

A Guide to Induced Spawning and Larval Rearing of Milkfish *Chanos chanos* (Forsskal)

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International Development Research Centre

**A GUIDE TO INDUCED SPAWNING
AND LARVAL REARING OF MILKFISH
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Foreword

Milkfish is an important food fish in Indonesia, Taiwan and the Philippines. Although it has been cultured for centuries in brackishwater ponds, fishfarmers are still entirely dependent on fry collected along the coastline during the breeding season. The main constraint to the intensification and expansion of the milkfish industry is the inadequate supply of fry.

At present, there has been growing interest in breeding milkfish in captivity because of its importance as food and the potential of milkfish fingerlings as baits for Tuna and the Skipjack Industries. For this reason, we have written this guide so we could share the technique we have developed at SEAFDEC for the artificial propagation of milkfish. The detailed results of our study on the induced breeding and larval rearing of milkfish, which have been partially supported by the International Development Research Centre of Canada from 1976 to date, have been published elsewhere. It is our hope that this guide will be useful to the fisheries sector most especially in the Philippines, Taiwan and Indonesia.

This guide is definitely not a recipe for success in the artificial propagation of milkfish fry. We envision this to be refined and periodically revised as soon as additional data will be known, especially those related to the mass production of milkfish fry in larger tanks. We request, therefore, our colleagues and readers/users of this guide to give their comments, suggestions, and where necessary, make corrections to ensure its usefulness.

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A GUIDE TO INDUCED SPAWNING AND LARVAL REARING OF MILKFISH *CHANOS CHANOS* (FORSSKAL)

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The artificial propagation of milkfish has been attempted in Hawaii, Indonesia, Taiwan, Tahiti and the Philippines; but so far, success has only been achieved in the Philippines (Vanstone et al., 1977; Chaudhuri et al., 1977, 1978, Liao et al., 1979; Juario and Natividad, 1980) and in Taiwan (Tseng and Hsiao, 1979). The induced spawning and larval rearing techniques discussed in this paper have given us consistently good results with both wild and captive spawners during the 1978 to 1981 spawning seasons.

I. Capture and transport of spawners

Catch by means of a scoop net lined with a fine-mesh net the adult milkfish that enters the fish corral. If the trap is near the research station, transfer the fish

to a floating cage hitched underneath the outrigger of a pumpboat and pull the cage slowly towards the shore. When only one or two fish are caught, directly transfer each of them to doubled plastic bags provided with seawater and aeration and transport them to the shore aboard the pumpboat. As soon as the pumpboat reaches the shoreline, carry the fish to the experimental tank by a stretcher. For milkfish caught in traps far away from the experimental station, transfer to a 2-m diameter canvas tank installed aboard a pick-up truck and 1/3-full of 18-20 ppt seawater (Vanstone et al., 1976). Aerate the water continuously during transport. Upon reaching the station, transfer the fish immediately to the experimental tank by means of a stretcher. (Fig. 1). A maximum of 4 fish at a time may be transported in a 2-m diameter canvas tank, but only 1 in a plastic bag.



Fig. 1. Transport of milkfish spawners by land. A spawner within the doubled plastic bag is being transported to the experimental tank by means of a stretcher.

Catch with a hook-and-line the adult milkfish from a captive broodstock reared in floating cages. This method minimizes stress in other spawners. This is desirable because stress due to handling will cause atresia in maturing or mature females (Lacaniño and Marte, 1982). In order that the fish will take the bait, do not feed for a day prior to catching (Marte, personal communication). Transfer the fish in aerated seawater contained in one section of the hull of the pumpboat. In the absence of aeration, change 1/3 to 1/2 of the water every 30 minutes.

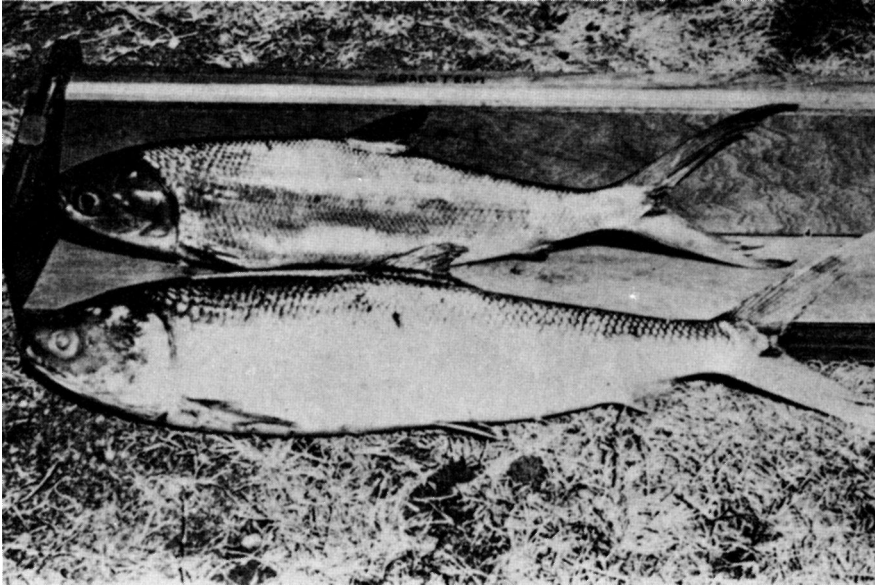


Fig. 2. A male (top) and female (below) wild adult milkfish.

II. Determination of sex and weight of fish

From the external appearance alone it is not possible to distinguish a male from a female milkfish (Fig. 2). Although generally, males are smaller than females, size is not a reliable criterion for distinguishing males from females since there are females that are smaller than males, and vice-versa. During the spawning season, determine the sex of the fish caught from the wild or from the captive stock by aspirating gametes through a fine polyethylene cannula (Clay Adams, PE 160) with an inner diameter of about 1.1 mm.

Before aspirating gametes, anesthetize the fish completely by placing it in a doubled plastic bag with seawater containing 100 ppm 2-phenoxyethanol (ethyleneglycol monophenyl ether, Merck). When the fish becomes immobile, carefully turn it ventral side up. Cautiously insert about 15 to 20 cm of the cannula into the gonads of the fish through the region of the urogenital pore located below the anal opening (Fig. 3). Sample gametes by aspirating them as the cannula is

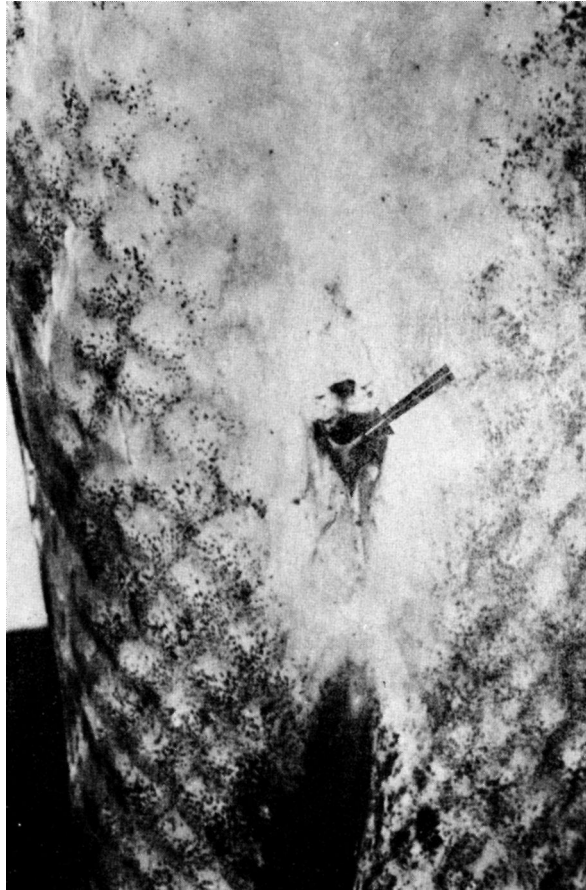


Fig. 3. Ventral view of the posterior portion of a wild adult milkfish showing urogenital region (arrow).

slowly withdrawn (Fig. 4). After egg sampling, measure the fork length of the fish and estimate its weight from a length-weight curve; or weigh the fish rapidly but carefully on a platform balance. We usually prefer to estimate only the weight to prevent further stress on the fish.

III. Determination of maturity

Determine the stage of maturity of individual females from the average dia-

meter of their eggs. Milkfish eggs with a diameter of 0.65 mm or bigger is at the tertiary yolk globule or more advanced stage.

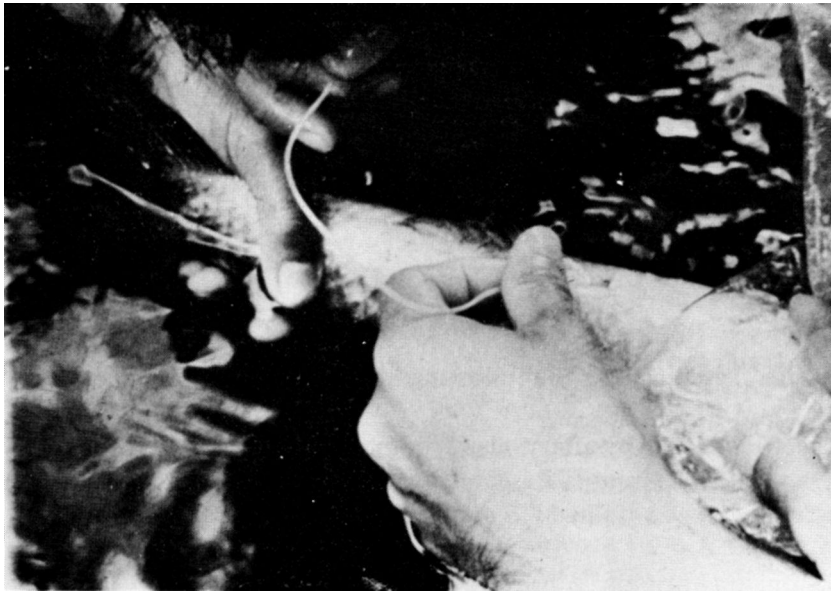


Fig. 4. Sampling of milt/eggs by means of a polyethylene cannula.

Collect some eggs by means of a polyethylene cannula. Place the eggs in a petri dish containing 1% formalin in 0.9% NaCl solution. Measure the diameter of at least 50 eggs under a compound microscope by means of a micrometer eyepiece. If the eggs are not perfectly round, measure the biggest and the smallest diameter, then get the average. If the average diameter is equal to or greater than 0.65 mm, the fish is induced to spawn by hormone injection. Fish with an average egg diameter equal to or greater than 0.65 mm could be induced to spawn by hormone injections, while those with an average egg diameter lower than 0.65 mm, though already at the tertiary yolk globule stage, usually die before completing maturation.

Males are ripe if milt oozes out from the urogenital pore when the abdomen is pressed. If the degree of maturity is right, then the milt will be white and creamy; poor milt is watery and curdled. Some mature males caught during the early part of the season may have a very thick viscous milt (especially true to the captive stock) so that milt does not come out even after pressing the abdomen several times. If this is the case, insert a polyethylene cannula through the urogenital pore to withdraw some milt. Then assess sperm motility and vitality as

described by Mounib (1978; Appendix 1). If in the very viscous milt less than 30% of the sperm are motile, induce seminal thinning by hormone injection.

IV. Induced spawning

To induce spawning, use the following hormones:

- SPH — acetone dried pituitary gland homogenate of coho salmon prepared by the British Columbia Research Council at Vancouver, Canada; one gram of the powder contains 17.6 mg gonadotropin.
- HCG — human chorionic gonadotropin, manufactured by Ayerst Laboratories, New York.
- DF — Durandron Forte "250", a long-acting androgen manufactured by N.V. Organon, Holland. This is an oily solution containing 30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg testosterone isocaproate and 100 mg testosterone decanoate.

4.1 Preparation of the injection

Dissolve HCG using its accompanying diluent. The volume of the diluent to be used is determined by the desired concentration of HCG in the resulting solution, so that 3 ml of this when used for injection will contain the required

international units (IU). It is recommended that not more than 3 ml of this solution is used for injection to avoid possible damage to the muscle tissue. The necessary amount of acetone-dried pituitary gland is homogenized in this solution before it is finally administered. In cases wherein it becomes necessary to inject more than 3 ml of HCG solution, administer the injection in divided quantities at two different sites on the dorsal musculature, a few centimeters below the dorsal fin (Fig. 5).



Fig. 5. Intramuscular injection of gonadotropins. Injection is administered a few centimeters below the dorsal fin.

4.2 Females

Only fish with eggs having an average diameter of at least 0.65 mm should be induced to spawn. Anesthetize the fish completely by immersing it in seawater containing 100 ppm 2-phenoxyethanol. For the first injection, give the fish a

combination of 10 mg SPH/kg body weight + 1,000 IU HCG/kg body weight; for the second, 10 mg SPH/kg body weight + 2,000 IU HCG/kg body weight. The time interval between injections is 9-12 hours both for wild and captive milkfish. This interval is used to ensure that final maturation of the oocytes (eggs) is completed before the fish dies or before the eyes become completely covered with an opaque substance. Newly caught fish, if subjected to excessive handling, will die in 2-3 days.

Usually, only 2 injections are needed to induce both captive and wild adult milkfish to spawn, as long as the dosage and time interval mentioned above are followed. However, badly injured fish may need a third injection. In such cases, use for the third injection the same dose as that of the second. When a third injection is necessary, very often the fish dies before ovulation takes place; if ever ovulation takes place, usually the fertilization and hatching rates are very low.

It should be borne in mind that the dosages mentioned here were found to give consistently good results in spawners caught from the wild with a salinity of 32-35 ppt, and in spawners from the captive stock that were reared to sexual maturity in floating cages in seawater with a salinity ranging from 28-35 ppt. Furthermore, the experiments were done at ambient temperature (28°-30°C) and at a salinity of 32-34 ppt. The same dosages may not be effective in inducing spawning in spawners reared to sexual maturity in earthen ponds or in concrete tanks at lower salinities. (Liao and Chen, 1979; Tseng and Hiao, 1979; Kuo et al., 1980).

4.3 Males

One of the problems in the artificial fertilization of eggs has been the viscous, non-dispersing milt of milkfish caught during the natural breeding season. This problem can be solved by injecting newly caught males with viscous milt with 1 ml DF. A day after the injection, the viscous milt will become more fluid, copious and disperses easily when mixed with seawater; moreover, the percentage of motile sperms as well as sperm motility will increase (Juario et al, 1980). It is more advantageous to use DF rather than HCG because, aside from its longlasting effect which minimizes handling stress, it is cheaper.

V. Fertilization and incubation

Between 10 to 12 hours (usually 10 hours) after the second injection, the fish will start to spawn eggs. This does not mean, however, that the eggs are already fertilizable. It is still necessary, when the fish starts spawning eggs, to sample eggs from the gonads by cannulation and examine them under the microscope. Strip the fish of its eggs only if at least 30% -40% of the eggs are transparent (not glassy transparent); otherwise, insert a plug through the genital pore to prevent further loss of eggs. The best time to spawn/strip the fish is 10½-12 hours after the second injection.

Spawning/stripping is done as follows: the holders, two persons with bath towels in their hands, grasp the fish, one holding at the caudal peduncle while the other handles the head region. A third person immediately covers the genital pore with a small towel to prevent further loss of eggs (Fig. 6). He also helps the holders wipe the fish dry. When the fish is dry, turn it upside down and hold the tail down so that the ripe eggs will flow naturally toward the genital pore. The

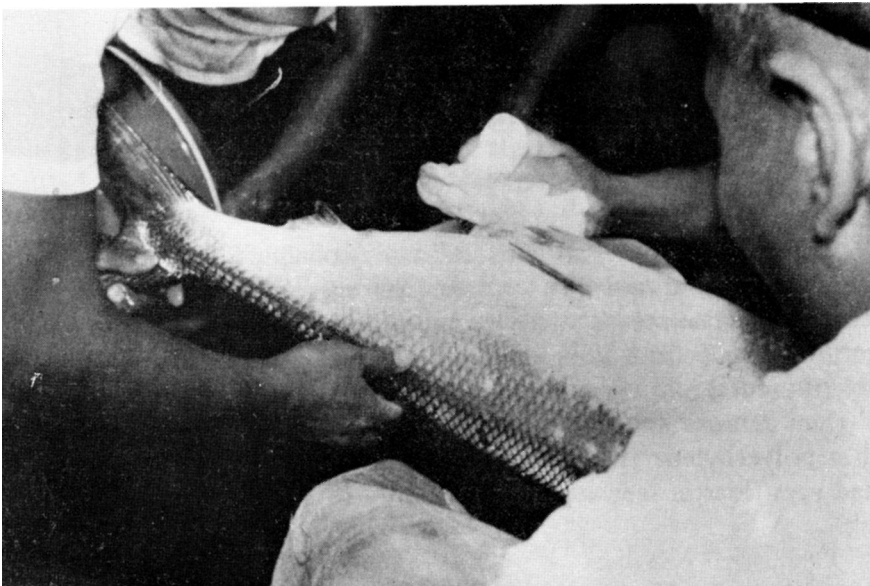


Fig. 6. Genital pore or anal region of the female about to be covered by white cloth to prevent further loss of eggs.

third person holds the spawning basin beneath the genital pore. A fourth person, the spawntaker, gently presses out the eggs with the thumb and forefingers, beginning pressure just in front of the genital pore. (Fig. 7). The hand is then moved nearer the head of the fish and further gentle pressure is applied as necessary to assist the natural flow of eggs until all that would freely come out of the fish are obtained. Collect eggs in separate arbitrary batches. As soon as one batch is collected, fertilize it following the dry method with the milt taken by hand-stripping from a hormone-treated male. Mix the eggs and milt gently but thoroughly by using turkey or chicken feathers. After at least 3 minutes, add seawater (34 ppt salinity) to the mixture while stirring it. After another 3 minutes transfer the fertilized eggs to a scoop net (mesh size, not less than 500 micra) and wash tho-

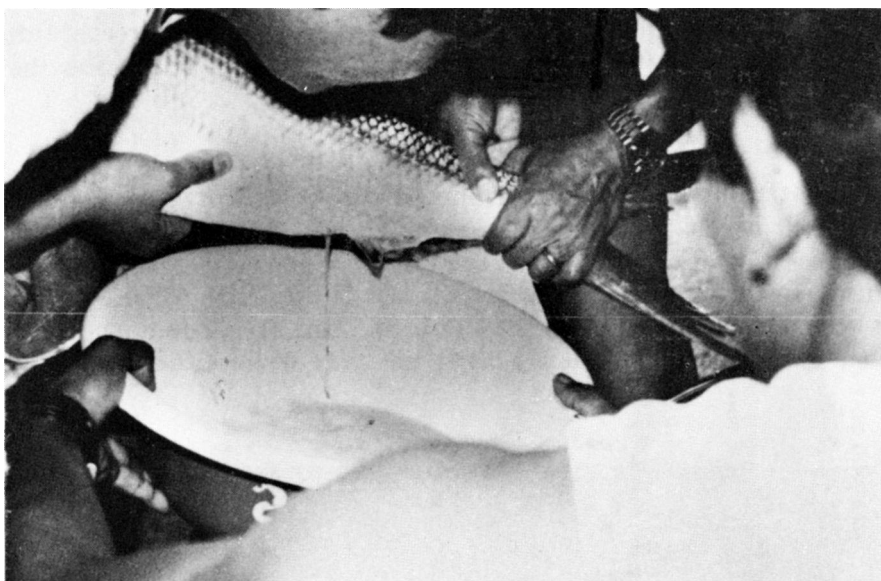


Fig. 7. Stripping the female milkfish of its eggs.

roughly with seawater of the same salinity (isohaline) as that in the incubation tanks (Fig. 8). After washing, transfer the eggs to the incubators. Aerate the incubators strongly to prevent the eggs from clumping. Incubate the eggs at ambient temperature ranging from 25°-30°C and at a salinity of 34 ppt. Six hours after the start of incubation, change 1/3 of the water in the tank with new filtered seawater. Then remove the dead eggs from time to time by siphoning them out through a polyethylene tube after the aeration has been stopped for about 5 min. Fertilized eggs float in seawater with a salinity of at least 34 ppt while unfertilized eggs sink.

The embryonic development and hatching of milkfish is presented in Fig. 9 and Table 1. Depending upon the ambient temperature at incubation, the eggs will hatch in 24-35 hours.



Fig. 8. Fertilized eggs in the scoop net are washed thoroughly with filtered seawater before introducing them into the larval rearing tank.

VI. Larval rearing

About five hours before hatching time, transfer the developing eggs to 600-liter fiberglass tanks. Provide the tanks with strong aeration until the larvae start to hatch, i.e., 24-25 hours after fertilization. Stock in each tank about 20 developing eggs/liter. When the eggs are hatched, you will have a stocking density of about 5-10 larvae/liter or even more, depending upon the hatching rate.

The feeding schedule for milkfish larvae is shown in Figure 10. Add only 4-6 day old *Chlorella* and *Tetraselmis* and 4-day old *Isochrysis* cultures to the rearing tanks. Concentrate and wash thoroughly with aged and filtered seawater the roti-

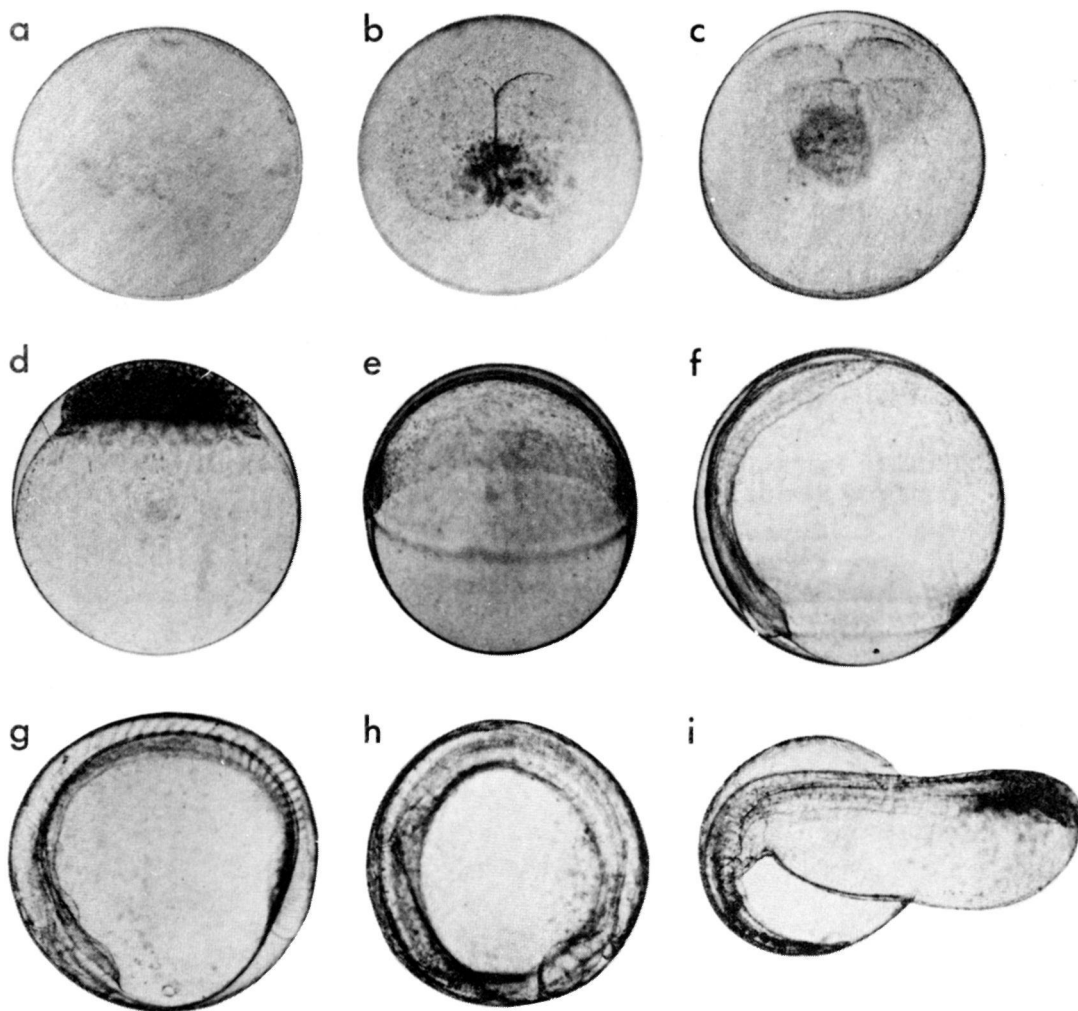


Fig. 9. The embryonic development and hatching of milkfish. a) Fertilized egg b) 2-cell c) 4-cell d) Blastula e) Late gastrula f) Late gastrula; yolk plug and embryonic streak apparent; g) C-shaped embryo h) Late embryonic development i) hatching

Table 1. The embryonic development and hatching of milkfish at ambient temperature (28° - 32°C) and at a salinity of 34 ppt.

| TIME AFTER FERTILIZATION (h min) | STAGE OF DEVELOPMENT | PHOTO NO. IN FIG. 9 |
|-------------------------------------|--|------------------------|
| 0.00 | Fertilized egg; this is spherical, non-adhesive and transparent; yolk is granulated and has a yellow tinge; no oil globule | a |
| 1.10 | 2-cell | b |
| 1.16 | 4-cell | c |
| 5.40 | Blastula | d |
| 8.00 | Late gastrula; yolk invasion 50% complete | e |
| 10.45 | Late gastrula; yolk plug and embryonic streak apparent | f |
| 14.45 | Embryonic differentiation; C-shaped embryo with somites; optic and otic vesicles formed | g |
| 21.40 | Embryonic differentiation; embryo starts to show twitching movement | h |
| 25.45 | Hatching; fully formed embryo emerges head first out of egg shell | i |

fers, harpacticoid copepods and newly hatched brine shrimp nauplii before adding them to the rearing tanks. Decapsulate *Artemia* cysts following the method of Sorgeloos, *et al* (1977; Appendix 2) before incubation.

Change at least one-third of the water in the rearing tanks with aged and filtered seawater after Day 3 and daily thereafter until Day 21. Provide continuous mild aeration in each rearing tank. If equipment are available, monitor dissolved oxygen, salinity and temperature once a day. Dissolved oxygen should be maintained between 6-7 ppm and salinity between 32-34 ppt.

The development and behavior of milkfish larvae are presented in Figs. 11-13 and Table 2.

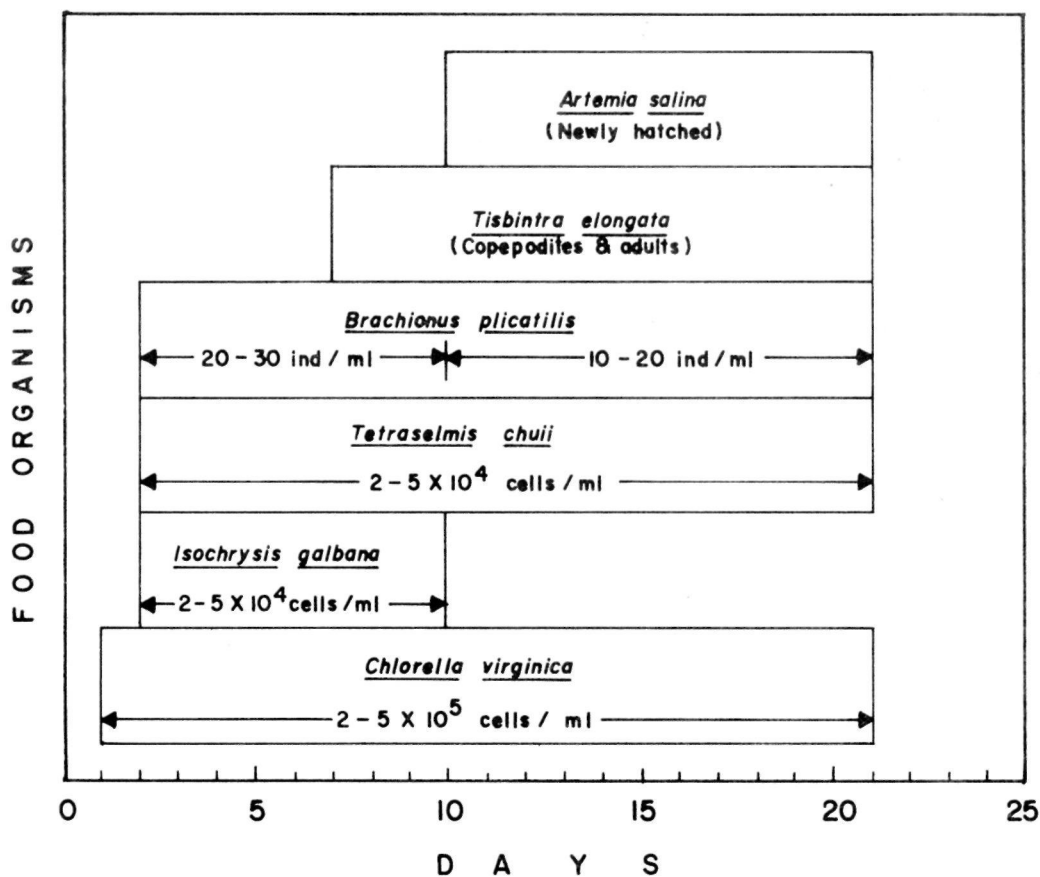


Fig. 10. Feeding schedule for milkfish larvae during the 21-day rearing period.

VII. Mass production of larval food

7.1 *Chlorella virginica* and *Tetraselmis chuii*

The following agricultural fertilizers are used for the mass production of *Chlorella virginica* and *Tetraselmis chuii*:



Fig. 11. Newly hatched larva.

1. Urea (46-0-0) at 10 gm/ton of filtered seawater
2. Ammonium sulphate (21-0-0) at 100 gm/ton of filtered seawater
3. Ammonium phosphate (16-20-0) at 10 gm/ton of filtered seawater

Dissolve the fertilizers in 5 liters of filtered seawater and broadcast in the culture tanks containing filtered seawater with algal starters. The starting density should be about $0.5-1.0 \times 10^6$ cells/ml for *Tetraselmis* and $0.5-2.0 \times 10^6$ cells/ml for *Chlorella*. Aerate vigorously the culture tanks throughout the culture period to prevent cells from clumping. The average peak density of 14.0×10^6 cells/ml for *Chlorella* and 1.0×10^6 cells/ml for *Tetraselmis* is usually attained between days 4-8. At this time, harvest the culture and introduce it gradually into the larval rearing tanks. On day 10-12, the population density of *Chlorella* and *Tetraselmis* will start to decline. In order to have a continuous supply of green water, prepare a series of culture tanks in such a way that daily harvest is possible.

7.2. *Isochrysis galbana*

For the mass production of *I. galbana*, only triple 14 (14-14-14) is used as

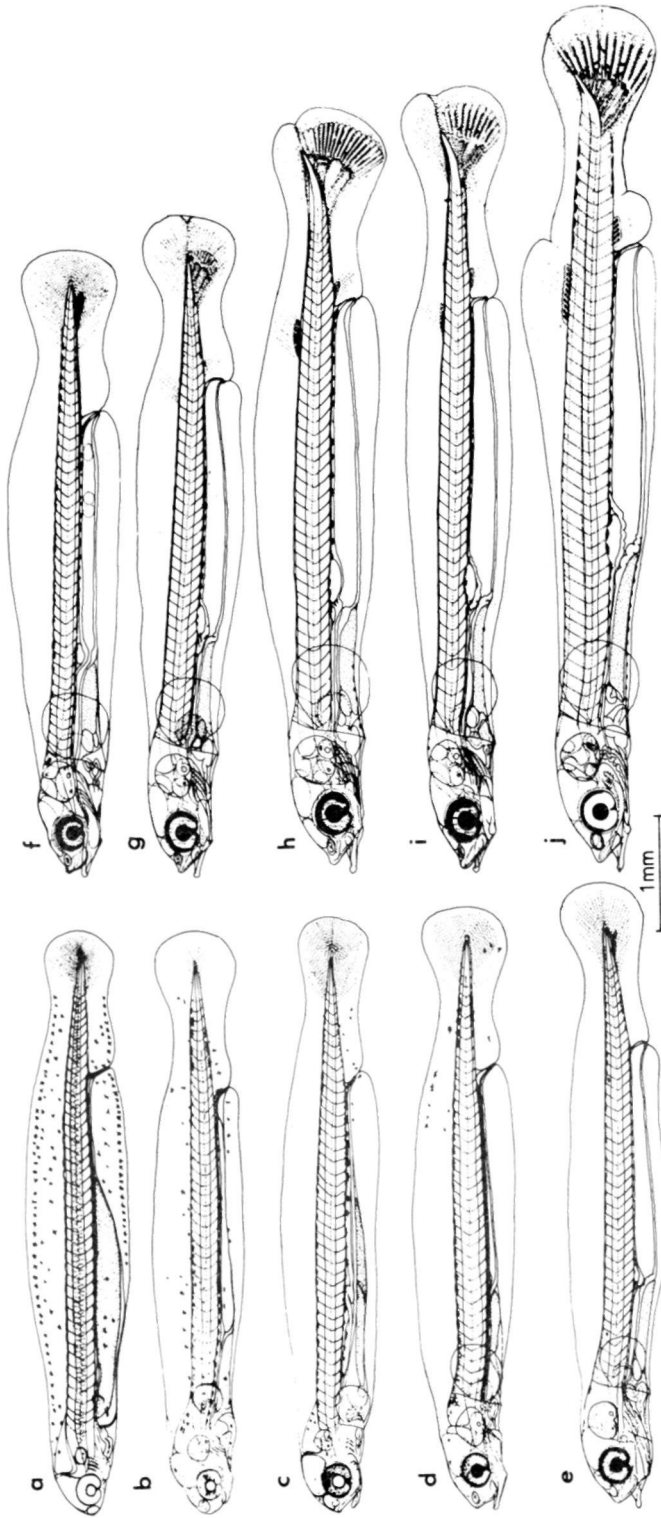


Fig. 12. The development of milkfish larvae. a) 1 day old b) 2 days c) 3 days d) 4 days e) 5 days f) 6 days g) 7 days h) 8 days i) 9 days j) 10 days.

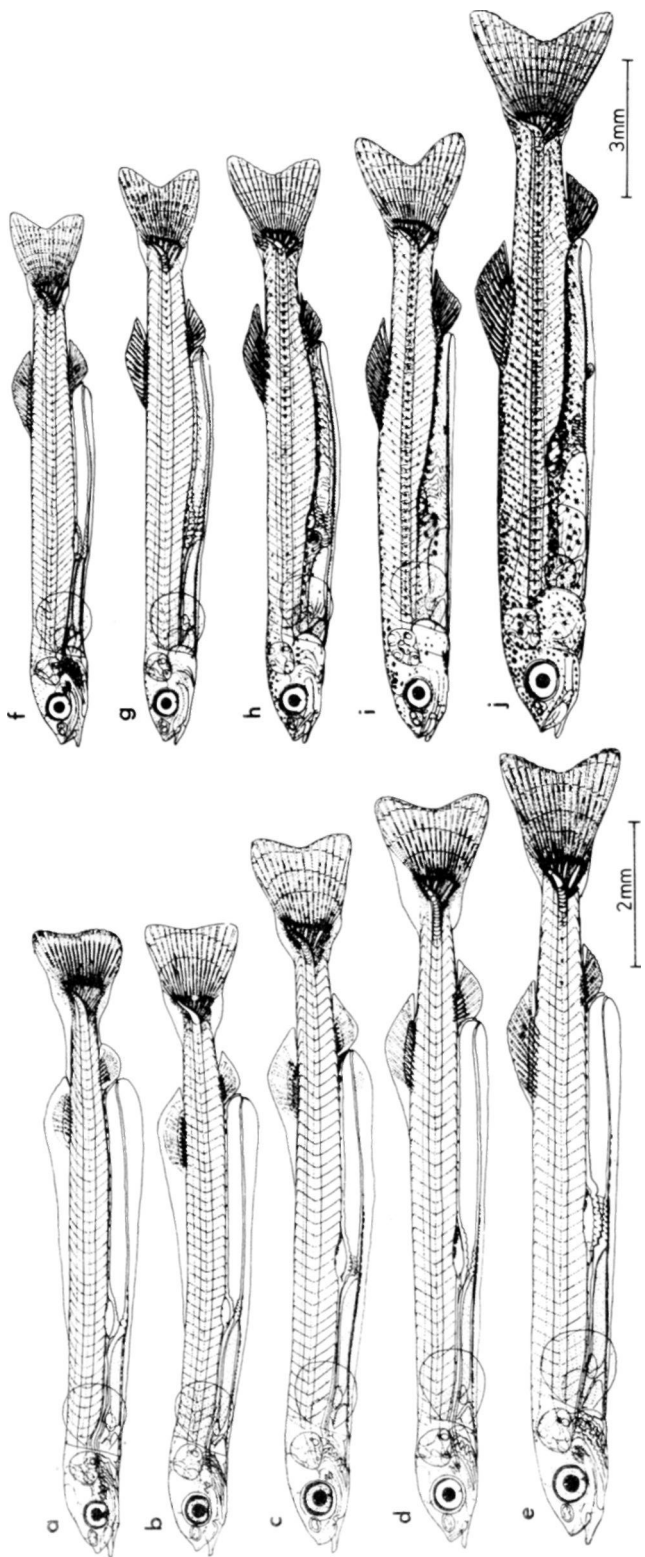


Fig. 13. The development of milkfish larvae (continued). a) 11 days old b) 12 days c) 13 days
d) 14 days e) 15 days f) 16 days g) 17 days h) 18 days i) 19 days j) 21 days

Table 2. The development and behavior of milkfish larvae.

| DAYS AFTER HATCHING | MEAN TOTAL LENGTH (mm) | FIG. NO. | DEVELOPMENT AND BEHAVIOR |
|---------------------|------------------------|----------|--|
| 0 | 4.27 ± 0.11 | 11 | Newly hatched larva slightly curved with unpigmented eyes; mouth not formed, anus closed and located posterior to the yolk mass; yolk sac broad and extends anteriorly near the head end of the larva. Pigments sparsely scattered in the yolk mass and on the head end of the larva. Larva remains suspended in the water column with head down and belly up and sink slowly in an oblique position; then it makes a quick 360° upward turn and swims to the surface. |
| 1 | 5.14 ± 0.11 | 12a | Eyes still unpigmented; yolk sac is reduced; pectoral fins start to develop; mouth and anus still closed. |
| 2 | 5.18 ± 0.12 | 12b | Eyes starting to be pigmented; mouth and anus opened; yolk very much reduced. |
| 3 | 5.22 ± 0.12 | 12c | Eyes well pigmented; yolk completely absorbed; feeding behavior apparent. The larvae exhibit phototaxis during the day but drift at night. |
| 4 | 5.29 ± 0.26 | 12d | Heart chambers well developed; healthy larvae feed actively. |
| to 5 | 5.31 ± 0.24 | 12e | Critical period starts at Day 4. |
| 6 | 5.51 ± 0.24 | 12f | Pectoral fins well developed; a bilobal caudal fin becomes apparent; critical period ends at Day 7. |
| to 7 | 5.62 ± 0.46 | 12g | |
| 8 | 6.33 ± 0.42 | 12h | Operculum is starting to form; growth starts to accelerate. |
| to 9 | 6.38 ± 0.14 | 12i | |
| 10 | 6.72 ± 0.42 | 12j | Dorsal and anal fin start to differentiate; caudal fin more differentiated; body becomes very transparent; larvae swim in school and show strong rheotaxis during the day. |

| | | | |
|----|--------------|-----|--|
| 11 | 7.52 ± 0.41 | 13a | Dorsal and anal finfold separate from caudal finfold; body transparent and assumes the pigmentation pattern of wild fry. |
| 12 | 7.00 ± 0.57 | 13b | Caudal finfold is separated completely from the dorsal and anal finfolds; pigmentation on the dorsal portion of trunk increases; but it is reduced on the dorsal peritoneal membrane except in the region above the air bladder and the posterior portion of the anus; variability in size of larvae becomes apparent. |
| 13 | 7.96 ± 0.85 | 13c | |
| 14 | 8.26 ± 1.15 | 13d | Fin development resembles that of wild fry; larvae swim circularly and rapidly during the day; larvae show strong phototaxis at night. |
| 15 | 10.51 ± 0.73 | 13e | |
| 16 | 10.67 ± 0.66 | 13f | Pigmentation on head region increases; intestinal wall folds increase and become distinct; rudimentary gill rakers apparent; larvae are very active and react to sudden movements. |
| 17 | 11.87 ± 0.66 | 13g | |
| 18 | 12.44 ± 0.63 | 13h | Intestinal folds distinct; rudimentary pelvic fins apparent; pigmentation pattern differs from that of wild fry; larvae are no longer sensitive to direct exposure to sunlight; larvae feed on algae growing on the wall of rearing tank. |
| 19 | 13.36 ± 0.53 | 13i | |
| 20 | 13.63 ± 0.27 | | |
| 21 | 14.09 ± 0.60 | 13j | Pelvic fins developing; pigments scattered over the upper half of the body and dense on the peritoneal wall; larvae now resistant to handling and could be transported to and stocked in nursery ponds. |

fertilizer at 40 gm/ton of filtered seawater. The starting density should be at least 1.0×10^4 cells/ml. The highest density of $0.5-1.0 \times 10^6$ cells/ml is reached between day 3-4. At this time, harvest *Isochrysis* and introduce it gradually to the rearing tanks. On day 4-5, the population density of *Isochrysis* will start to decline. If Walne's Medium (Appendix 3) is used to culture *Isochrysis*, an average density of $2.0-3.0 \times 10^6$ cells/ml is reached between day 5-7. On day 8-9 the population density of *Isochrysis* will start to decline.

It should be borne in mind that at the outbreak of contaminants, algal cultures should be discarded right away. The culture tanks should be disinfected with the commercially available "Chlorox" or "Purex", washed and brushed thoroughly and dried before re-use.

7.3 *Brachionus plicatilis*

A density of 2 ind/ml or even as low as 0.5 ind/ml is sufficient to start a culture of rotifers in one-ton tank containing 100 L of filtered seawater. Feed rotifers with *Chlorella* at a density of about $1.5-3.0 \times 10^5$ cells/rotifer/day. Aerate moderately the rotifer culture tanks throughout the culture period. Once the culture tank reaches its full capacity, harvest the rotifers and concentrate by filtering them through a bolting cloth with a mesh size of 150 and 48 micra. As in the mass culture of *Chlorella*, prepare a series of culture tanks so that there is sufficient food for the fish larvae throughout the rearing period.

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APPENDIX

Appendix 1. Assessment of sperm motility

Sperm motility is assessed in two ways. (1) The number of motile spermatozoa is expressed as a percentage of the total number of spermatozoa, and (2) the degree of progression of the motile spermatozoa is calculated on a scale of 0-10 in which 1 refers to a very feeble spermatozoon and 10 refers to a spermatozoon with good progressive motility.

Source: Mounib, Jr. S. (1878). Cryogenic preservation of fish and mammalian spermatozoa. *J. Reprod. Fert.*, 53:13-18.

Appendix 2. Decapsulating Artemia cysts

1. Dry cysts are hydrated in a funnel-shaped container with tap water or sea water and kept in continuous suspension by aeration from the bottom.
2. After 1 hr, the suspension is diluted with an equal volume of commercial hypochlorite to obtain a final concentration of active ingredients of 2.12% (oxidation process starts immediately and, as the chorion dissolves, a gradual colour change is observed in the cysts from dark brown via white to orange). Decapsulation processes must be kept below 40°C.
3. Within 7-10 min, the chorions disappear completely and the decapsulated cysts should then be filtered immediately and thoroughly washed with tap water or sea water in order to remove all traces of hypochlorite.
4. The treated cysts are now either incubated directly for hatching or, after immediate dehydration in a brine solution, stored for later use.

Source: Sorgeloos, Patrick, E. Bossuyt, E. Laviña, M. Baeza-Mesa, & G. Persoone. (1977). Decapsulation of Artemia cysts: A simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture*, 12: 211-315.

Appendix 3: Walne's Medium

Solution A

| | |
|---|---------|
| NaNO ₃ | 100.00g |
| Na ₂ EDTA | 45.00g |
| H ₃ BO ₃ | 33.60g |
| NaH ₃ PO ₄ ·2H ₂ O | 20.00g |
| (or NaH ₂ PO ₄ ·H ₂ O) | 17.69g |
| FeCl ₃ ·6H ₂ O | 1.30g |
| MnCl ₂ ·4H ₂ O | 0.36g |

Dissolve Solution A in 1 liter of distilled water.

Solution B (Trace metal stock)

| | |
|--|------|
| ZnCl ₂ | 2.1g |
| CoCl ₂ ·6H ₂ O | 2.0g |
| (NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O | 0.9g |
| CuSO ₄ ·5H ₂ O | 2.0g |

Dissolve Solution B in 100 ml of distilled water and acidify using 1N HCl until the solution clears. Use 2 ml of Solution B per liter of Solution A

Solution C (Vitamin Stock Solution)

| | |
|---------------|---------|
| B12 (Biotin) | 10.0mg |
| B1 (Thiamine) | 200.0mg |

Dissolve Solution C in 200 ml of distilled water. Use 0.1 ml of Solution C per liter of Solution A.

N.B.

Walne's Medium is given at a rate of 1 ml per liter of culture.

Source: Walne, P.R. 1974. Culture of Bivalve Molluscs. Surrey Fishing News (Books), Whitefriars Press, London. 173 pages.

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