

Effect of Social Environment on 11-KT and Cortisol Production in Pair Bonded Convict Cichlids

C Alhadeff, B Arbasi, D Castillo, Y Davis, H Nguyen, MS Grober, & EW Rodgers
Department of Biology, Georgia State University, Atlanta, GA, USA



INTRODUCTION

Social behaviors are a product of evolution, with the environment being the critical selective force. These social behaviors are a result of evolution acting on organisms within a complex environment. Cooperation is a complex social behavior exhibited by a pair or group of organisms in which they assist one another to increase overall fitness. Convict cichlids (*Amatitlania siquia*, *kanna*, *nigrofasciata*) are a serially monogamous, cooperatively parenting, freshwater fish native to Central America. Previous research on pair bonded convict cichlids revealed an increase of behaviors (affiliative and aggressive) in dynamic tanks when compared to isolated tanks [1].

In this experiment, hormones were taken from pairs (male and female) of monogamous convict cichlids held in two different environments: dynamic and isolated. The dynamic environment contained the focal fish alongside conspecifics, closely mimicking their natural environment. Isolated tanks contained pair bonded focal fish and eggs/fry if the pair had any. To understand the hormonal effects of social environment and thereby behavior, levels of 11-Ketotestosterone (11-KT) and cortisol were measured. Known as the most potent fish androgen, 11-KT is associated with aggression, and courting while cortisol is released in response to stress. Convict cichlids are ideal models for behavioral studies due to their extreme levels of social behaviors. In fact, elevated levels of aggression are necessary to provide proper territory and nest defense [2].

Due to the social nature of convict cichlids, it was hypothesized that steroid hormone levels would be significantly higher in dynamic social environments when compared to isolated social environments. Elevated 11-KT levels in dynamic tanks were expected, as the dynamic environment permits aggression against conspecifics. So too, the stress of mate protection and brood care was predicted to correlate with an increase in cortisol. Conversely, pairs in isolated tanks were predicted to have low steroid hormone levels. Decreased 11-KT levels were expected due to lack of conspecifics to aggress toward. Similarly, due to the absence of predators and expected decline in parental care and mate competition, cortisol hormone levels were predicted to be lower.



METHODS

We established three fifty-five gallon tanks and two thirty gallon tanks as dynamic environments. Each tank was sub-species specific: *A. nigrofasciata*, *siquia*, or *kanna*. Pairs were allowed to form naturally in groups of 10-15 conspecifics. Sign of pair forming includes defending the nest (flowerpot) together. Behavioral data consisted of three 10-minute observations. The rates of behavior (aggressive, affiliative, and reproductive) were recorded. Hormones were collected after the third observation.

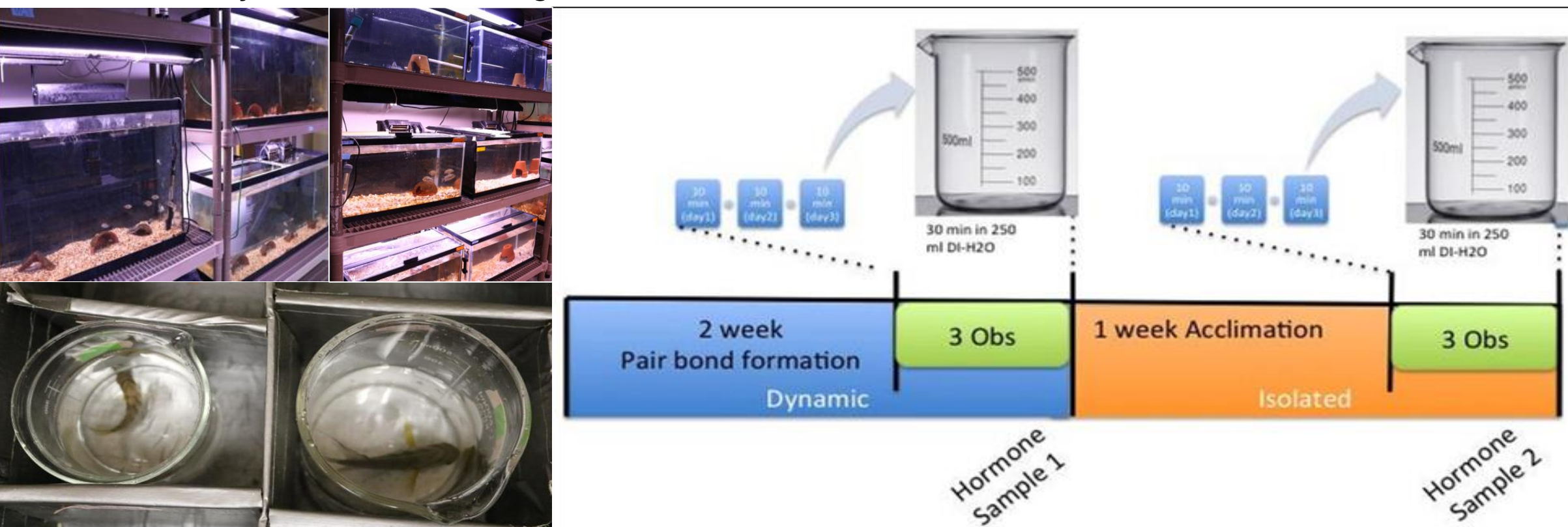
Hormones were extracted by leaving the focal fish in a 250 mL beaker of water for 30 minutes to allow for hormone release.

Established pairs were moved from the dynamic environment into isolated ten gallon aquariums, and were acclimated for 7-10 days. Hormones were collected after performing three 10-minute behavioral observations.

Mouth locks, bites, and displacement were recorded as aggressive behavior. Lateral displays, tail waggles, nips, and approaches without displacement were recorded as affiliative behavior. Any behavior directed towards fry was recorded as reproductive behavior. Inter-observer reliability was established and maintained by simultaneous observations of groups of two individuals and a correlation analysis was conducted. Correlation coefficients of 0.9 or greater ensured a high fidelity of observer accuracy.

The fish were fed on a regular schedule, twice a day, with brand flake food. Fish were not fed within one hour of observations. Fifty percent water changes occurred once a week using de-chlorinated tap water. Treatment of the fish corresponds with IUCAC Protocol A16050.

Data analysis was done using Prism Software Excel.



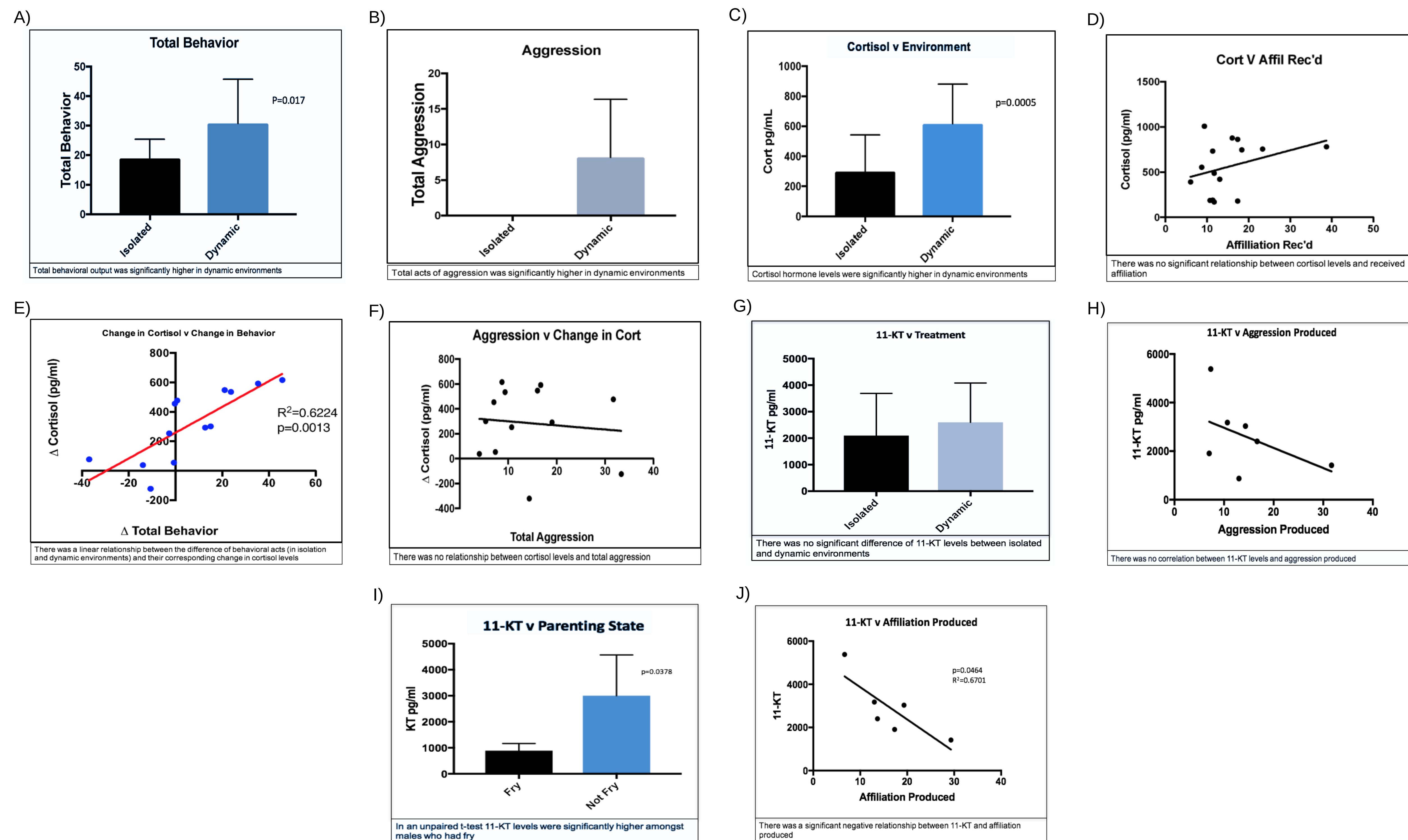
Acknowledgements

The researchers would like to thank the Undergraduate Stem Research at GSU for financial support. We would also like to thank the GSU Biology Department and the Undergraduate Research Center (URC).

References

1. Bruinjes R, Lynton-Jenkins J, Jones JW, Radford AN (2015) Out-group threat promotes within- group affiliation in a cooperative fish. The American Naturalist, online in advance of print. <http://dx.doi.org/10.1086/684411>
2. Wisenden, Brian D. (1994) Factors affecting reproductive success in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). Canadian Journal of Zoology. <http://dx.doi.org/10.1139/z94-291>
3. Gabor C. R, Contreras A, (2012) Measuring water-borne cortisol in *Poecilia latipinna*: is the process stressful, can stress be minimized and is cortisol correlated with sex steroid release rates? Journal of Fish Biology, <http://dx.doi.org/10.1111/j.1095-8649.2012.03411.x>
4. Schweitzer C, Melot G, Laubu C, Teixeira M, Motreuil S, Dechaume-Moncharmont FX. (2016) Hormonal and fitness consequences of behavioral assortative mating in the convict cichlid (*Amatitlania siquia*). General and Comparative Endocrinology. <http://dx.doi.org/10.1016/j.ygcen.2016.10.010>

RESULTS



CONCLUSIONS

- ◆ The results support our hypothesis that cortisol is higher in dynamic vs isolated social environment. We could not link the increasing cortisol to higher rates of aggression produced toward conspecifics in the dynamic social environment, nor could we link it to higher rates of affiliation received. We did find a positive correlation between behaviors produced over time and higher levels of cortisol produced.
- ◆ The results do not support our hypothesis that 11-ketotestosterone was higher in dynamic vs isolated social environment. We could not link 11-KT to aggression. We saw a negative correlation between affiliation produced and 11-KT--the more affiliation the male produced, the lower levels of 11-KT the male exhibited. However, the level of 11-KT is significantly lower in individuals with fry compared to without fry, showing the relationship between 11-KT and courting behavior.
- ◆ This semester, we mimicked a natural environment by establishing pairs in the dynamic social environment with multiple natural stimuli present compared to the isolated social environment. In the previous semester, we attempted to establish pairs in both isolated and dynamic social environments. The pairs in the dynamic social environment had conspecifics (a natural stimulus) to aggress towards to defend the nest, pair bond, and fry. The pairs in the isolated social environment did not truly establish a pair bond due to the absence of conspecifics, and therefore never exhibited breeding behavior or high levels of overall behavior.
- ◆ In this experiment, our set up better replicated what was observed in their natural habitats. Pairs in nonbreeding schools establish a territory, defend against conspecifics (early stage), and then isolate themselves in order to breed, raise, and defend fry (late stage). Among other predators, convicts cannibalize fry of other pairs. We secluded them into isolated environments to mimic their natural environment, as they would in the wild. For this reason, we believe the data gathered was more accurate. This is why we see similar levels of cortisol and 11-KT between the dynamic and isolated social environments.
- ◆ We did not control for the stages of pair bonding. Only one pair out of eight had fry at the time of sampling in the dynamic social environment, while three pairs out of seven had fry at time of sampling in the isolated social environment. This implies that the stages of pair bonding may have more of an effect on steroid production than social environment, therefore affecting behavior exhibited.
- ◆ Future experiments should focus more on the stages of pair bonding--courting, breeding, and parenting stages of pair bonds may show significant changes in behavior and steroid output.