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Studies on the Maturation and Spawning of Milkfish *Chanos chanos* Forsskal in a Photoperiod-Controlled Room

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Abstract

Progress in propagation of milkfish has been impeded by the shortage of mature broodstock. This study was a first step toward revealing the environmental cues for the maturation of milkfish. Experiments were carried out during 1978–1985 in an indoor tank (5.2 × 4.8 × 1.3 m) under photoperiod control and in an outdoor tank (6.3 × 5.4 × 1.3 m). In two controlled, long-photoperiod regime experiments, 86 and 83% of the fish matured, the highest percentages yet reported. In these experiments, milkfish matured one month earlier than the normal spawning season, two months after the fish were exposed to the long daylight regime. Only 21% of the fish under a natural photoperiod regime in the outdoor tank matured. Results of the current study suggest that a change and increase in photoperiod are necessary for the onset of maturation.

The importance of milkfish (*Chanos chanos* Forsskal) as a food or bait fish has been reported by Kuo et al. (1979); Liao et al. (1979); and Juario et al. (1984). Demand for milkfish fry has motivated research on artificial propagation, however, progress on spawning and propagation of milkfish has been constrained by the lack of mature broodstock (Kelley and Lee 1986). The establishment of a captive broodstock would provide a reliable source of mature individuals for production of hatchery-reared milkfish fry.

Environmental cues which trigger the maturation of fish are often absent from holding facilities. Various environmental stimuli influence activity in the hypothalamic-pituitary-gonadal axis and consequently control the secretion of reproductive hormones in teleosts (Crim 1982). Therefore, environmental manipulation and exogenous hormone treatments have long been used to facilitate maturation in many fish species.

The relation of environmental factors to fish reproduction varies among species and has recently been reviewed by Lam (1983) and Stacey (1984). Environmental cues which initiate milkfish maturation have not been defined (Lee 1985). Milkfish that achieve sexual maturity are usually larger than 2 kg in body weight and 5+ years old (Liao 1974; Liao and Chen 1979). Because of their size, a larger holding facility is required to accommodate the fish and to avoid the stress of overcrowding. Conducting environment-related experiments requires a more complex facility. Consequently, experiments in these areas have not been conducted.

In this study, we examined the effects of photoperiod alone and in conjunction with luteinizing hormone-releasing hormone (LHRH) implants on the maturation of milkfish prior to and during the normal breeding season.

Materials and Methods

Two separate experiments were conducted in which adult milkfish were exposed to a specific photoperiod regime. Experiment I utilized a single, photoperiod-controlled holding facility. Experiment II was conducted in the same holding facility, but a control outdoor tank, exposed to ambient light levels, was added. The photoperiod control portions of this study were carried out in an indoor rectangular concrete tank, 5.2 × 4.8 × 1.3 m deep. Two channels, 4.8 × 0.8 × 0.6 m deep, were built into

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each side of the tank bottom to facilitate capture and handling of fish. Four sets of 2 m long, double-lined fluorescent lights were hung 1.1 m above the surface of the water. Light intensity at the water surface ranged from 4.2×10^{14} to 1.05×10^{15} quanta/sec cm. The light regime was controlled by an automatic timer. Throughout all experiments, water temperature and salinity ranged between 25–27 C and 30–36 ppt, respectively.

In Experiment I, 12 three-year-old fish (fork lengths 55–70 cm) were obtained from local Hawaiian fish ponds and stocked into the tank in 1978. The primary diet was Ralston Purina Catfish Chow. Photoperiod was constant at 18L:6D until August of 1981. It was then adjusted to 6L:18D for three months, followed by 12L:12D for another three months, and finally 18L:6D until September of 1982.

The controlled photoperiod portion of Experiment II repeated the same photoperiod regime as in Experiment I. The photoperiod was set to 10L:14D in August, 1984, was adjusted to 12L:12D in November, and was finally changed to 18L:6D on 28 February 1985. The 11 fish (67–83 cm in fork length) were fed Purina Trout Chow #6 exclusively. On February 28, five of the eleven fish were implanted intramuscularly with a hormone cholesterol pellet containing 200 μg of des-Gly^{10} (D-Ala^{6})-LHRH Ethylamide (Sigma Chemical Company). Pellet preparation and implantation techniques were described by Lee et al. (1985). Each fish received one cholesterol pellet every month from March through June. The other fish received no hormone treatments.

Fourteen fish in the ambient photoperiod portion of Experiment II ranged between 66 and 82 cm in fork length and received the same diet as those in the indoor tank with the exception of algae which grew in the outdoor tank. The tank measured 6.3 × 5.9 × 1.3 m and was covered by a translucent fiberglass roof 2.5 m above the tank. Water temperature and salinity ranged between 25–28 C and 35–39 ppt, respectively. Ten of the fourteen fish were implanted with the same LHRH-a cholesterol pellets described above. The four remaining fish were implanted with placebos.

Milkfish maturation was assessed each year during the normal spawning season in Hawaii, i.e., June through August (Kuo and Nash 1979). Female maturational stage was assessed by inserting a cannula through the urogenital pore to sample gametes as described by Shehadeh et al. (1973). Males were considered mature if pressure on the abdomen resulted in milt being expelled from the gonadopore. Females with oocytes larger than 700 μm in diameter were removed to an outdoor 10,000 L tank for induced spawning trials. Hormones used to induce spawning in Experiment I included acetone-dried salmon pituitary homogenate (SPH), acetone-dried carp pituitary homogenate (CPH), and human chorionic gonadotropin (HCG). Hormone dosages were based on data developed by Kuo et al. (1979). LHRH-a (250–300 μg/fish) was used to induce spawning of fish in Experiment II.

Results

Experiment I—Controlled Photoperiod

None of the seven fish remaining in the photoperiod room in August 1981 had matured, but six of the seven matured in May 1982. This constitutes an 86% success rate in maturation induction. The sex of the remaining fish could not be determined. Of the mature fish, four were female and two were male. Table 1 lists the average egg diameters for mature females in this experiment that were used in induced spawning trials. The two mature males were not discovered until June 14.

The first two mature females were found on May 21, moved immediately to an outdoor tank and injected with HCG and fish pituitary (Table 1). Nineteen hours later, hydrated eggs were released by one of the fish. Eggs were stripped from both females 22 hours after the first injection, and were

TABLE 1. *Summary of hormone dosage and injection frequency to four milkfish held under a photoperiod regime with 4 months at 10L:14D, 3 months at 12L:12D and 1–5 months at 18L:6D.*

Fish no.	Initial egg diam. (m)	Body weight (kg)	Injection dose	Hours after cannulation	Remarks
1	858	6.8	100 mg SPH[a] + 10,000 I.U. HCG[b] 50 mg SPH + 500 g LH-RH[c]	1 18	1. Hydration of eggs was proceeding. 2. No fertilization was achieved due to a lack of males.
2	840	7.3	100 mg CPH[d] + 10,000 I.U. HCG 50 mg CPH + 20,000 I.U. HCG	1.5 18.5	1. Eggs were found in the water 19 hours after 1st injection. 2. The eggs were stripped at 22 hours after 1st injection. 3. No fertilization was achieved due to a lack of males.
3	940	9.1	110 mg CPH + 10,000 I.U. HCG 50 mg CPH + 20,000 I.U. HCG	3 21	1. Seven hours after 1st injection hydration of eggs was confirmed. 2. No eggs were obtained from the stripping at the 25th hour after injection.
4	920	8.2	100 mg SPH + 10,000 I.U. HCG 50 mg SPH + 20,000 I.U. HCG	3 21	1. Fish released some eggs spontaneously after 18 hours of 1st injection. Stripping was followed 3 hours later. Eggs were fertilized.

[a] Salmon pituitary homogenate.
[b] Human Chorionic gonadotropin.
[c] Luteinizing release hormone.
[d] Carp pituitary homogenate.

fertilized with the sperm of *Mugil cephalus*, as there were no available male milkfish. False fertilization was observed.

Three weeks later, another two females with egg diameters of 920 and 940 μm were injected with hormones (Table 1) even though the eggs were partially atretic. One of these fish did not develop further and, although handstripping was attempted, no eggs were obtained. The other fish was stripped approximately 21 hours after the first injection. The eggs were fertilized with milt from one of the males. An estimated 79,200 eggs were obtained from this female and about 1% were fertilized. An estimated 70% of the fertilized eggs hatched. Many larvae had deformed vertebrae and died before the absorption of the yolk sac was complete.

Experiment II—Controlled Photoperiod

Four of the five fish in the implant group and five of the six in the non-implant group matured during the experiment, constituting an overall maturation rate of 82.8% (Table 2). Fish that did not mature were the smallest in the experiment. Two of the three females in the implant group possessed eggs larger than 800 μm in diameter at the beginning of April, while the two females in the non-implant group did not have eggs larger than 750 μm before May 6. Thus, fish in the implant group matured one month earlier than fish in the non-implant group. Only one male in either group possessed running milt during the monthly sampling in June (from the implant group) and July (from the non-implant group).

TABLE 2. *Egg diameters and running condition of milkfish held under a photoperiod regime with 4 months at 10L:14D, 3 months at 12L:12D and 1–5 months at 18L:6D.*

Fish no.	Sex	FL (cm)	Gonads egg diameters or milt condition	April 5	May 6	June 3	July 1	August 2
				Fish receiving implants				
1	F	76.0	mean ± SD	0.85 ± 0.04	0.04 ± 0.08	0.84 ± 0.16	0.37 ± 0.07	0.70 ± 0.08
			range	0.75–0.95	0.30–0.70	0.25–1.05	0.20–0.60	0.45–0.85
			no. of eggs	79	100	100	114	112
2	F	71.5	mean ± SD	0.95 ± 0.03	0.30 ± 0.03[a]	0.35 ± 0.04	0.85 ± 0.08	0.31 ± 0.03
			range	0.90–1.00	0.25–0.40	0.25–0.45	0.70–1.00	0.25–0.40
			no. of eggs	4	100	100	114	100
3	F	72.0	mean ± SD	—	0.73 ± 0.11	0.70 ± 0.26	0.86 ± 0.88	0.72 ± 0.11
			range	—	0.45–0.90	0.25–1.00	0.30–1.05	0.30–0.90
			no. of eggs	—	101	102	54	106
4	M	80.0	milt rating[b]	1	0	1	1	0
5	?	69.5		—	—	—	—	—
				Fish not receiving implants				
6	F	83.0	mean ± SD	—	0.83 ± 0.04	0.69 ± 0.08	0.43 ± 0.06	0.78 ± 0.08
			range	—	0.75–0.95	0.25–0.90	0.25–0.55	0.25–0.95
			no. of eggs	—	55	96	100	105
7	F	71.5	mean ± SD	0.51 ± 0.07	0.79 ± 0.06	0.43 ± 0.05	0.80 ± 0.05	0.33 ± 0.05
			range	0.30–0.65	0.60–1.00	0.25–0.55	0.60–0.95	0.20–0.45
			no. of eggs	78	74	110	102	102
8	M	76.5	milt rating	0	0	0	3	1
9	M	71.5	milt rating	0	0	0	0	1
10	M	70.5	milt rating	0	0	0	1	0
11	?	67.5		—	—	—	—	—

[a] Fish was not reimplanted at this time.
[b] Milt rating—0 = no milt observed, 1 = traces of milt, 2 = small amounts of milt, 3 = copious running milt.

The observed frequency distribution of egg sizes (Table 3) indicates that all five females were capable of multiple spawning during the season. Each female possessed eggs larger than the critical size required for hormone-induced spawning (Juario et al. 1984) at least every other month. Spontaneous spawning occurred four times from April to August. The first two spawnings were obtained from implanted Fish #2 and 3, which spawned two days and eight days after hormone implantation, respectively. Each spawn produced only a few eggs and none were ferilitized in the absence of mature males. The last two spawnings came from unidentified females. The third spawning resulted in the release of 300,000 eggs, a fertilization rate of 83% and a hatching rate of 24.1%. The fourth spawning produced 100,000 eggs, but these were not fertilized even though mature males were present.

In August, induced spawning by injection of 250 μg of LHRH-a in liquid form was attempted without success on Fish #1 and 3. Mean egg diameters for Fish #1 and 3 were 722 and 700 μm, respectively.

Experiment II—Ambient Photoperiod

Three of ten fish in the implant group matured and no fish in the non-implant group matured (Table 3). The two females that matured fully were the two largest fish in the tank. Fish #2 had egg diameters larger than 800 μm in May and August and was therefore capable of multiple spawning during the season.

TABLE 3. *Egg diameters and running condition of milkfish exposed to ambient photoperiod in Experiment II.*

Fish no.	Sex	FL (cm)	Gonads egg diameters or milt condition	April 4	May 5	June 6	July 5	August 2
				Fish receiving implants				
1	F	78.5	mean ± SD	—	0.64 ± 0.11	0.47 ± 0.14	0.47 ± 0.18	0.85 ± 0.05
			range	—	0.35–0.80	0.20–0.85	0.30–0.95	0.65–0.95
			no. of eggs	—	55	137	14	100
2	F	82.0	mean ± SD	small eggs	0.90 ± 0.07	0.58 ± 0.12	0.44 ± 0.15	0.82 ± 0.01
			range	0.2–0.3	0.75–1.05	0.30–0.80	0.25–1.05	0.30–0.95
			no. of eggs		114	111	93	102
3	F	67.5	mean ± SD	—	—	—	—	Previtellogenic oocytes
4	F	73.5	mean ± SD	—	—	—	—	Previtellogenic oocytes
5	M	70.0	milt rating[a]	3	2	1	3	3
6	?	77.5		—	—	—	—	—
7	?	73.0		—	—	—	—	—
8	?	66.0		—	—	—	—	—
9	?	69.5		—	—	—	—	—
10	?	77.0		—	—	—	—	—
				Fish not receiving implants				
11	F	68.0	mean ± SD	—	0.32 ± 0.02	—	Previtellogenic oocytes	0.28 ± 0.02
			range	—	0.25–0.35	—		0.20–0.30
			no. of eggs	—	100	—		20
12	F	76.0	mean ± SD	—	—	—	—	0.43 ± 0.05
			range	—	—	—	—	0.25–0.50
			no. of eggs	—	—	—	—	108
13	?	68.0		—	—	—	—	—
14	?	75.0		—	—	—	—	—

[a] Milt rating—0 = no milt observed, 1 = traces of milt, 2 = small amounts of milt, 3 = copious running milt.

Spontaneous spawnings were observed twice, on March 26 and May 8. The first spawn occurred 27 days after hormone implantation, and the second spawn took place 3 days after hormone implantation. Both spawns produced only a small number of eggs, and fertilization did not take place in either case, even though a mature male was present.

Two fish with mean egg diameters of 853 μm for Fish #1 and 823 μm for Fish #2 were each injected with 300 μg of LHRH-a in liquid form in August. Fish #1 spawned one day after injection and Fish #2 began dribbling eggs after one day, but did not complete spawning until that night. Fertilization did not occur with either spawning attempt.

Discussion

Maturation of milkfish was not observed in Experiment I, either before or during 1981, under a constant long-daylight regime. When daylight was increased in 1982, however, milkfish began to mature. To our knowledge, this is the first incidence of milkfish maturation under artificial lighting conditions. This result implies that a change and increase in daylight may be required to stimulate the onset of the maturation process in milkfish. A point to consider is that fish that matured in 1982 were a year older. The difference in age of milkfish that matured should also be considered as a factor in maturation. Lin (1984) demonstrated that

older milkfish showed a higher percentage of maturity in captivity. Further experimentation on the effect of age is necessary.

Under the same photoperiod regime, females in both Experiments I and II had eggs larger than the critical size for induced spawning by May and as early as April in Experiment II. In Experiment II, a much greater percentage (83%) of fish exposed to a controlled photoperiod in the indoor tank reached full maturity than fish exposed to ambient photoperiods in the outdoor tank (21%). An increase in photoperiod seems to trigger the maturation of milkfish. A longer photoperiod is an important factor in the control of maturation of summer-spawning fish (Lam 1983). If this is true, it will be possible to control the maturation of milkfish by manipulating photoperiod. Indeed, milkfish in this experiment matured in April, a month before the normal spawning season (Kuo and Nash 1979). Milkfish in the outdoor facilities also matured by April through hormone implantation and were, in fact, the first to spawn. The percentage of mature fish was, however, much lower. The increased maturation rate of indoor milkfish may be directly attributed to photoperiod adjustment.

In Experiment II, milkfish in the indoor tank, implanted with exogenous hormones, matured at least one month earlier than fish receiving no hormones. Milkfish that did mature in the outdoor tank also received hormone implants. LHRH-a cholesterol pellet implantation has been proven to be an effective method for enhancing the maturation of milkfish (Lee et al. 1986*a*).

In this study, milkfish were induced to spawn by LHRH-a pellet implants, LHRH-a liquid injection or CPH and HCG injections (Table 2). Ovulation was achieved by a single application of exogenous hormone to fish which possessed eggs larger than 823 μm in diameter. Fish which possessed eggs with diameters less than 823 μm did not ovulate as a result of the single hormone injection of LHRH-a. More than one injection was necessary for the final maturation of milkfish with small eggs (Kuo et al. 1979; Juario et al. 1984; Lam 1984). Our recent studies also indicate that the critical egg diameter for inducing spawning by single hormone injection is 800 μm (Lee et al. 1986b).

In conclusion, milkfish matured in an indoor tank of 25 m^3 under a long daylight regime. Maturation occurred in a larger percentage of fish when exposed to long photoperiods in an indoor tank than in an outdoor tank with exposure to the ambient photoperiod. Implantation of LHRH-a pellets caused milkfish to mature one month earlier than untreated fish. Milkfish spawned in both tanks.

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