

**Student Internship Report
to the University of Hawaii Marine Option Program**

**Influence of 17-alpha-methyltestosterone on *Oreochromis mossambicus*
behavior in cultured stocks.**

DURATION

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PROJECT LEADER

Scott Bloom

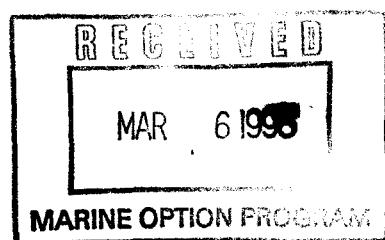
ADVISORS

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FINAL REPORT DEADLINE

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Introduction:

In the spring of 1995, Scott Bloom and Adam Shilling, under the guidance of Dr. George Losey (*Professor of Zoology, University of Hawaii*), set out to gain an understanding of the motivational effects of rearing conditions on cultured tilapia (*Oreochromis mossambicus*). The premise of this study stemmed from the casual observation by researchers at the Hawaii Institute of Marine Biology, Coconut Island, Hawaii, that the rearing conditions for hormone-treated tilapia used in their research were producing fish that were behaving differently from the control fish. An extensive literature search showed that Johnsson and Bjornsson (1994) found "striking changes in the behavior of rainbow trout (*Onchorhyncus mykiss*) that had been reared with growth hormone treatment." They discussed the influence of hormone treatment on the aggression due to appetite in fish and that this finding could have important implications in aquacultured stocks. Among the things they recorded were growth rates, distance from the food source, competitive dominance for that food source, and defense of territory. According to personal communication with Dr. George Losey, in his review of that paper, "It is clear that, with the use of these measures, they could not discriminate between increased boldness, offensive aggression, inhibited fright, or even increased activity due to feeding motivation. Their suggestions that hormone treatment thus might produce 'high gain - high risk' phenotypes and that increased feeding motivation may elevate aggression levels are premature." Thus serving as our premise, we hypothesized that methyl-testosterone treated tilapia would show a larger value for the coloration and aggression index, calculated by the BEAST system (described later in Methods), than the control fish fed a general Purina* trout diet.

Methods:

In designing our experiment, we used past reports of tilapia behavior (Neil 1964, Barlow and Ballin 1976, Losey 1982, and Watanabe et al. 1988) to aid us in our methods. We used contests between pairs of individuals of approximately the same size and sex to assess their motivational status. Fish were allowed three days to acclimate to the test aquarium conditions. We later reduced the acclimation time to 24 hours. Upon suggestion by Dr. George Losey, we finally settled on allowing only overnight isolation and acclimation to the observation tanks. Using this new method, we saw a little more activity among the fish, and on occasion, aggression was seen, but again there were no significant differences among groups.

Two fish were taken from the same rearing tank and were closely matched for size. They were placed in 30 gallon aquaria separated by a P.V.C. opaque barrier. The barrier was removed one day after acclimation, and the behavior was recorded for 15 minutes. The observers were blind to the treatment groups from which the fish were taken. Twenty fish from each treatment were used. The results from the observation were typed directly from the observation in a computer program called the Behavioral Analysis System Technique (BEAST). Using the numerical keys on the keyboard as a means of representing movements, Dr. Losey created an Aggressive Index calculated from the observed behavior as the sum, over both fish, of 2* Approach duration, the time one fish approaches another; 3* Tilt duration, an aggressive indication of defense; 4* Number of Rams/Bites, an offensive aggressive action; 5* Gular duration, another defense mechanism; 6* Tail Wag duration, an offensive attack; and 7* Jaw Gape, a territorial defense mechanism. A paper by Neil (1964) aided in creating a Coloration Index which was calculated to reveal gradation from fright to attack as the sum of the Neutral

duration, 2* Stripe of Bar duration and 3* Dark duration. The results from the experiment remain with Dr. Losey; I never actually saw the data myself.

Results/Discussion:

Our biggest problem in our results was that none of the aquacultured fish behaved as literature suggested. Most of our results ended in both fish being frightened, refusing to feed, showing timid behavior, or freezing on the bottom for hours.

Dr. Losey reported that a two-way ANOVA failed to show significant differences in Sea vs. Fresh Water or untreated vs. methyltestosterone (MT) treated ($p>.4$) for coloration and aggression index. We hypothesized the order of results and the insignificant differences for Coloration Index between the means were in the predicted direction (Sea > Fresh Water and MT > Untreated). Dr. Losey also showed that "if each of the four treatment groups is considered as a qualitatively different treatment, a one-way ANOVA still indicates no overall differences, but Sea Water Controls are indicated as having a higher coloration index than Fresh Water Controls ($p<.05$ by Duncan's multiple Range Test and t-test for Least Significant Difference)." The hormone-treated group did not differ from either control group.

Most of the fish used in prior experiments were from U.C. Berkeley stocks. It is possible that there are genetic differences between the Hawaii stocks and these other fish; we do not know. It is also possible that the aquacultured tilapia densely stocked on Coconut island contributed to the differences between the expected behavior cited in literature and those we observed.

Scott Bloom led the study under the guidance of Dr. Losey to fulfill his certificate requirement at the Marine Option Program, the University of Hawaii at Manoa. Mr. Adam Schilling, a student from Long Island University aided Mr. Bloom in the spring of 1995. Both were involved in literature review, experimental design, fish handling, data collection, and analysis of behavior.

Neither methyl-testosterone treated fish nor the salinity of the rearing environment is indicated as having a significant effect on offensive social aggression. Losey suggests that the differences that were found in coloration could be related to overall levels of arousal as well as to aggression. Continued study will likely reveal the source of effects in tilapia.

ACKNOWLEDGMENTS:

I would like to thank the Marine Option Program of the University of Hawaii for funding the study, Dr. George Losey for his guidance in system design and data analysis, and Adam Schilling for helping out whenever possible.

EVALUATION:

This was a fantastic opportunity for me to work with two accomplished scientists, namely Dr. Losey and Dr. Grau. Although Dr. Gordon Grau wasn't directly involved in the behavioral studies, he provided the space and atmosphere for good fundamental science. It was a learning experience all the way through the ups and downs. Cichlids are a very social class of fish, and understanding their behavior is a lot more difficult than previously thought. I was impressed with the data analysis system developed and designed by Dr. George Losey. The Behavioral Analysis

System Technique (BEAST) system he designed was used during this experiment as a preliminary trial of his new software, and that in itself was exciting. Overall, I learned some good fundamental science in an area where I would otherwise have been lacking. I learned that there are more components to an aquaculture system than just fish production.

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