Aquaculture, 56 (1986) 53—62
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Effects of Salinity and Photoperiod on 17α-Methyltestosterone-Induced Spermatogenesis in the Grey Mullet, *Mugil cephalus* L.

CHENG-SHENG LEE and GREG M. WEBER

Oceanic Institute, Makapuu Point, Waimanalo, Hawaii 96795 (U.S.A.)

(Accepted 8 May 1986)

ABSTRACT

Lee, C.-S. and Weber, G.M., 1986. Effects of salinity and photoperiod on 17α-methyltestosterone-induced spermatogenesis in the grey mullet, *Mugil cephalus* L. *Aquaculture*, 56: 53—62.

Male grey mullet, *Mugil cephalus*, can be maintained at a mature stage by feeding diets containing 17α-methyltestosterone (MT). The potency of MT in the maturation of grey mullet at different salinities and photoperiod regimes has not been studied. In this study, the MT was administered in the diet at a dosage of 12.5 mg per kg body weight per day. Salinities ranged from freshwater to 25 ppt. Photoperiod was composed of 8L:16D, 12L:12D and 16L:8D or 6L:18D, 12L:12D and 18L:6D.

Fish were induced to mature in all salinities. There were no significant differences in the rates of maturation for fish in 25, 20 and 10 ppt treatments. The freshwater fish suffered high mortalities, so the results from this group cannot be compared to those of the other groups. One fish, however, did mature, indicating that mullet can be induced to mature in freshwater using MT.

Short photoperiods (less than 12 h of light) favored maturation in both the prespawning and postspawning periods. There did not appear to be any effect of photoperiod on the rate of testicular regression once the fish were taken off the MT diets. The smallest male which matured in this study was about 114 g in body weight.

INTRODUCTION

The grey mullet, *Mugil cephalus*, is a euryhaline fish cultured in freshwater, brackishwater and seawater. In nature, grey mullet matures and spawns during the winter months. These fish have also been observed to mature in freshwater ponds and lakes. However, they do not spawn in these environments (Eckstein, 1975); gonadal atresia occurred in both male and female mullet shortly after they matured in a freshwater lake (Shireman, 1975). Mature mullet from freshwater were acclimated to full seawater over a 12–24-h period and induced to spawn using injections of carp pituitary and luteinizing hormone (Yashouv, 1969). Salinity is therefore an important factor controlling the maturation and

0044-8486/86/$03.50 © 1986 Elsevier Science Publishers B.V.

spawning of grey mullet. Photoperiod is another important factor. Kuo et al. (1974) demonstrated that a 6L:18D photoperiod could be used to stimulate vitellogenesis in grey mullet out of season. There was no study on induced maturation of male mullet through photoperiod manipulation.

It has been demonstrated that MT is an effective steroid for inducing spermatogenesis in the grey mullet. Shehadeh et al. (1973b) used injections of MT to induce spermatogenesis in the spawning and prespawning seasons. Fish were maintained in this condition for 6 weeks. Spermatogenesis was also induced during the postspawning season with oral administrations of MT and these fish were maintained in this condition for 55 weeks under ambient photoperiod conditions (Weber and Lee, 1985).

This study examines the ability of MT to induce spermatogenesis of grey mullet maintained under different salinities and photoperiods at different times of the spawning season. The objective of the study is to induce spermatogenesis of grey mullet more efficiently through the application of MT and the manipulation of environmental conditions.

MATERIALS AND METHODS

Salinity

The observed salinities of water in four 5000-l fiberglass tanks deviated slightly from the designated salinities of 25 ppt, 20 ppt, 10 ppt and 0 ppt despite volume adjustments of incoming seawater and freshwater. The mean observed salinities were 26.8 ppt, 20.4 ppt, 8.0 ppt and 0 ppt. Forty mullets (average body weight of 402 g) were randomly distributed in the above four tanks without sex determination, one tank for each salinity. Mullet are not sexually dimorphic so it is difficult to determine sex out of the spawning season. At the end of the experiment it was determined that 6, 8 and 7 males were in the waters of 25 ppt, 20 ppt and 10 ppt salinity, respectively. Fish in each tank were gradually acclimated to their assigned salinities over a period of 10 days before the inception of the experiment. Individual fish were identified by fin clips on the caudal and/or anal fins. Regenerated fins were clipped again during weekly routine checking.

All fish diets contained the same amount of MT, mixed with codliver oil and added to Purina Catfish Chow #5144 as described by Weber and Lee (1985). The dosage of MT was 12.5 mg/kg body weight per day. The experimental diets were given in the morning and additional regular feed was provided in the afternoon. The fish were checked weekly for the presence of milt by applying gentle abdominal pressure. The criteria for the scoring of maturation are 0 for no milt, 1 for traces of viscous milt, 2 for small amount of running milt and 3 for copious running milt. These mullet ranged from 0 to 3 in maturity. Sperm

V

samples were also collected in polyethylene cannulas and tested for motility in different salinities. The experiment began on 30 June and ran for 5 weeks.

Photoperiod

Two experiments were conducted to determine the effect of photoperiod on the potency of MT for inducing spermatogenesis in mullet at different times during the annual reproductive cycle. Experiments were carried out in three rooms, each containing two 1200-l fiberglass tanks (2 × 1 × 0.6 m in depth). Artificial light was provided by six 48-inch fluorescent lamps hung 2.2 m above the surface of the water. The light intensity at the water surface ranged from 2.2×10^{14} to 3.6×10^{14} quantum s^{-1} cm^{-2}. The photoperiod was controlled by automatic timers. The experimental diet containing MT was given to the mullet at a dosage of 12.5 mg/kg body weight per day. The MT was dissolved in 95% ethanol and then sprayed on the trout chow which was stored in a covered container at 5°C. The control diets were also sprayed with the same amount of 95% ethanol. The diet was fed in the morning and additional plain trout chow was provided ad libitum in the afternoon. Mullet in this study came from a group of fish propagated in early 1983.

The first experiment was initiated in September 1984, during the natural prespawning period for grey mullet in Hawaiian waters. The fish (160.69 ± 41.23 g average body weight) were just under 2 years old. Sixteen fish were held in each of the two tanks in each of the three photoperiod rooms (six tanks in all). One of the two tanks in each room served as a control, the other tank received the MT-supplemented diet. The photoperiod regime in each room was either 8L:16D, 12L:12D or 16L:8D. The fish were acclimated to their respective photoperiods for one month before the experimental diet was given to the treatment groups. The maturational stages of the fish were checked both at the start of the acclimational period and on the day the fish were first fed the experimental diets. During the 4-week experimental period, fish were checked for milt weekly. Then, the fish were dissected and measured for body weight, gonad weight and liver weight. Gonadosomatic index [GSI = (gonad weight/body weight) × 100] and hepatosomatic index [HSI = (liver weight/body weight) × 100] were calculated. Water temperature ranged from 25.8°C to 26.8°C and salinity from 32 to 34 ppt.

The second experiment was conducted in April 1985, during the natural postspawning season of grey mullet in Hawaiian waters (Kuo and Nash, 1975). The fish were 2 years old and weighed 126.83 ± 39.17 g. None of the fish checked had milt at the beginning of the experiment. There were two tanks under each of the three photoperiod regimes; 6L:18D, 12L:12D and 18L:6D. Each tank held 15 fish. One tank of each pair served as a control and the other received MT. The fish were fed the designated diet and experienced the specified photoperiod regime after one week of acclimation in the tank. The fish in the treatment group received the MT for 11 weeks. Then all fish were fed on a

3

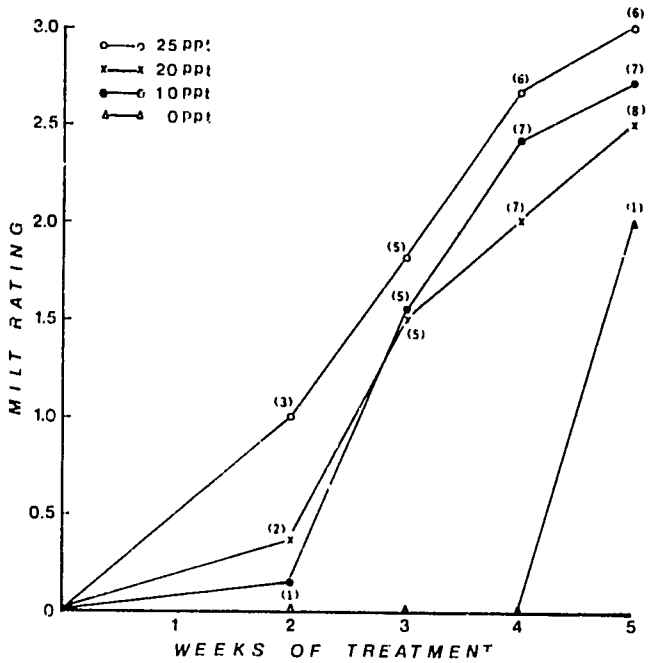

Fig. 1. Maturation of male mullet fed a diet supplemented with MT at different salinities. Figures in parentheses indicate the number of mature fish.

regular diet without MT. All the fish were checked for milt every week until they did not possess any trace of milt. At that time, body weight and standard length were recorded. The fish that did not produce milt throughout the experiment were sacrificed and their gonads were fixed in Bouin's solution for histological examination to determine the sex. Average milt rating for mullet under different photoperiods was calculated thus: average milt rating = sum of score of all males/number of mature males. During the experiment, water temperature was 25.8 to 26.8 °C and salinity was 32 to 35 ppt.

RESULTS

Salinity experiment

Maturity of fish in this experiment was checked and scored according to the condition of milt every week except the first (Fig. 1). In the second week of the experiment, 3, 2 and 1 mature males were found in the waters of 25 ppt, 20 ppt and 10 ppt salinity, respectively. These results represent maturation rates of 50%, 25.0% and 14.3% in each of the above salinities. A fish with copious running milt was found in the water with 25 ppt salinity. No male matured in freshwater at that time. By the fourth week, all the males in the waters of 25 ppt and 10 ppt matured, but one out of eight males in 20 ppt did not mature

TABLE 1

Average rating for milt condition, number of males and percentage of mature male mullet induced by MT during the prespawning period

Photoperiod	16L:8D		12L:12D		8L:16D	
	treated	control	treated	control	treated	control
Average rating for milt condition (mean ± SD)	1.75 ± 1.23	0.36 ± 0.78	2.33 ± 0.71	0.56 ± 0.80	2.53 ± 0.64	0.71 ± 0.82
Number of mature males	10	6	12	9	12	10
Number of males in group	12	11	12	12	12	14
Mature males (%)	83	54.5	100	75	100	71

until one week later (Fig. 1). Fish in the freshwater tanks experienced high mortality from undefined causes after the second week in the tank. One of the remaining three fish was a male and graded as a "2" in week 5.

Sperm from the fish in 10 ppt were not activated in water of 10 ppt but were activated in the 32 ppt seawater. The motility of sperm from the fish in 20 ppt seawater was very weak in seawater of the same salinity. Those sperm were activated in 32 ppt seawater.

Photoperiod experiment

After the fish in the first experiment were exposed to various photoperiod regimes for one month, some of the fish showed milt before receiving MT. There were 2 mature males out of 13 males under a 16L:8D photoperiod, 13 out of 24 under 12L:12D and 9 out of 26 males in 8L:616D.

The average milt ratings for treated and control groups are listed in Table 1. The average rating for the treated group was 3.5 to 4.5 times greater than that of the control group under the same photoperiod. The smallest size of mature male mullets in this experiment was 114 g in the treatment group and 119 g in the control group. Gonadosomatic indices (GSI) and hepatosomatic indices (HSI) of treated and control groups are shown in Table 2. Both GSI and HSI were higher in the treatment groups than in the control groups. The difference in GSI was significant ($P < 0.05$) for all three photoperiod conditions (Table 2). There was no significant effect of photoperiod on the values of GSI and HSI. All the males in the treatment group matured by the end of the experiment with the exception of the 16L:8D photoperiod in which 83% matured.

In the third week of the second experiment, 55.6% of the males matured in the 6L:18D photoperiod, 40% in 12L:12D and 33.3% in 18L:6D (Fig. 2). All

TABLE 2

Gonadosomatic indices [(gonad weight/body weight) × 100] and hepatosomatic indices [(liver weight/body weight) × 100] for mullet fed a diet supplemented with MT for 4 weeks during the prespawning period

Photoperiod	Group	Male			Female		
		Mean ± S.D.	Range	Sample size	Mean ± S.D.	Range	Sample size
			Gonadosomatic index				
16L:8D	Treatment	0.54 ± 0.27**	0.05–0.94	12	0.28 ± 0.11	0.13–0.44	4
	Control	0.18 ± 0.22	0.04–0.86	12	0.28 ± 0.05	0.23–0.34	4
12L:12D	Treatment	0.45 ± 0.20**	0.16–0.74	12	0.26 ± 0.23	0.22–0.28	4
	Control	0.26 ± 0.16	0.10–0.58	12	0.40 ± 0.09	0.26–0.51	4
8L:16D	Treatment	0.41 ± 0.23*	0.15–0.60	12	0.22 ± 0.06	0.15–0.29	3
	Control	0.23 ± 0.17	0.04–0.57	14	0.36 ± 0	0.36	2
			Hepatosomatic index				
16L:8D	Treatment	1.30 ± 0.23**	0.77–1.68	12	1.24 ± 0.18	1.03–1.47	4
	Control	0.98 ± 0.14	0.71–1.19	12	1.00 ± 0.10	0.88–1.14	4
12L:12D	Treatment	1.62 ± 0.27**	1.24–2.20	12	1.50 ± 0.11	1.36–1.67	4
	Control	1.06 ± 0.13	0.85–1.28	12	1.04 ± 0.16	0.88–1.29	4
8L:16D	Treatment	1.17 ± 0.29	0.40–1.64	12	1.60 ± 0.25	1.35–1.94	3
	Control	1.11 ± 0.13	0.90–1.42	14	1.16 ± 0.06	1.10–1.22	2

*Significant difference at $P < 0.05$.
**Significant difference at $P < 0.01$.

these mature fish were sacrificed at this time. The average size of mature fish was 151.3 ± 42.4 g or larger than that of all the remaining male fish in the treatment groups (112.6 ± 21.1 g). The average GSIs of the sacrificed treatment and control fish are listed in Table 3. There were significant ($P < 0.05$) differences between control and treatment groups under the photoperiod of 18L:6D. All of the males receiving MT in the 12L:12D photoperiod matured

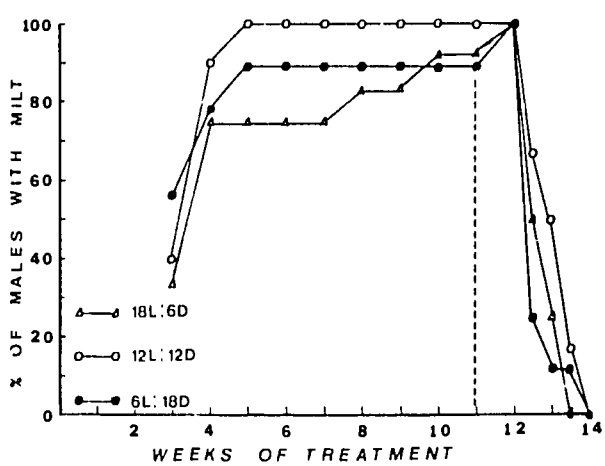

Fig. 2. Percent of mature male mullet after feeding a MT-supplemented diet under various photoperiod regimes during the postspawning period. MT was withdrawn from the diet after 11 weeks.

TABLE 3

Gonadosomatic indices and average body weight for male mullet producing sperm in response to 3 weeks of 17α-methyltestosterone treatment and male mullet in control groups during the postspawning period

Photoperiod	Group	Gonadosomatic index		Body weight (g)		Sample size
		Mean ± S.D.	Range	Mean ± S.D.	Range	
18L:6D	Treatment	0.14 ± 0.05*	0.09–0.22	153.82 ± 55.36	79.85–228.95	4
	Control	0.05 ± 0.01	0.03–0.05	155.80 ± 56.16	87.51–243.31	4
12L:12D	Treatment	0.23 ± 0.15	0.09–0.47	140.53 ± 25.22	110.71–180.27	4
	Control	0.06 ± 0.02	0.03–0.09	129.87 ± 28.85	88.82–163.18	4
6L:18D	Treatment	0.14 ± 0.08	0.06–0.30	157.77 ± 39.53	108.38–204.75	5
	Control	0.07 ± 0.03	0.04–0.10	156.25 ± 49.25	107.00–205.49	2

*Significant difference at $P = 0.05$.

by the fifth week of treatment (Fig. 2). However, one male in each of the other treatment groups did not mature until one week after MT was removed from the diet (Fig. 2). Throughout the experiment, the condition of mature males in the three photoperiods did not differ from the average rating (Fig. 3). There was also no difference in the regression time for the mature males under different photoperiods after MT was withdrawn from the diet (Fig. 3). The time for complete regression of the testes of mature males was 2.5 to 3 weeks. No

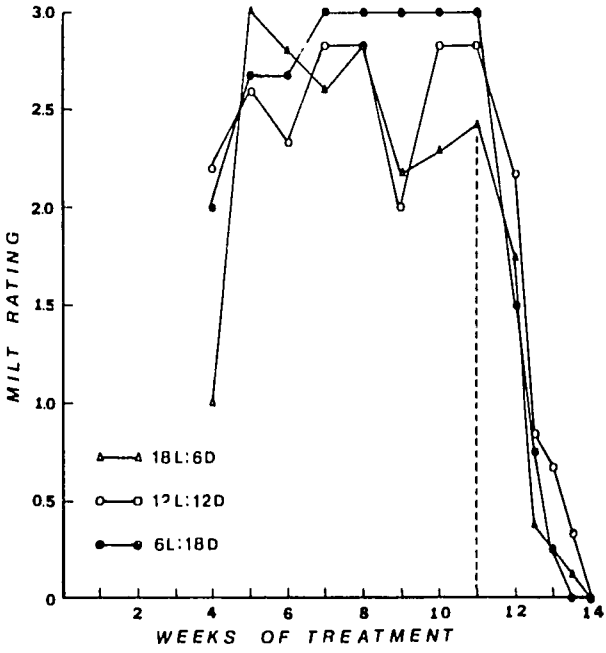

Fig. 3. Gonadal maturation (expressed as milt rating) of male mullet during feeding of a diet supplemented with MT and after withdrawal of the hormone (week 11).

males matured in any of the control groups except one male in the 6L:18D room which showed traces of viscous milt in the last week of the experiment.

DISCUSSION

The experiment related to the salinity factor was conducted about 4 or 5 months before the beginning of natural spawning of grey mullet in Hawaii which occurs from mid-December to the beginning of March (Kuo and Nash, 1975). The gonadal maturation of males in this experiment could be attributed solely to the administration of MT. From the condition of mature males under different salinities, the males at 25 ppt appeared to be in slightly better running condition than those at the other salinities throughout the experiment (Fig. 1). Also, there was a higher percentage of males that matured earlier at 25 ppt than at the other salinities. However, the average water temperature at 25 ppt was 1 C higher than the average temperature in 10 ppt and in freshwater. Although the effect of water temperature on the potency of MT is not known, it seems that there is no significant difference in the potency of MT to male mullets at the salinities from 10 ppt to 25 ppt.

This study also showed that male mullet will mature in freshwater through the application of MT in the diet. However, the high mortality in freshwater made the comparison with mature males in the other conditions impossible. In our other study (Weber and Lee, 1985), we found that male mullet from 4 ppt experienced high mortality in freshwater. However, they possessed milt for 11 weeks in freshwater. Eckstein (1975) indicated that male mullets in freshwater could synthesize the necessary steroids for testicular development. In another experiment (Shireman, 1975) it was shown that mature mullets of both sexes undergo atresia shortly after transfer to a freshwater lake, controverting our findings. Therefore, further study on inducing male maturation in freshwater is required.

Our results showed that sperm from fish in 10 ppt were not mobile in 10 ppt. We did not test sperm fertility with mature eggs. However, the study conducted by Hu and Liao (1981) obtained fertilization and no pigmented embryos in salinities as low as 7.0 ppt. Eckstein (1975) reported that the infertility of grey mullet in freshwater is limited to female fish. The motility of sperm in water where mullets mature needs to be verified.

The first photoperiod experiment was conducted about 3 months before the spawning season in Hawaiian waters. Some of the male mullets were induced to mature after being in various photoperiods for one month. At the same time, there were few mature males in the outdoor ponds. This indicates that maturation of male mullets was accelerated by the manipulation of photoperiod. The percentages of mature males in each photoperiod regime were 8.7%, 54.2% and 34.6% with the shortening of daylength. This indicates that short daylight, less than 12 h, favors the maturation of male mullet. After 8 weeks, the percentage

of males which matured in this experiment was higher under shorter photoperiod regimes in both treatment and control groups (Table 1). The treatment groups which received MT had more mature males than the control groups, regardless of photoperiod regime (Table 1).

The second photoperiod experiment was conducted during the postspawning season. Males in the control groups did not mature during the experiment, with the exception of one fish. In the treatment groups, male mullets showed an earlier response under shortened daylight conditions (Fig. 2). Therefore, it required less time to mature male mullets by the application of MT if the daylength was shortened.

The average ratings for milt condition were higher in the treatment groups than in the control groups and increased with the shortening of daylight in Experiment 1 (Table 1). Average ratings of mature males in the second experiment did not differ between daylight regimes (Fig. 3). There was no difference in the GSI of male mullets under different photoperiods in both Experiments 1 and 2 (Tables 2 and 3). However, GSIs in some treatment groups of both experiments were significantly higher than in the control groups (Tables 2 and 3).

In both treatment and control groups, GSIs in Experiment 1 were about three times higher than those in Experiment 2 (Tables 2 and 3). This was explained by the timing of Experiment 1 during the prespawning season and Experiment 2 during the postspawning season. Theoretically, GSIs should not relate to fish size. Differences in size were, therefore, not considered. The results also suggested that MT initiated spermatogenesis and spermiation but not the growth of testes. Thus, the GSIs were much smaller than those reported by Shehadeh (1973a).

This experiment was not designed to define the minimum size of male mullets which could be induced to mature by the application of MT. In the second photoperiod experiment, however, larger mullets matured earlier than smaller ones. Experimental fish in this study were about 2 years old, the minimum age for maturation in many locations (Brusle, 1981). Consequently, the effect of size on maturation became critical. Additional experiments are required to confirm this conclusion. The smallest mullet to mature in this study was about 114 g in body weight.

In conclusion, 17α-methyltestosterone can be used to induce maturation of male mullets in freshwater and under different photoperiod regimes. However, fish respond earlier to the treatment if conditions are optimal for the maturation of mullet.

ACKNOWLEDGEMENTS

Part of this work was supported by a grant from the United States Agency for International Development (DAN-4161-A-00-4055-00). We wish to thank

9

J. Thomas for his assistance in the salinity experiment and K.M. Cho in the first photoperiod experiment. Our thanks also to A. Belanger for preparation of the manuscript.

REFERENCES

Brusle, J., 1981. Sexuality and biology of reproduction in grey mullet. In: O.H. Oren (Editor), Aquaculture of Grey Mullets. Cambridge University Press, Boston, MA, pp. 99–154.

Eckstein, B., 1975. Possible reasons for the infertility of grey mullets confined to fresh water. Aquaculture, 5: 9–17.

Hu, F. and Liao, I.-C., 1981. The effect of salinity on the eggs and larvae of grey mullet, *Mugil cephalus*. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, 178: 460–466.

Kuo, C.-M. and Nash, C.E., 1975. Recent progress on the control of ovarian development and induced spawning of the grey mullet (*Mugil cephalus* L.). Aquaculture, 5: 19–29.

Kuo, C.-M., Nash, C.E. and Shehadeh, Z.H., 1974. The effects of temperature and photoperiod on ovarian development in captive grey mullet (*Mugil cephalus* L.). Aquaculture, 3: 25–43.

Shehadeh, Z.H., Kuo, C.-M. and Nash, C.E., 1973a. Establishing broodstock of grey mullet (*Mugil cephalus* L.) in small ponds. Aquaculture, 2: 379–384.

Shehadeh, Z.H., Madden, W.D. and Dohl, T.P., 1973b. The effects of exogenous hormone treatment on spermiation and vitellogenesis in the grey mullet, *Mugil cephalus*. J. Fish Biol., 5: 479–487.

Shireman, J., 1975. Gonadal development of striped mullet (*Mugil cephalus*) in fresh water. Prog. Fish Cult., 37: 205–208.

Weber, G.M. and Lee, C.-S., 1985. Effects of 17α-methyltestosterone on spermatogenesis and spermiation in the grey mullet, *Mugil cephalus* L. J. Fish Biol., 26: 77–84.

Yashouv, A., 1969. Preliminary report on induced spawning of *M. cephalus* (L.) reared in captivity in freshwater ponds. Bamidgeh, 21: 19–24.