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## Experiments on the induced spawning and larval rearing of the milkfish, *Chanos chanos* (Forsskal) in 1979\*

J. V. Juario, M. Natividad, G. Qunitio and J. Banno

This paper summarizes the results of the experiments on the induced breeding and larval rearing of milkfish during the 1979 season.

Milkfish spawners caught in the fish corrals in Tigbauan and in Hamtik were transported to the laboratory and sexed by examining the gametes obtained by inserting a polyethylene cannula through the region of the urogenital papilla. The developmental stage of the oocytes was determined from their mean diameter. The spawning agents used for these experiments were the acetone-dried pituitary gland homogenate of the coho salmon (SPH) prepared by the British Columbia Research Council, Vancouver, Canada, and human chorionic gonadotropin (HCG) from Ayerst Laboratories, New York.

The response of the newly caught spawners to the hormone treatments is presented in Table 1. One female, having oocytes with a mean diameter of  $0.67 \pm 0.06$  mm and receiving a total dose of 280 mg SPH and 31,250 HCG in four injections, successfully completed the hydration process. The eggs were stripped and fertilized by sperms of males held captive in rubberized canvass tanks for 3 days. The eggs were not however, fertilized. Another female, having oocytes with a mean diameter of  $0.77 \pm 0.04$  and receiving a total of 140 mg SPH and 20,000 IU HCG in two injections, was also successfully induced to ovulate in captivity. The eggs were stripped 10.5 hrs after the 2nd injection and these were fertilized by sperms from males caught at the same time as the female and each injected with 5,000 IU HCG. The number of eggs stripped was about 1.4 million, the biggest number so far, and the fertilization rate was 59.4%.

The fertilized eggs were thoroughly washed and incubated in 600-liter fiberglass tanks. The hatching rate was estimated to be 39.7%. A total of about 25,492 larvae were reared in six 600-liter circular fiberglass tanks for mass production experiments at a temperature of 25.8 to 29.8°C and a salinity range of 28-32 ppt. *Chlorella* and rotifers were added to all rearing tanks from the 2nd day onwards. *Chlorella* was maintained at a density between 200,000 to 300,000; the rotifers, at a density between 20 to 30 individuals per mL throughout the rearing period. Copepod nauplii were given in addition to the rotifers from the 10th day and brine shrimp nauplii from the 15th day onwards. On day 22, a total of 1,234 fry were harvested from the rearing tanks representing a survival rate of 4.8%.

Results of the present work indicate that milkfish larvae could be reared successfully without feeding them with the trochophore larvae of oysters during the first few days. Liao *et al.* (1979) could not demonstrate a significant difference in the survival rate of milkfish larvae fed with *Brachionus* alone or with *Brachionus* and trochophore larvae of oysters during the first four days of rearing.

In the present work also, the total dose of human chorionic gonadotropin used to induce ovulation in wild adult milkfish is higher than those previously used by Vanstone *et al.*, (1977) Juario *et al.*, (1978) and Liao *et al.*, (1979) to successfully induce ovulation in newly caught adult milkfish. Similar and even higher doses were used by Kuo (1978) and Kuo *et al.*, (1979) to successfully induce ovulation in wild and pond-reared milkfish. Newly caught females, having oocytes with a mean diameter less than 0.65 mm, were not successfully induced to ovulate in captivity despite a very high total dose of 490 mg SPH and 50,000 IU. HCG.

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\*This work was done in collaboration with the Milkfish Breeding Team of the Oceanic Institute, Waimanalo, Hawaii. The members of the team include: Dr. C.M. Kuo (Leader), Dr. C.S. Lee, Messrs. W. Watanabe and C. Paulsen.

Table 1. The Response of Female Spawners to Hormone Injection

Fish No.	Estimated (actual) body weight (Kg)	Initial egg diameter (mm)	Total dose per injection SPH (mg) + HCG (IU)	Date & time of injection	Remarks and observations
790327	6.5 (8.4)	0.77 ± 0.06 (N=106)	<u>1st</u> 39 ± 2,500 <u>2nd</u> 59 ± 5,000 <u>3rd</u> 0 ± 10,000 <u>4th</u> 0 ± 10,000	3/27/79 – 0550 hrs 3/27/79 – 1710 hrs 3/28/79 – 0615 hrs 3/28/79 – 0850 hrs	(1) Fish was relatively in good condition; three ripe males were placed together with the female after the <u>2nd</u> injection; egg diameter increased to 1.00 ± 0.07 mm 12 hrs after the <u>2nd</u> injection; fish became very weak and could not swim well immediately after the <u>4th</u> injection; there was no change in the mean oocyte diameter 3 hrs after the <u>4th</u> injection; at 1210 hrs on 3/28/79, the fish died.
790401	7.0 (*6.7)	0.67 ± 0.06 (N=68)	<u>1st</u> 70 ± 1,250 <u>2nd</u> 70 ± 10,000 <u>3rd</u> 70 ± 10,000 <u>4th</u> 70 ± 10,000	4/1/79 – 0933 hrs 4/1/79 – 1745 hrs 4/2/79 – 0245 hrs 4/2/79 – 0900 hrs	(2) Fish was relatively in good condition but swam sluggishly; 2 ripe males caught 3 days ago were each injected with 5,000 IU HCG and placed together with the female immediately after the <u>2nd</u> injection; prior to the <u>4th</u> injection, the mean oocyte diameter was 0.99 ± 0.07 mm; 2.5 hrs after the <u>4th</u> injection, it started releasing hydrated eggs with a mean diameter of 1.05 ± 0.05 mm; the mean diameter of the released eggs increased further to 1.08 ± 0.05 mm 4.5 hrs after the <u>4th</u> injection; at 1510 hrs the fish was stripped of its eggs; eggs were fertilized by sperms of injected males using the wet method because there was difficulty in extracting milt from the males; the eggs were not fertilized.
790403	7.0 (*8.3)	0.77 ± 0.04 (N=113)	<u>1st</u> 70 ± 10,000 <u>2nd</u> 70 ± 10,000	4/3/79 – 0830 hrs 4/3/79 – 1800 hrs	(3) The fish was only slightly injured when brought to the laboratory; 3 newly caught ripe males were placed together with the female immediately after the <u>1st</u> injection; all males were injected each with 5,000 I.U. HCG when the female was injected with hormone for the <u>2nd</u> time; the fish was stripped of its eggs 10.5 hrs after the <u>2nd</u> injection when it started releasing fully hydrated eggs; eggs were fertilized by the dry method by sperms of the 3 injected males placed in the same tank; fertilization rate was 59.4% and the hatching rate was 39.7%.
790408	4.0 (5.3)	0.49 ± 0.04 (N=106)	<u>1st</u> 10 ± 2,500 <u>2nd</u> 10 ± 2,500 <u>3rd</u> 0 ± 5,000	4/8/79 – 0910 hrs 4/9/79 – 0830 hrs 4/10/79 – 0830 hrs	(4) Fish was relatively in good condition upon arrival; immediately after the third injection, oocytes were sampled; the mean oocyte diameter increased from 0.49 ± 0.04 mm to 0.54 ± 0.04 mm; prior to the third injection the fish became very weak; it died at 1100 hrs on 4/11/79.
790416	7.0 (6.5)	0.63 ± 0.05 (N=71)	<u>1st</u> 70 ± 10,000 <u>2nd</u> 140 ± 10,000 <u>3rd</u> 140 ± 15,000 <u>4th</u> 140 ± 15,000	4/16/79 – 0835 hrs 4/16/79 – 1745 hrs 4/17/79 – 0430 hrs 4/17/79 – 1645 hrs	(5) The fish was moderately injured during capture; the oocytes sampled were oval in shape and 2 size groups were observed; 12 hrs after the 4th injection, the mean oocyte diameter for the bigger size group was only 0.76 ± 0.19 mm while the smaller size groups were still opaque; at 0600 on 4/20/79, the fish died.

\*without gonads

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