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Effectiveness of Chronic LHRH-Analogue and 17α-Methyltestosterone Therapy, Administered at Different Times Prior to the Spawning Season, on the Maturation of Milkfish (*Chanos chanos*)

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ABSTRACT

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In this study, 100 milkfish were divided into two groups. In each group, 40 individuals were chronically exposed to the LHRH-a plus 17α-methyltestosterone therapy and the 10 remaining individuals were implanted with placebos. The first group (Experiment I) was implanted in March 1986, the second (Experiment II) in April 1986.

There was no significant difference between the percentage of males that matured in the treated and control groups. A significantly higher percentage of mature females was found among fish receiving hormone implants, however, by the onset of the natural spawning season. Eighty-five percent of the hormone-implanted females (34 of 40) reached a stage of maturity in which induced spawning trials could be initiated. Only 33.3% of the females receiving placebo implants matured to this stage.

The data also suggested that the response of individuals to the therapy exhibits seasonal variation. Chronic exposure to the hormonal therapy appeared to be more effective when initiated closer to the start of the natural breeding season.

INTRODUCTION

Research on the culture of milkfish *Chanos chanos*, has included a substantial effort in the area of reproduction. Even so, only a small proportion of these captive fish have ever spawned. A lack of females which complete vitellogenesis may be one major reason that a standardized method in inducing final maturation and spawning has yet to be achieved (Lam, 1984; Kuo, 1985). Past attempts at using hormones to stimulate maturation have been disappointing (Juario et al., 1980; Lam, 1982; Lacanilao et al., 1985; Lee et al., 1986b).

Several recent reports have provided some ground for optimism. Natural

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maturation and spawning have taken place in floating sea cages (Lacanilao and Marte, 1980; Marte et al., 1984; Marte and Lancanilao, 1986) and more notably, in earthen marine ponds (Lin, 1985). In addition, Lee et al. (1986c) tested five chronic hormone therapies and found that a combination of 200 μg LHRH-a cholesterol pellets and 250 μg 17α-methyltestosterone (17-MT) capsules effectively stimulated maturation of milkfish to the point where they could be induced to spawn. Although these results are encouraging, many questions and problems remain (reviewed by Kelley and Lee, 1986).

In this study, the effectiveness of chronic LHRH-a plus 17-MT therapy was evaluated using a large number of individuals. We also investigated whether the response of the milkfish to the hormone therapy would differ if treatment was initiated at different time intervals prior to the onset of the spawning season.

MATERIALS AND METHODS

A total of 100 adult milkfish was used during this experiment. Individual fork lengths and body weights were obtained at the start of the experiment and the condition of each fish was estimated by calculating the condition factor index (CFI) as described by Ricker (1975). All fish were both fin-clipped for immediate visual identification and implanted intramuscularly with a glass-encased transponder (Identification Devices, Boulder, CO) which provided a more permanent means of identification.

The milkfish were maintained in ten 30 000-l round fiberglass tanks (10 fish per tank). Eighty of the fish had been in these tanks since January 1985 and had been used in a previous experiment (Lee et al., 1986c). The sex of these individuals was known at the start of the experiment. Although the sex of the other 20 fish was not known, they were evenly distributed among the tanks so that, in most cases, a 1:1 sex ratio was achieved in each tank.

Water flow in each tank was adjusted to provide an average water exchange rate between 1 and 1.5 times the volume of the tank per day. Temperature and salinity for each tank were measured daily. All fish were fed Purina trout chow # 6 floating pellets twice a day.

One hundred fish were divided into two groups for Experiment I and Experiment II. Each experiment consisted of 10 control fish receiving placebo implants while 40 treated fish received a 200-μg LHRH-a cholesterol pellet every month plus a 250-μg 17-MT silastic capsule every other month. The procedure for making and administering the two types of hormone implants was previously described (Lee et al., 1985; Lee et al., 1986d). The only difference between the two Experiments was the date on which therapy began. Experiment I was started on 4 March 1986, and Experiment II on 2 April 1986.

The state of maturity for each fish was monitored monthly. The fish were anesthetized with 300 ppm of 2-phenoxyethanol. Mature males were staged by

applying pressure to the abdomen. If sperm was extruded from the gonadopore, the male was scored as mature. Samples of oocytes from each female were obtained via cannulation, fixed in 10% formalin and measured according to the procedure described by Shehadeh et al. (1973) which provides a good indicator of the maturational state of the milkfish ovary (Lee et al., 1986c). A female was considered to be mature when she possessed oocytes averaging 700 μm or more in diameter.

Although all hormone therapies were terminated in June, monitoring of gonadal maturation continued until August which is considered to be the end of the natural spawning season for milkfish in Hawaiian waters (Kuo and Nash, 1979).

All statistical analyses were conducted using the *t*-test (Sokal and Rohlf, 1969).

RESULTS

Temperature and salinity in the maturation tanks remained relatively constant throughout the study (Fig. 1). Temperature ranged between 25.9 and 27.2°C and salinity between 34 and 37‰.

The combined mean fork length, body weight, and CFI (×1000) of the male and female control and treated fish were 64.0±3.4 cm, 4.6±0.8 kg and 1.8±0.1, respectively. There were no significant differences in these measurements between the sexes or treatment groups.

The cumulative percentages of females in each of the two experiments that matured during the course of the study are presented in Fig. 1. The percentage

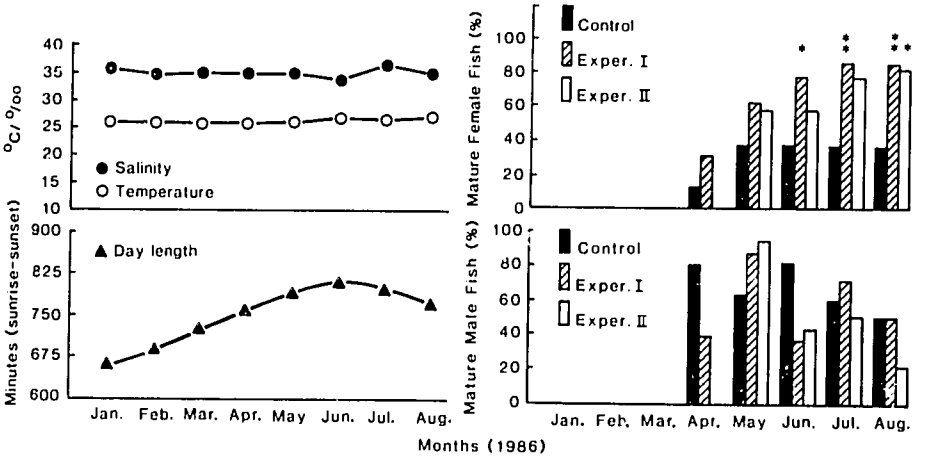

Fig. 1. Salinity, temperature, photoperiod and the cumulative percentage of mature females and males observed during the 1986 maturation study. See text for explanation of the treatments. * – $P<0.05$ ** – $P<0.01$

of fish in each group already maturing at the start of the study is not indicated because of the staggered start of the two experiments. April was the first month in which both control tanks were staged simultaneously, thereby allowing a meaningful statistical analysis between control and treated individuals. Therefore, Fig. 1 records results beginning in April.

In Experiment I, although a small percentage of females was already mature in March (10%), the number increased to 31.6 ± 16.0% after 1 month of therapy. The percentage of treated females that matured at this point was twice that of the control fish (12.5 ± 17.7%), however, there is no statistical difference. This trend continued until July and August when an average of 86.7 ± 16.3% females had reached maturity at least once as opposed to only 32.5 ± 10.6% among the control females ($P < 0.01$).

A similar pattern was observed in Experiment II females. In April, when this group was first checked and implanted, 5% of the females were already mature. One month later, 58.3 ± 16.6% of treated females were mature in contrast to 32.5 ± 10.6% of the control females, but this difference is not statistically significant. The number of females that matured in this group increased steadily and by August, the percentage of females which had matured peaked at 83.3 ± 23.6%. The percentage of mature fish was significantly higher than in the control group at the $P < 0.05$ level. It should be noted that although there was a significantly higher percentage of treated females that reached full maturity (late vitellogenesis), all females (both treated and controls) had primary yolk globule stage oocytes (i.e., early vitellogenesis) at some time during the course of the experiment.

Only three control females reached full maturity (i.e., possessed a clutch of eggs with a mean diameter of 700 μm or larger) during this study and they did so, initially, in April and May. Two of the three rematured two more times by August. Experiment I females also matured an average of three times during the course of the experiment. The variation was much larger, however, ranging between one and five times. Experiment II females matured an average of two times during the course of the experiment, ranging between one and three times. It should be noted that this group was under therapy 1 month less than the corresponding group in Experiment I. If the duration of hormone therapy is taken into account, no significant difference can be found between controls and all other groups in the frequency with which an individual matured.

The monthly percentage of males that were scored as mature during the course of the experiment are presented in Fig. 1. One month after receiving the first treatment, the percentages of mature males in Experiment I and the control group were 38.8 ± 19.3% and 80.0 ± 28.3%, respectively. The percentage of mature males in the control group was either greater than or equal to the combined treated groups every month, except May. Even in May, the difference was not statistically significant.

The size distribution of fish that matured and those that did not is presented

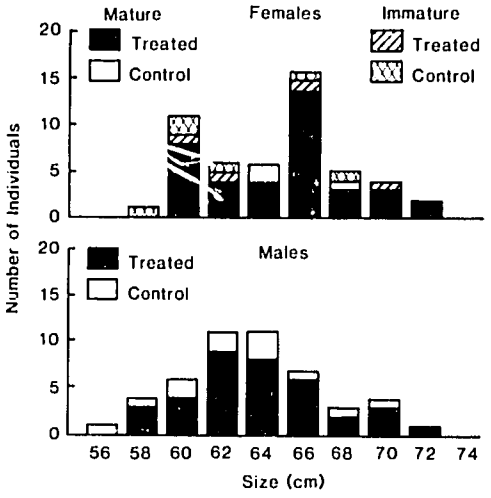

Fig. 2. Size distribution of treated and control fish of both sexes that matured or did not.

in Fig. 2. Control females that matured fell in the middle of the size range of all the individuals that matured. There appeared to be a higher percentage of treated females from the smaller size classes that matured as opposed to untreated individuals. The size distribution of the treated and control males that were found to be mature showed a high degree of overlap. Only one fish did not mature during the course of the experiment. This individual was in Experiment II and was the smallest (in fork length) of all the fish in the experiment.

DISCUSSION

The design of the experiment did not permit determination of the critical size at which milkfish will respond to LHRH-a/17-MT therapy. The number of fish was too small to be conclusive. Within the size range tested, maturation of treated and control fish of both sexes did not show any significant size-related patterns. It should be mentioned, however, that among females in the smaller size classes, a higher percentage of treated fish reached full sexual maturity in comparison to similar sized controls. Furthermore, the one individual which never began maturing was also the smallest fish. The possibility exists that there may be a critical size at which milkfish will respond to this particular therapy. Captive milkfish, like other cultured organisms, often exhibit stunting of growth. Studies on milkfish have shown that although individuals reach the appropriate age to be sexually mature, they are often undersized (Liao and Chen, 1984; Marte and Lacanilao, 1986). We are currently examining the effectiveness of the chronic hormone therapy used in this experiment on such a group of milkfish.

The response of the males to hormonal therapy conflicts with our previously reported results (Lee et al., 1986c). During that trial, a high percentage of similarly treated males matured from April and remained in mature condition until August. In the present experiment, though the same trend began to appear in the month of May, it did not continue for the duration of the experiment. In view of our previous result, it appears that maintenance of spawning conditions by treated males may have been inhibited during the months of June through August. The performance of the control males was similar to the treated males, however, which suggests that the response was not solely due to hormonal therapy. One possible explanation for the reduced period of maturity of the males during the months of June through August is that they were subjected to a higher degree of handling during this time. Peak spawning activity occurred during these months and the fish were repeatedly used for spawning trials. The maturation of male milkfish remains a topic for further investigation.

The performance of control females in this study was found to be consistent with past reports; low numbers of females mature in captivity, even during the peak reproductive season (Lam, 1984; Liao and Chen, 1984; Kuo, 1985; Lee et al., 1986c). In contrast, many treated females matured. This was consistently observed in a relatively large number of individuals, regardless of when the therapy was initiated. Overall, 34 of 40 females (85%) that received LHRH-a plus 17-MT matured. This result was consistent with our previous report (Lee et al., 1986c).

Organs responsible for regulating gonadal maturation in fish reportedly exhibit seasonal changes in sensitivity to their respective hormones (Crim et al., 1983; Kagawa et al., 1983; Kobayashi et al., 1986). There was no significant difference, however, between Experiment I and II in the percentage of females that matured in May, even though fish in Experiment I were treated for a month longer. This implies that our therapeutic treatment can elicit maturational responses at the target tissue level early in the reproductive season. The responsiveness of the individual is presumed to be dependent on the physiological state of the organ systems involved in the maturation process. This particular state of receptivity appears to exhibit some seasonal variation in the milkfish, although the environmental cues milkfish may use are unknown (Lam, 1984; Lee, 1985). The responses observed in this study coincided with increases in daylength; variations in temperature and salinity did not appear sufficient to provide an obvious environmental cue. This is consistent with the hypothesis that photoperiod alone may be the pivotal influence on milkfish maturation (Lee et al., 1986e).

A major issue arising from this experiment is whether the therapy can stimulate the onset of vitellogenesis or is more important in its completion. Every female in the study, whether control or treated, began to mature (i.e., possessed primary yolk granule stage oocytes; Tan, 1985) at least once. However, a much

larger proportion of treated females than control females completed maturation. The capacity of milkfish for multiple spawning during a single spawning season has only recently been demonstrated (Lee et al., 1986a,f; Marte and Lacanilao, 1986). In this study, the average number of times that a female reached full maturity did not differ between treated and untreated groups. Since all females were observed to initiate vitellogenesis during the study, these observations suggest that the hormone therapy may not have affected the number of times vitellogenesis was initiated but rather influenced its completion. This appears paradoxical because all treated fish that matured obviously had to begin vitellogenesis. One possible means of clarifying the issue is to treat milkfish during their normal refractory period. For milkfish in Hawaiian waters this would occur during the months of October through February (Lee et al., in prep.). A positive response at that time would indicate that vitellogenesis can be initiated using this treatment. This topic is currently under investigation.

The effect of hormone therapy on egg quality was not studied, but females that reached the proper state of maturity (Lee et al., 1986a) were induced to spawn. The results of these experiments will be presented in a future report.

In summary, chronic exposure to LHRH-a plus 17-MT substantially increased the number of mature female milkfish. The significantly larger numbers of treated females that matured demonstrate the effectiveness of this particular therapy for influencing maturation in female milkfish. This was not, however, the case with the males.

The effectiveness of the hormone therapy in inducing maturation appears to be dependent on the time when treatment is initiated. In particular, the therapy seems to be more effective when it is begun closer to the onset of the natural spawning season.

In this experiment, a larger percentage of smaller sized females exposed to the chronic hormonal therapy, as opposed to control fish in the same size classes, tended to reach full sexual maturity. The size dependence of response to hormone therapy needs to be more rigorously examined.

Future investigations will focus on clarifying if this particular therapy functions to initiate vitellogenesis, or is more important to its completion.

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