VERY LATE	G6 #5 MALE	G5 3-21-19 #4 TUBE 17	[illegible] M G2 LATE
B	BLK	S2	S2	S1 9-27-18 NO FRY	T2 9-3-18 NO FRY	G4 11-15-18 EARLY	T2 10-1-18 POST EARLY FRY DIED	G3 9-13-18 MIDDLE	G4 11-29-18 VERY LATE	G6 #5 MALE	G5 3-21-19 #4 TUBE 17	[illegible] M G2 LATE
C	NSB	S3	S3	G1 11-13-18 NO FRY	T1 9-3-18 NO FRY	G3 9-13-18 EARLY	T2 9-19-18 POST EARLY FRY DIED	S1 10-25-18 LATE- NO FEMALE	G2 #1 MALE	G5 3-21-19 #2 FISH 1	[illegible] M G2 NO FRY	CONTROL MIDSTREAM H2O 3/21/19
D	NSB	S4	S4	G1 11-13-18 NO FRY	T1 9-3-18 NO FRY	G3 9-13-18 EARLY	T2 9-19-18 POST EARLY FRY DIED	S1 10-25-18 LATE- NO FEMALE	G2 #1 MALE	G5 3-21-19 #2 FISH 1	[illegible] M G2 NO FRY	CONTROL MIDSTREAM H2O 3/21/19
E	BO	S5	S5	G4 9-28-18 NO FRY	S1 10-9-18 EARLY	T2 10-25-18 EARLY	S1 10-8-18 MIDDLE	G4 11-8-18 LATE	G2 #2 MALE	G5 3-21-19 #3 TUBE	[illegible] M G2 EARLY	T1 9-13-18 POST EARLY ALL FRY DIED
F	BO	S6	S6	G4 9-28-18 NO FRY	S1 10-9-18 EARLY	T2 10-25-18 EARLY	S1 10-8-18 MIDDLE	G4 11-8-18 LATE	G2 #2 MALE	G5 3-21-19 #3 TUBE	[illegible] M G2 EARLY	T1 9-13-18 POST EARLY ALL FRY DIED
G	BO	S7	S7	G3 9-3-18 NO FRY	G1 11-20-18 EARLY	G1 12-3-18 POST-EARLY	G4 10-25-18 MIDDLE	G1 9-28-18 LATE	G2 #4 MALE	G5 3-21-19 TUBE 8	[illegible] M G2 MIDDLE	
H	TA	S8	S8	G3 9-3-18 NO FRY	G1 11-20-18 EARLY	G1 12-3-18 POST-EARLY	G4 10-25-18 MIDDLE	G1 9-28-18 LATE	G2 #4 MALE	G5 3-21-19 TUBE 8	[illegible] M G2 MIDDLE	

DATE: EXP:

Figure 3.19 Supplemental Data- KT Plate Layout Key

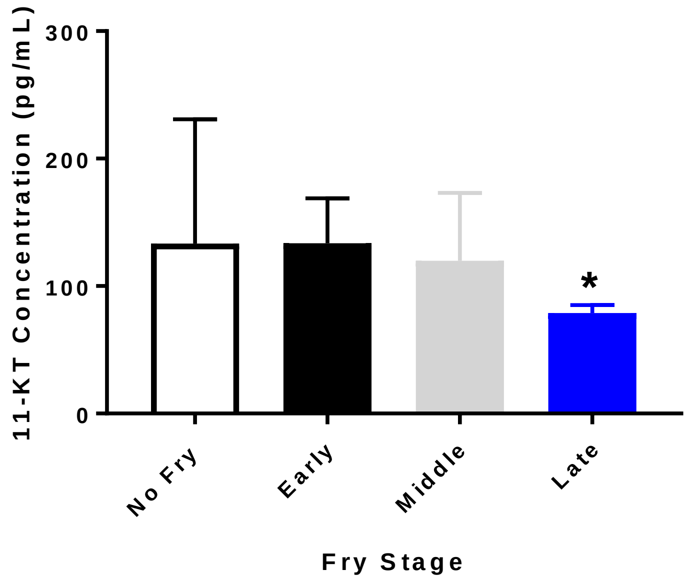

Figure 3.20 11-KT concentrations in male convict cichlids over fry development (Lab)

Data was analyzed with a two-way ANOVA statistical test. 11-KT is significantly lower for males with late (4) stage fry. The no fry (4), early (4), and middle (4), groups appear to have similar 11-KT concentration. There is the most variation in the no fry group. There is very little variation in the late group. Error bars represent the standard deviation.

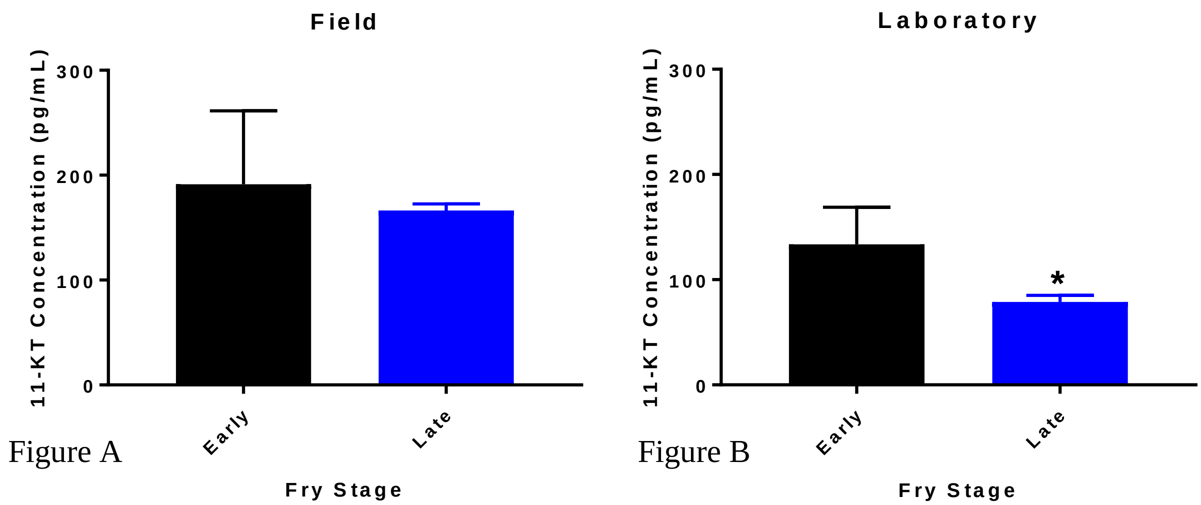

Figure 3.21 11-KT concentration for males with late and early stage fry in the field (left) and the lab (right).

Data was analyzed with a two-way ANOVA statistical test. For figure A. There appears to be no significant difference in 11-KT concentration for males that have early and late stage fry in the field though the trend suggest early is higher in the field though not significantly. There were 8 fish sampled (3 excluded due to processing errors; 2 early/3 late). For figure B there is a significant difference $p<0.05$. The same 4 fish were sampled at different time points in figure B. Error bars represent the standard deviation.

4 DISCUSSION

Pair bonding dynamics are not fully understood in any species. Convict cichlids exhibit pair bonding and the associated behaviors can be observed and quantified in both a laboratory and a field environment. The purpose of this experiment was to determine whether the disparity in investment in any given clutch would result in differences in the behaviors of the males and females in the pairs.

Experiments were conducted in both the lab and the field in order to provide a comparative view of how the social context affects the behaviors of these organisms. The results indicate that fry size is related to the behaviors that the male and female direct toward each other, toward the fry, and toward brood predators. Overall when comparing the field and lab results, the rate of behavior observed in the laboratory environment is higher than the rate of behavior observed in the field environment. We suspect that lab animals are exhibiting more total behavior due to the fact that the tanks were small (20 gallon), and the pairs were constantly subjected to small fry predators/ territorial intruders (tetras and juvenile conspecific), despite aggressive interactions with the intruder, the intruder could not leave. In the field context, the members of the pair also spend time away from each other for feeding. In the laboratory context, the pair is provided with food and as they do not need to allocate food in this way and more time is spent within the pair and with the fry in turn; this difference may also impact behavioral rates in the lab. We found that there is a significant correlation between the behaviors of the male and the female during the early and middle stages for pairs found in the field environment. This suggests that the relationship of the male and female within a pair, their relationship toward the fry and toward one another are different when the fry are older than when the fry are younger. Further research in the laboratory environment was conducted to determine whether the phenomenon would persist in the absence of a variety of predatory stimuli. The results support the fact that this mechanism is context dependent and it does not appear in the lab environment.

Lab and field data also display different trends with regard to stage related rates of behavior; this supports the idea that the social context can impact the dynamics of the pair bond. Both the lab and field experiment showed that there were differences between the behaviors of the males and females. Females displayed higher rates of behavior overall in both lab and field condition. This reflects the greater investment of the female in that clutch. In laboratory conditions, there was a trend for males to have lower rates of behavior when their fry are late stage. Total behavior decreased for males as the fry got larger. Aggression was lowest for pairs that had no fry and highest for parents. Female aggression was the same throughout fry development but, males with late-stage fry had the lowest rates of aggression. Females are also more parental and parental behaviors decrease with the fry stage as the fry get older. Reproductive (parental) investment also seems to change in a stage-dependent manner in that males and females exhibit similar rates of investment behaviors during the middle and early stage but during the late stage, the male shows significantly less behavior. Affiliation seems to be the same for males and females throughout fry development. This data suggests that the age of the fry does have an impact on the behaviors of the parents and that females are similarly invested throughout fry development but that males are less invested when their fry are in the late stage. It could be the case that low rates of predation have left the male with very little to do in terms of nest defense during that late stage, it could also be possible that the male is conserving his energy in order to prepare to reproduce again or other unknown factors could be responsible for this phenomenon. For pairs in the field, females had had higher rates of behavior for all behaviors sampled except affiliation. Interestingly, the only stage deference in behavior in the field environment seems to be early stage affiliation. Early stage affiliation is significantly higher than the middle and late for both males and females. Furthermore, there is no significant difference between the behaviors of the female and the behaviors of the male in terms of affiliation. This could mean that affiliation is important in the field when the fry a very small. In the field, all observed behaviors (aggression, affiliation, and parental), of the male correlates to the behavior of the female for early stages and often middle stages but not late stages. Early stage affiliation produced by the female also correlates to the early stage aggressive behaviors produced by the male and early stage reproductive investment of the pair is also correlated. Overall, for all field pairs included regardless of stage, female affiliation is significantly correlated with male aggression. Data suggests that the more aggressive a male displays, the more affiliative the female is. Taken altogether it appears that there are differences in the early stage that could mean that the early stage is important for the reproductive success of these organisms. Males in the field did not display lower rates of behavior in response to late-stage fry as they showed in the lab samples. This suggests that the social context also has an impact on the reproductive investment of males in this species. Results suggest that convict cichlids may be able to adapt their behaviors based on the context in order to maximize their investment behaviors and possibly increase their reproductive success.

The results of sampling 11-kt from parental males from both the lab and the field show that there is no difference between the hormone concentrations of males that have early and late stage fry in the field environment. In the lab there appeared to be higher androgen concentration for males with late-stage fry. The result of previous studies suggests that there is an inverse relationship between male androgen and parental care and the results of this study do not support that idea. Instead, it seems that the 11-kt concentrations are related to the activity level of the male. In the field there is no difference in reproductive investment for males with early and late stage fry however; in the lab, the reproductive investment is higher for males with early-stage fry. The hormone concentration of males in the lab is also higher for males with early-stage fry. Future study is necessary to determine if the male androgen levels should increase near the late stage of fry development.

Overall we found that there is an effect of fry stage on the behavior of the parent. Data also suggests that there is some effect of fry stage on androgen levels in males. Our prediction that female affiliation would be highest during the late stage of fry development was not supported by the data collected in this experiment. The behavioral dynamics of the pair was different at different stages of fry development especially in the case of aggressive behaviors and parental behaviors directed towards the fry. Also consistent with our hypothesis is the finding that females generally have higher rates of behavior than males. The data does support the hypothesis that female affiliation is correlated to higher rates of male investment. Some male behaviors did decrease as the fry aged in the lab environment. In conclusion, the results show that there is an effect of fry stage on the behavior of the parent, longitudinally and more studies should be conducted in the future on the dynamics of behaviors within the pair bond throughout parenting and as the fry age in order to provide more insight into this interaction.

REFERENCES

1. van Breukelen, N. A., Snekser, J. L., & Itzkowitz, M. (2015). Male convict cichlid 11-ketotestosterone levels throughout the reproductive cycle: An exploratory profile study in laboratory and field populations. *PeerJ,3*. doi:10.7717/peerj.949
2. Santangelo, N. (2015). Female breeding experience affects parental care strategies of both parents in a monogamous cichlid fish. *Animal Behaviour,104*, 31-37. doi:10.1016/j.anbehav.2015.03.004
3. Wisenden, B. D. (1994). Factors affecting reproductive success in free-ranging convict cichlids (Cichlasoma nigrofasciatum). *Canadian Journal of Zoology,72*(12), 2177-2185. doi:10.1139/z94-291
4. Wisenden, B. D. (1995). Reproductive behaviour of free-ranging convict cichlids,Cichlasoma nigrofasciatum. *Environmental Biology of Fishes,43*(2), 121-134. doi:10.1007/bf00002480
5. Grens, KE, Fernald, RD, Clement, Tricia S. (2005). Female affiliative preference depends on reproductive state in the African cichlid fish, Astatotilapia burtoni. Behavioral Ecology, 16(1), 83-88.
6. Godin, Jean-Guy J., Lee-Jenkins, Stacey SY (2013). Concurrent effects of familiarity and kinship on social affiliations in convict cichlid (Amatitlania siquia) young. Behaviour., 150(8), 895-919.
7. Barlow, G. W. (1992). Is Mating Different in Monogamous Species? The Midas Cichlid Fish as a Case Study. American Zoologist, 32(1), 91-99. doi:10.1093/icb/32.1.91
8. Snekser, J., & Itzkowitz, M. (2019). Serial monogamy benefits both sexes in the biparental convict cichlid. PeerJ., 7, E6535.

9. Sowersby, Will, Lehtonen, Topi K, & Wong, Bob B M. (2018). Threat sensitive adjustment of aggression by males and females in a biparental cichlid. Behavioral Ecology, 29(3), 761-768.
10. Tittaferrante, Stephanie, Way, Gregory P., Fuller, Ashlei, Sullivan, Nicole, Moss, Sarah . . . McRobert, Scott P. (2015). Interactions between aggression, boldness and shoaling within a brood of convict cichlids (Amatitlania nigrofasciatus). Behavioural Processes, 121, 63-69.
11. Jones, R., Moscicki, M., Clotfelter, E., & Earley, R. L. (2014). Breeding convict cichlids (Amatitlania siquia) display differential aggression based on conspecific intruder coloration. Integrative and Comparative Biology., 54, E238.
12. Estela, V. J., Leese, J. M., Itzkowitz, M., Bloch, AN(2016). Male mate preference and size-assortative mating in convict cichlids: A role for female aggression? Behavioural Processes, 130, 81-85.
13. Ratikainen II, Haaland TR, Wright J (2018). Differential allocation of parental investment and the trade-off between size and number of offspring. Proceedings of the Royal Society B: Biological Sciences, 285(1884), http://doi.org/10.1098/rspb.2018.1074
14. Kleiman D. G. (1977). "Monogamy in Mammals," The Quarterly Review of Biology 52*(1)*, 39-69, https://doi.org/10.1086/409721
15. Oliveira, Rui F, Hirschenhauser, Katharina, Carneiro, Luis A, & Canario, Adelino VM. (2002). Social modulation of androgen levels in male teleost fish. Comparative Biochemistry and Physiology., 132(1), 203-215.
16. Carlisle, S, Marxer-Miller, SK, Canario, AVM, Oliveira, RF (2000). Effects of 11-ketotestosterone on genital papilla morphology in the sex changing fish Lythrypnus dalli. Journal of Fish Biology, 57(2), 445-456.

17. Pradhan, D S, Solomon-Lane, T K, Willis, M C, & Grober, M S. (2014). A mechanism for rapid neurosteroidal regulation of parenting behaviour. Proceedings., 281(1786), 20140239.

18. Rodgers, EW, Earley, RL, & Grober, MS. (2006). Elevated 11-ketotestosterone during paternal behavior in the Bluebanded goby (Lythrypnus dalli). Hormones and Behavior. 49(5), 610-614.

19. Trivers, R. L. (1972). Parental Investment and Sexual Selection. In B. Campbell (Ed.), Sexual Selection and the Descent of Man, 1871-1971 (pp. 136-179). Chicago, IL: Aldine.