

A GUIDE TO THE ESTABLISHMENT AND MAINTENANCE OF MILKFISH BROODSTOCK

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1. INTRODUCTION

Milkfish culture has been practiced for centuries in the Philippines, Indonesia and Taiwan. In the Philippines alone, about 195,000 hectares of brackishwater ponds and 30,000 hectares of freshwater fishpens are used for milkfish production. The fry needed to stock these ponds and fishpens still come exclusively from the coastal waters. Annual catches from the natural fry grounds, however, are uncertain due to fluctuations in climatic conditions. In addition, traditional fry grounds are threatened by increasing pollution.

Milkfish production in the Philippines has increased steadily through the years and is projected to continue over the next decade. With the recent improvements in culture operations through improved fertilization and pond management techniques resulting in higher stocking density per unit area, the demand for seeds is expected to increase. Furthermore, an estimated 62,000 hectares out of the 140,000 hectares of mangrove swamp are available for future expansion (Samson, 1983).

Research efforts to artificially propagate milkfish has resulted in successful induced spawning of wild adults (Kuo et al., 1979; Liao et al., 1979) and captive broodstock (Tseng and Hsiao, 1979; Juario and Natividad, 1980; Liao and Chen, 1979, 1983) as well as development of larval rearing techniques giving survival rates up to 70% (Vanstone et al., 1977; Juario et al., 1983). However, due to difficulties in obtaining wild spawners it becomes essential to establish and maintain captive broodstock. Recently, a 5 year old milkfish reared from wild (Lacanilao and Marte, 1980) and artificially-bred fry (Marte et al., 1983) have matured and spontaneously spawned. These developments underline the potential for increasing fry supply through controlled breeding.

Recognizing the need to accelerate development of milkfish breeding technology, President Marcos issued a Memorandum Directive on 27 December 1980 to the Minister of Natural Resources, the Director of the Bureau of Fisheries and Aquatic Resources (BFAR) and the Chief of the Southeast Asian Fisheries Development Center (SEAFDEC) Aquaculture Department to implement a "National Bangus Breeding Program". The program aims to: (a) accelerate the development of a simplified technology for propagating milkfish and test its economic viability for commercial application; (b) increase fry supply in selected natural fry grounds, particularly those close to productive fishponds; (c) establish hatcheries in fishpond areas far from natural source of fry; (d) eliminate or minimize transport cost and mortality of fry; and (e) produce excess fry for export.

The Ministry of Natural Resources through its implementing agency, the BFAR has initiated the establishment of experimental stations for rearing milkfish to maturity in various regions of the country. The procedures for establishing milkfish broodstock; the methods for assessing its reproductive performance and procedures for gathering data are uniformly carried out in all these breeding stations to facilitate evaluation of results.

The methods described in this manual were developed and followed in establishing milkfish broodstock at the SEAFDEC Aquaculture Department. Further improvement is foreseen through interaction with users of this manual during program implementation, especially with fish transport, maintenance of the stock, dietary requirements and egg collection. This will hopefully accelerate development of a simplified technology for breeding milkfish.

2. ESTABLISHING BROODSTOCK FARMS

The farm should be located near a protected coastal area such as coves or small bays. Accessibility of the farm site to major land transport routes is important to facilitate delivery of feeds and other farm supplies. Each site should have suitable areas for the following facilities: (a) two brackishwater ponds or two fishpens for rearing juvenile milkfish, (b) floating net cages for further rearing of broodstock to maturity, and (c) hatchery facilities for rearing larvae to fry.

3. REARING MILKFISH JUVENILES

Two 1-hectare milkfish ponds or two 400m² pens will be used for growing milkfish juveniles to 1-3 years. Brackishwater ponds or seawater pens should be accessible from the area where maturation cages are to be located. This will facilitate transfer of fish from the rearing ponds or pens to the cages.

3.1. Pond and Pen Construction

The rearing pond is similar in construction to ordinary brackishwater ponds currently used in milkfish culture. Each pond has an area of 1 hectare with maximum water depth of 0.5 m. Aside from the inlet gate, a water outlet consisting of an adjustable stand-pipe (15-cm diameter PVC) is recommended (Fig. 1). See Annex 1 for construction schedule.

Alternatively, two units of 20 x 20 m fishpens may be used for rearing milkfish juveniles (Fig. 2). It should be located in a well protected area, with sandy-muddy substratum and minimum water depth of 1 m at lowest low tide. The framework and slats are made of bamboo. Coal tar-treated Coralon netting (size of twine = 210 d/36) with 3.2 cm stretched mesh and hanging ratio of 30% is used for the net pen. (Refer to Annex 12 for calculating the length of netting needed to obtain a 30% hanging ratio). A free board of about 2 m above the highest high tide is provided to keep the stock from jumping out. The foot rope of the net pen is secured with bamboo pegs embedded 30 cm deep to the substratum to prevent escape of fish. Bamboo slats are installed around the pen to protect against predators and pouchers.

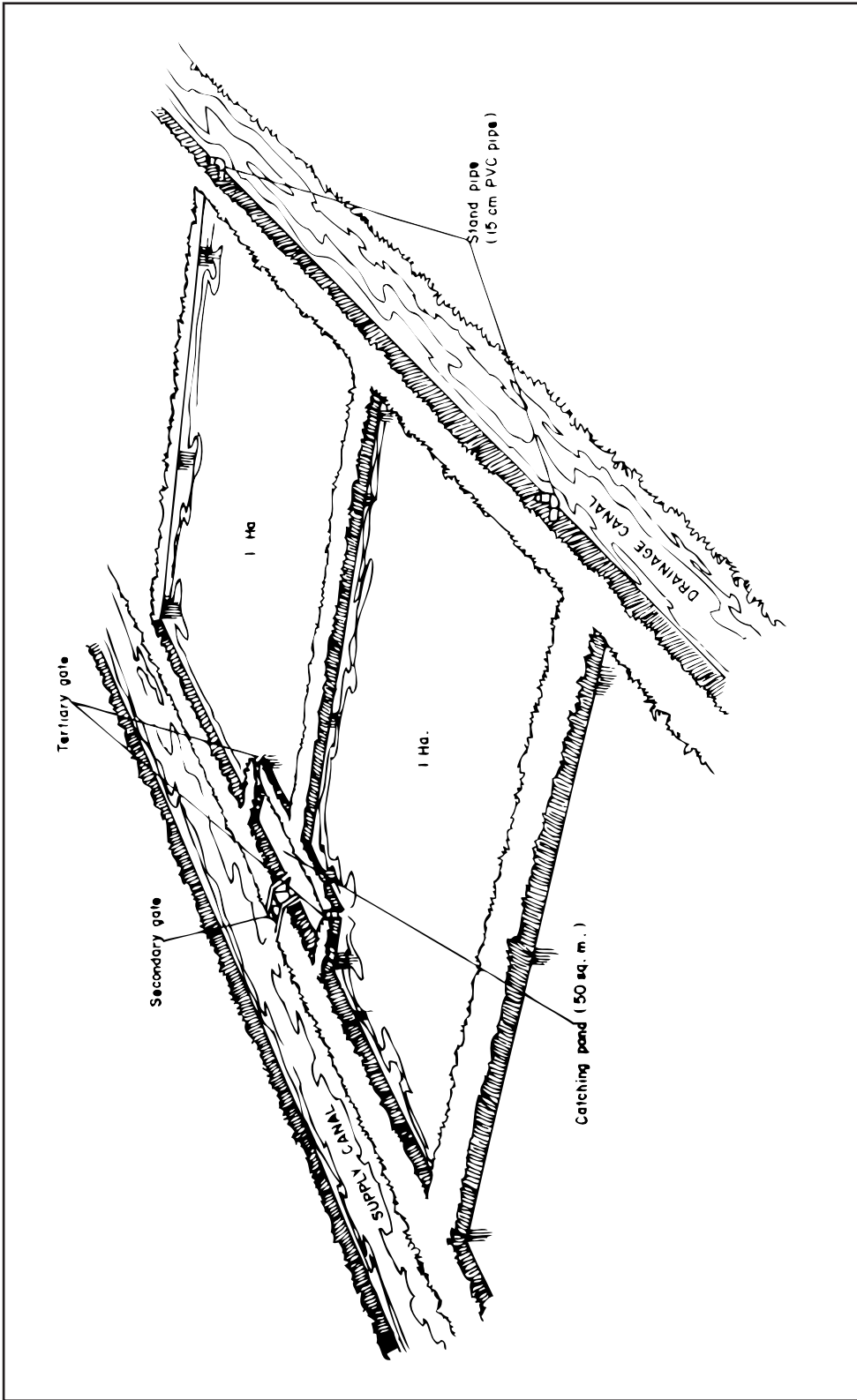


Fig. 1. A design of a rearing pond for milkfish broodstock.

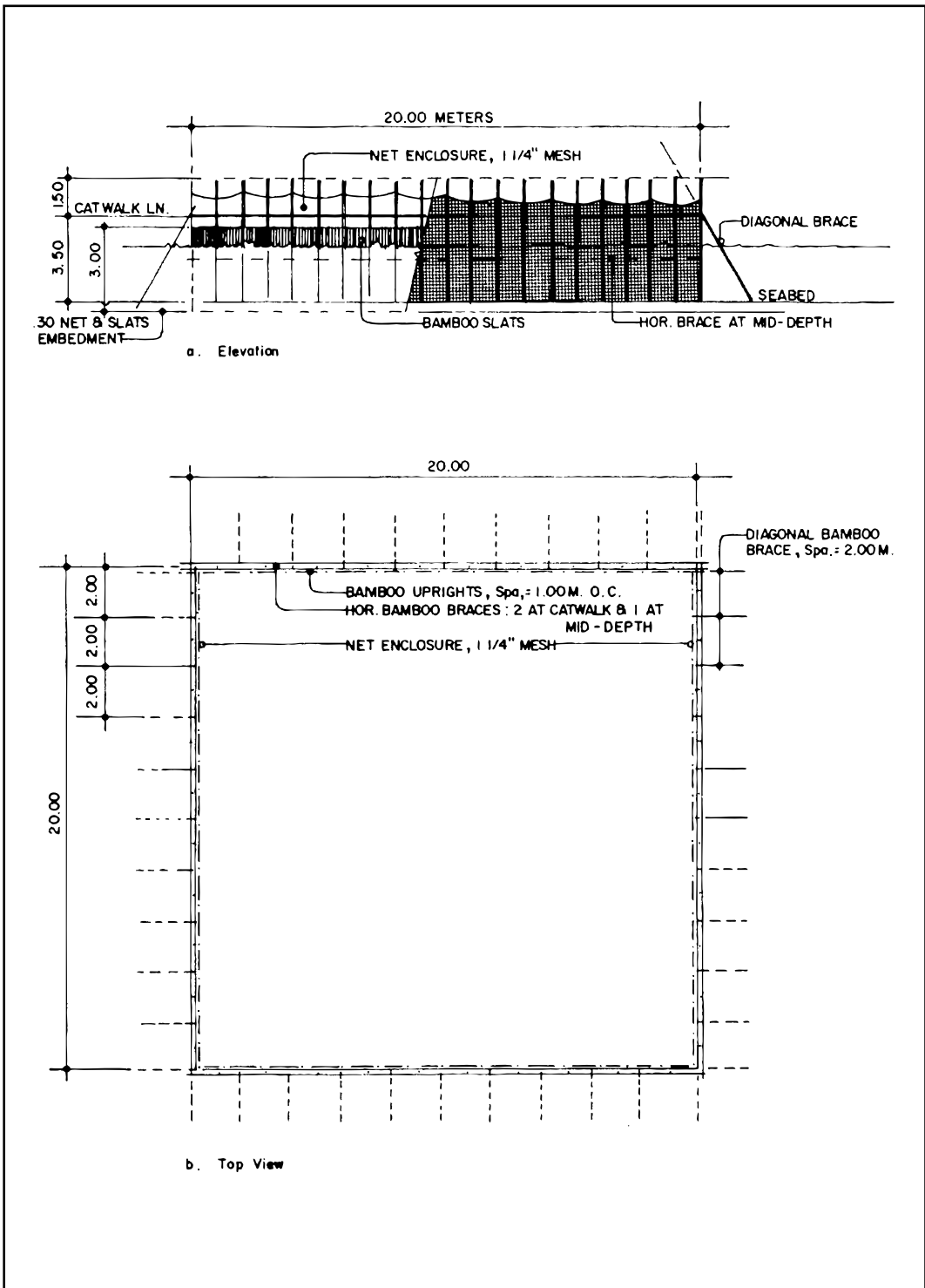


Fig. 2. Design and construction details of fish pen.

3.2. Pond Preparation

The pond is prepared about 1.5-2 months before stocking to ensure growth of lablab, a complex mat of benthic organisms that serve as natural food. The following procedure is recommended (Lijauco et al., 1979).

- a. Drain pond completely and allow to dry for 1-2 weeks until soil cracks.
- b. Apply chicken manure at 2 tons per hectare. Flood to a depth barely covering the pond bottom and apply 2-3 bags of 16-20-0 inorganic fertilizer per hectare. Urea (45-0-0) may be applied at the rate of 15 kilograms per hectare to speed up breakdown of chicken manure. Method of application is by broadcast.
- c. Increase water depth gradually over a period of 1-1.5 months, 3-5 cm each time until stocking depth of 50 cm is reached. An abrupt increase in depth causes lablab to detach and float.

3.3 Stocking

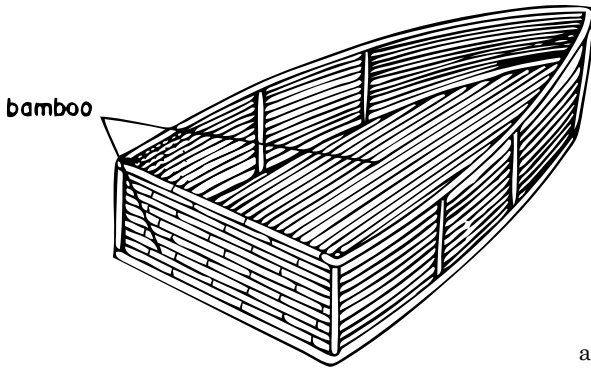
One thousand juveniles with body weight of about 250 g are stocked in one pond during the first year (Annex 2). (The fish to be stocked may be grown initially in rearing ponds to desired size or obtained from fish-pond operators in the vicinity). Fish are transported in oxygenated plastic bags or aerated tanks. If transport time exceeds 2 hours, the fish are first conditioned to the pond water temperature before they are released. This is done by floating the plastic bags containing the fish in the pond, or gradually replacing the water in the transport tank. Release the fish when the temperature of transport water reaches that of the pond water. Transport is best done early in the morning or late in the afternoon to minimize stress. (See Sec. 5.3.1 for sampling procedure to determine body weight before stocking.)

A similar stocking procedure in fish pens is followed. If fish pen can be reached by boat from the rearing pond, fish may be transported in bamboo or net barge (Fig. 3) supported by bamboo poles and dragged slowly by motorized banca to fish pen site.

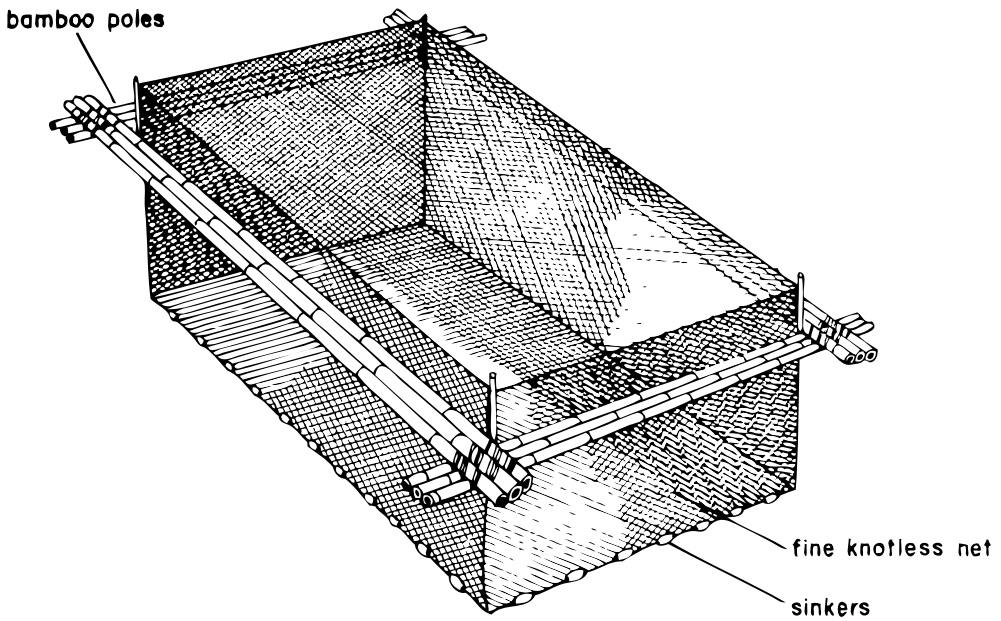
Subsequent stockings in the other pond or pen is done on the second year (Annex 2) following the methods described above.

3.4 Food and Feeding

Supplementary feeding is not required during the first 2-3 months of rearing when enough lablab grows in the pond. To maintain good growth of lablab, the pond is fertilized every 2 weeks with 50 kg per hectare of 16-20-0. Application may be modified depending on the quality of pond soil.



a. Boat-shaped fish cage made of bamboo rigged in between two pumpboats for transporting milkfish broodstock



b. A fish cage with bamboo poles as floats which could be dragged by a pumpboat for milkfish broodstock transport.

Fig. 3. Bamboo and net barge for fish transport.

From the fourth month or when lablab can no longer sustain good growth of the stock, commercial pelleted feed (20% protein) is given at 1.5% body weight per day. Refer to Annex 9 for computing daily feed ration. Feeding by broadcast method is preferred. Recommended feeding schedules are 9 AM and 3 PM. Feeding is done slowly and is stopped when fish refuse to eat or when daily ration is consumed. Fish stocked in fishpens are fed soon after stocking following the feeding procedure described for pond fish.

3.5 Water Quality Management

It is important to maintain optimum water condition to grow natural food. If tide (usually spring tide) allows, replenish about 1/3 of pond water before every fertilizer application. This is done by decreasing water level a few hours before the incoming tide can enter the pond. Tidal water is allowed to enter until the desired water level is reached. More frequent replenishment is necessary during hot months to compensate for evaporation, during rainy months to prevent sudden drop in salinity, and under stressful conditions (i.e. low dissolved oxygen, high water temperature, fish kill). If the tide is too low use water pump.

MATURATION CAGES FOR BROODSTOCK

The floating net cages for rearing broodstock fish to maturity should be located in protected seawater area with little wave action even under adverse weather conditions. There should be good water circulation, minimum water depth of 5 m at lowest low tide, and sandy-muddy substratum. Water conditions should include temperature range of 25-34°C, salinity of 28-35 ppt and minimum water transparency of 3 meters.

4.1 Design & Construction of Maturation Cages

The size of maturation cage is 10 m diameter x 3 m for circular or 10 x 10 x 3 m for rectangular cage. (Fig. 4a-4d). The choice will depend on available materials and suitability in the area. Coralon net (mesh size = 5.7 cm, size of twine = 210d/60) treated with coal tar is used for cage netting. A hanging ratio of 30% is provided to have good water exchange. The bottom should be provided with a fine-knotless net (mesh size = 3.0 mm) to retain feed that may sink to the bottom. A net cover should also be provided to keep fish from leaping out. The cages are supported by cylindrical styrofoam floats (0.6 m diameters x 1.0 m) fitted to cage frame but allowing for easy rotation to check fouling. Canvas cloth or fine net (mesh size = 1.0 mm) is wrapped around the floats for protection.

Only one cage is needed in the first year of the program. Additional cages will be set up in the second, third and fourth years (see construction schedule in Annex 1).

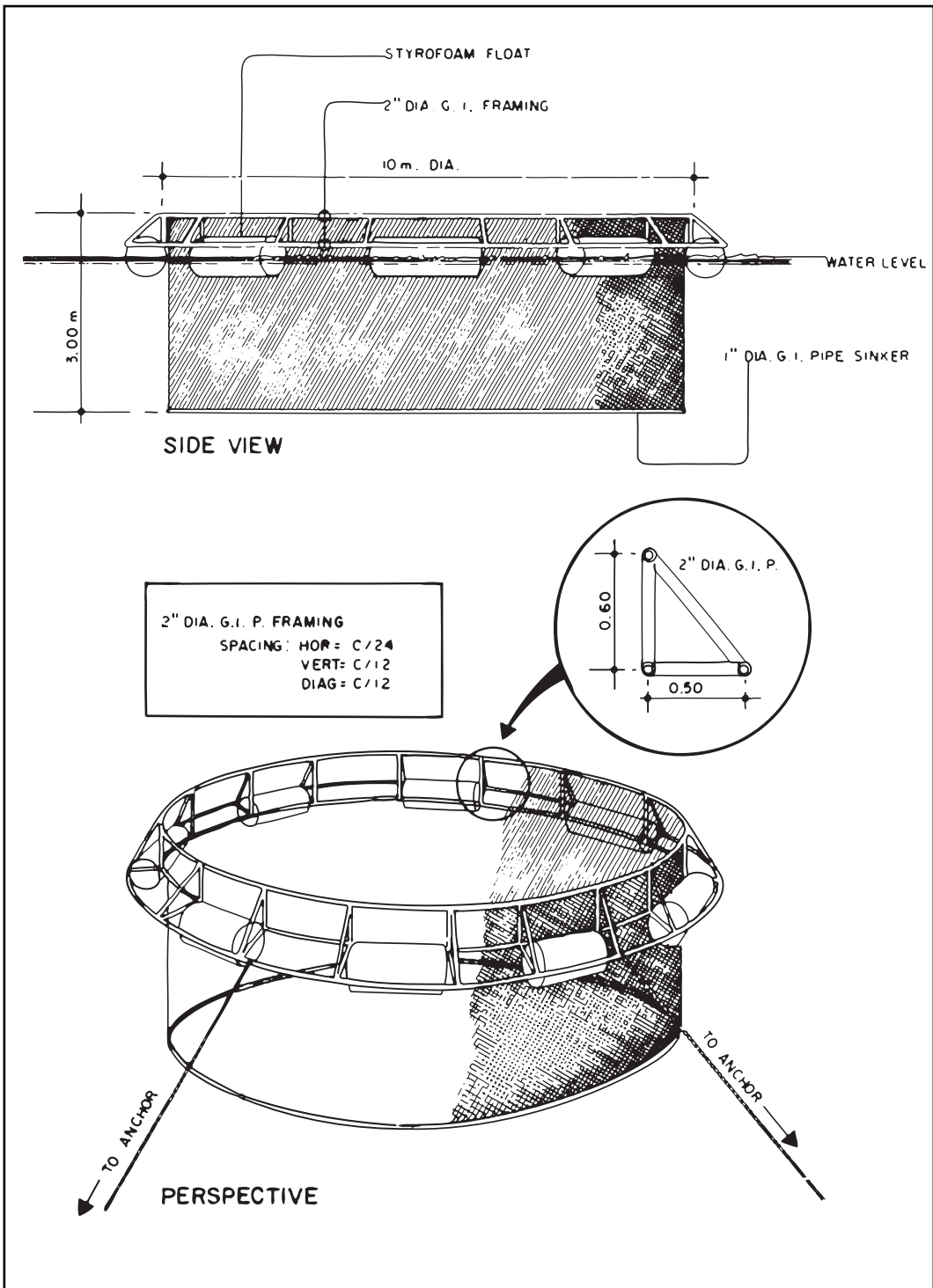


Fig. 4a. 10 m diameter x 3 m deep cage with G.I. pipe frame support.

Fig. 4. Design and construction details of floating cages.

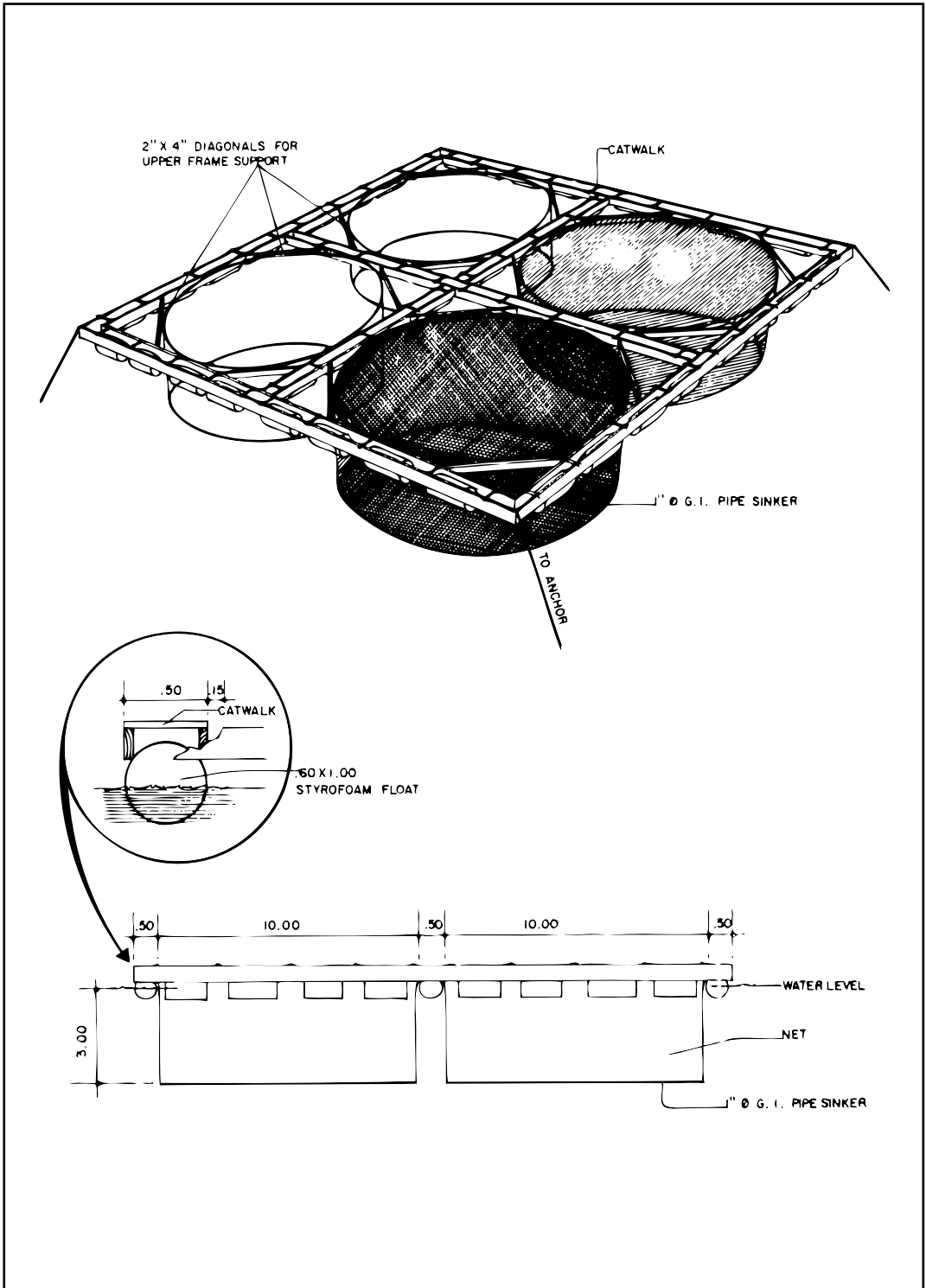


Fig. 4b. 10 m diameter x 3 m deep cage with G.I. pipe ring and wooden frame support.

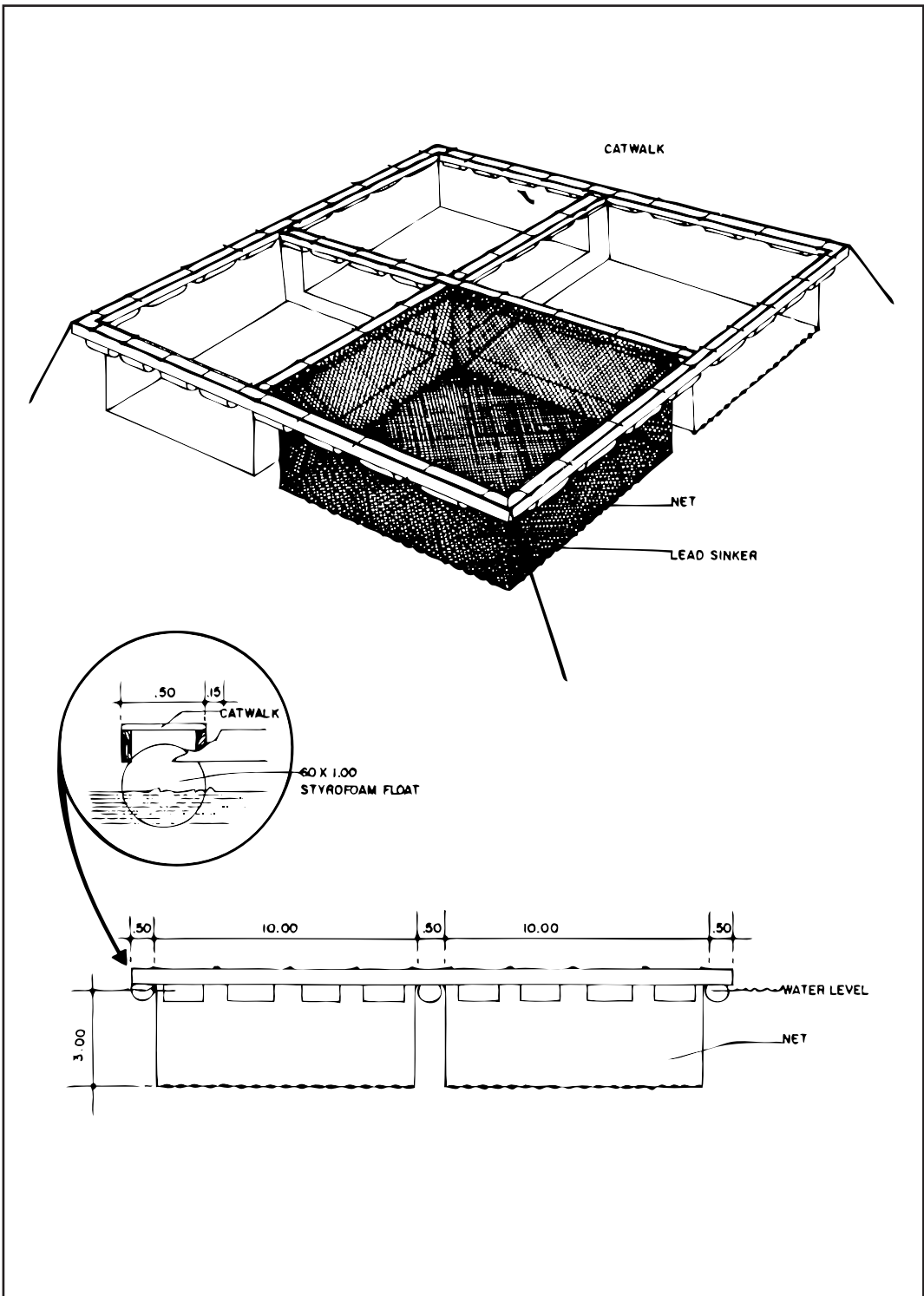


Fig. 4c. 10 m diameter x 3 m rectangular net cage with wooden frame support.

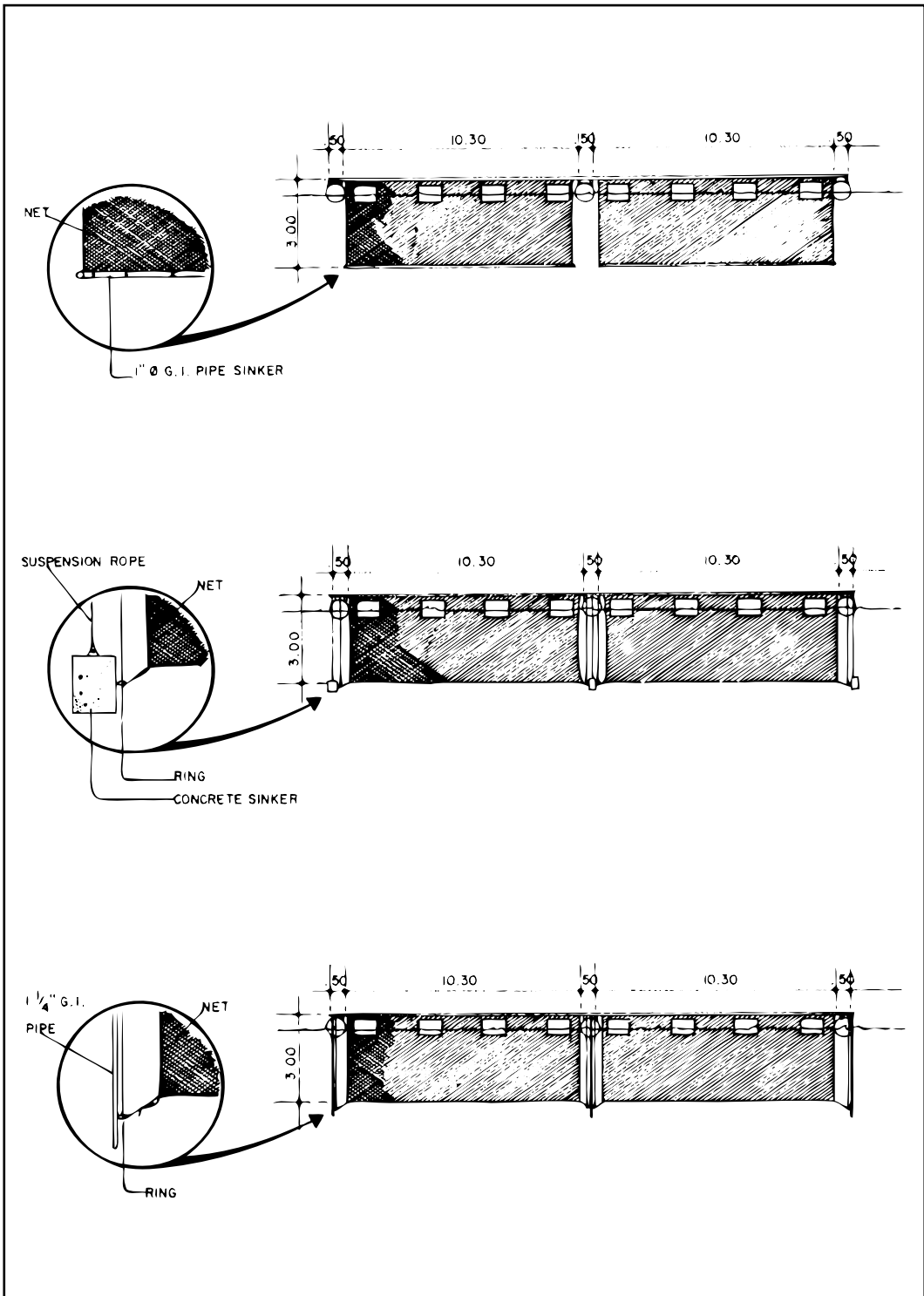


Fig. 4d. Alternative methods of rigging 10 x 10 x 3 m net cage.

4.2 Stocking

The stocking schedule is outlined in Annex 2. Transfers are to be done after one year of rearing in each holding structure.

- Year 1 — Stock 1000 fish in the first pond (Pond A) or pen (Pen A).
- Year 2 — Transfer 500 fish from Pond A (or pen A) to Cage 1.
- Year 3 — Transfer 250 fish from Pond A (or Pen A) to Cage 2 and 250 fish from Cage 1 to Cage 3.
- Year 4 — Transfer 125 fish from Cage 1 to Cage 1a and the remaining 250 fish from Pond A (or Pen A) to Cage 5 .

The schedule of transfers from the second pond (Pond B) or pen (Pen B) follows that for Pond A but shall start on the third year of the program. Fish are best transported in holding tanks with aerators or modified net barge (Fig. 3) towed slowly to cage site.

4.3 Feeding

Commercial pelleted feed (20% protein) is fed to the stock at 1.5-2.0% of body weight until the fish are 3 years old. On the fourth year of rearing or approximately one year before expected spawning feed shall consist of commercial pelleted feed (40% protein) at 2.0% body weight. Feeds are to be given twice daily at 9 AM and 3 PM. Refer to Appendix A for computing daily ration. Fish may be fed by broadcasting or by feeding trays suspended at 3-4 points within each cage.

4.4 Cage Maintenance

Cage maintenance is done regularly. This involves (a) daily inspection to detect tears in the netting and to remove debris and fouling organism, (b) periodic rotation of cylindrical styrofoam floats, repainting of wooden frame or GI pipe frame with coal tar, and (c) changing of nets every 2 months or as often as needed to protect from fouling organisms. Fouled nets are sundried, cleaned and repaired. Two or three months before breeding season, nets are no longer changed to avoid disturbing the fish which might inhibit maturation and spawning.

5. DATA GATHERING

Pertinent data on routine daily activities, existing stocks, sampled fish and environmental conditions are recorded.

5.1 Logbooks and Data Sheets

- a. Daily Activity Logbook (Annex 3) contains all day-to-day activities done during the entire course of the program. These include date and number of stocks, mortalities, repair and maintenance, etc.
- b. Stocking Logbook (Annex 4) monitors the number of stock present, transfers, mortalities, samples made on the stock, and feed ration for each fish enclosure. Each pond/pen and cage shall have its own stocking logbook.
- c. Sampling and Mortality Logbook (Annex 5) is used only for sampled fish collected during the maturation phase, beginning on the 4th year of the stock. Dead fish recovered before the 4th year may also be included here.
- d. Physico-chemical Data Logbook (Annex 6) contains data on salinity, temperature, etc. in the pond/pen or cage. Separate logbooks for pond/pen and cage are kept.
- e. Stocking Record Data Sheet (Annex 7) records all transfers made from an outside source to the rearing pond/pen, from pond/pen to cages, and from one cage to another. It contains information such as source, number, age, weight and length of stock.
- f. Length-weight Data Sheet (Annex 8) contains measurements on individual fish samples. Length-weight data are taken during stocking and transfer from rearing pond/pen to cage and from one cage to another. The Length-weight Data Sheet Number is entered under Cross Reference Number of the Stocking Record Data Sheet.

5.2 Physico-Chemical Parameters

The physico-chemical parameters to be monitored during the program are salinity, temperature, pH, dissolved oxygen and water transparency including weather and sea conditions. Enter data in appropriate logbook.

5.2.1 In Rearing Pond/Pen

- a. Salinity — measure salinity 2-3 times per week using handy refractometer.
- b. Temperature — measure surface water 2-3 times a week in the morning and 3 times daily (morning, noon and afternoon) for one week of each month.
- c. Dissolved Oxygen — with a Dissolved Oxygen meter, measure dissolved oxygen at specific sites in pond (e.g.

near gate and farthest point from gate) twice daily at 8 AM and 4 PM.

- d. pH — record pH of pond/pen water once a week using portable pH meter or pH paper with range of 7-9.

5.2.2 Maturation Cages

- a. Salinity — with a refractometer, measure salinity from surface and at 3 m (depth of cage), 2-3 times per week. Use water sampler (e.g. Nansen bottle or Rigosha water sampler) to obtain samples at 3 meters.
- b. Temperature — measure surface and bottom (3 m) temperature 2-3 times a week in the morning, and 3 times daily (morning, noon and afternoon) for one week of each month. Use water sampler to measure temperature from the bottom.
- c. Transparency — with Secchi disc, measure water transparency 2-3 times per week.

5.3 Fish Sampling Procedure

5.3.1 During Stocking and Transfers

During each stocking and transfer, and 6 months thereafter until stock is 3 years old, sampling for fish weight is done to determine feed ration. A sample consisting of 10% of the stock will suffice.

The sampling procedure below should be followed for each stocking and transfer:

- a. Fish reared in pond or pen may be caught by a small cast net. Those in cages are best obtained by carefully lifting cage and concentrating fish to one side of cage. Care should be observed while catching the fish to minimize stress and to prevent escape.
- b. Scoop out the fish and place in a large bag or plastic container containing 200 ppm of 2-(phenoxy) ethanol (Annex 10) until level of anaesthesia is reached (refer to stage III. phase 2, of Annex 11).
- c. Record weight and fork length in Length-Weight Data Sheet (Annex 8).
- d. Immediately return fish to rearing pond/pen or cage for recovery. Fill out also Stocking Logbook (Annex 4).

5.3.2 Sampling During Maturation

Beginning on the 4th year gonad development of stock is monitored by periodic samplings. Monthly to quarterly samples are obtained depending on progress of gonad development. At least 4 males and 4 females are sampled. More frequent sampling (monthly) is done when gonads are visibly maturing or when gonadosomatic index (GSI) is greater than 0.1 for males and 0.5 for females. (See Annex 12 for calculating GSI). Generally, maturing and mature ovaries appear yellow to yellow-orange while maturing and mature testes are white, creamy, or greyish.

Follow the Sampling Procedure below for determining gonad development.

- a. Sampling can be done by spear gun, aimed at the head or, preferably by hook and line. Immobilize the fish by pithing with a pointed knife just behind the operculum or, over-anaesthetize fish using 3 ml of 2 (phenoxy)-ethanol per 4 liters of seawater.
- b. Measure weight, total length, fork length, standard length, and pre-anal length (Fig. 5). Enter data in the Sampling and Mortality Log Book (Annex 5).
- c. Dissect out the gonads. Cut ventral abdominal wall from anus to pectoral girdle. Watch out for the gonads. Remove gonads carefully. Weigh, then record. Enter the data in the Sampling and Mortality Logbook.
- d. Obtain a sample from mid-portion of right gonad. A 2 cm x 3 cm section is sufficient. Fix section in Bouins fixative solution (Annex 13). The remaining gonads are fixed in 10% buffered formalin solution (Appendix E). Label all samples.
- e. For mature females, weigh 1 g sample from right ovary and fix in 10% buffered formalin or Gilson's fluid (Annex 13). This will be used for estimating fecundity.
- f. Calculate GSI and enter in Sampling and Mortality Log-book.
Determine fecundity of mature females as follows:
- g. Transfer the 1 g sample to a petri dish containing small amount of tap water.
- h. Under stereoscope, tease sample using dissecting needles to free eggs. Count total number of eggs in sample.

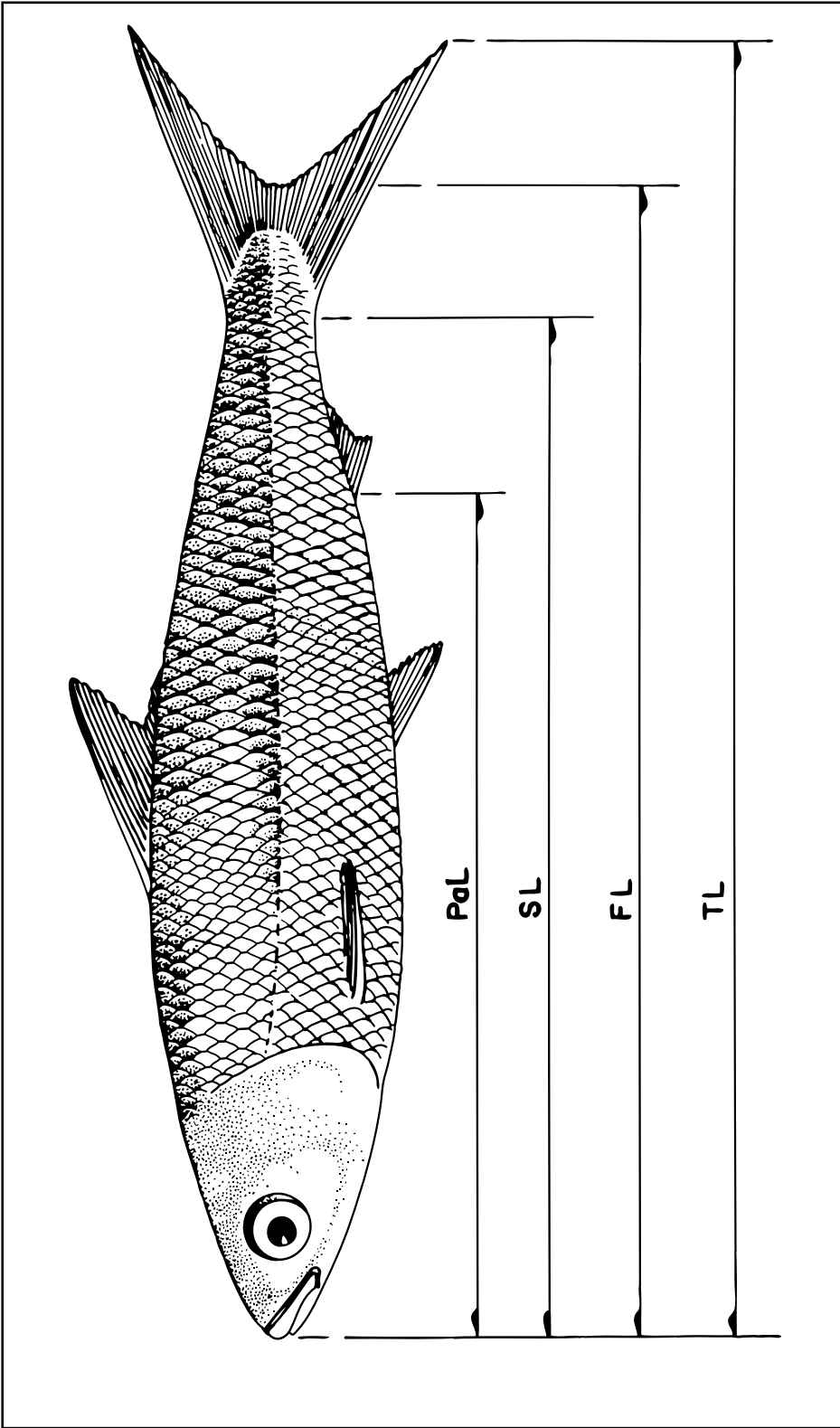


Fig. 5. Morphological measurements to be determined for milkfish during mortality and sampling.

Multiply this by gonad weight to determine fecundity (Annex 12). Enter data in the Sampling and Mortality Logbook.

5.3.3 Mortality Record

Record all mortalities as described in the previous section. Enter in Sampling and Mortality Logbook (Annex 5). Record gonad weight only when gonads are visibly maturing or mature.

SPAWNING AND EGG COLLECTION

During the spawning period, when the broodstock are 4-5 years old, some fish will show increased swimming and pronounced chasing behavior. Gonad maturation of the stock is confirmed when sampled fish have maturing or mature gonads (Refer to section 5.3.2 for sampling). Preparations for collecting spawned eggs should be completed before this time.

6.1 Collecting Spawned Eggs

Use plankton nets with 600-800 (0.6-0.8 mm) mesh size. Recommended design and specifications for egg collectors are shown in Fig. 6.

- a. Suspend egg collectors at 0.5-1 m below water surface, and position along prevailing water current (Fig. 7).
- b. Set up collectors for each maturation cage every afternoon.
- c. Lift collectors early each morning (6-7 AM) and examine sample.

6.2 Handling

- a. Place about 10 liters of seawater in 20-30 liter pail.
- b. Lift egg collector slowly and rinse by dipping the net a few times. Completely drain contents of net in the pail, splashing sides of net to dislodge eggs sticking on net. Aerate collected sample to keep eggs suspended.
- c. Examine sample in pail by scooping out one-half liter in glass beaker. Milkfish eggs are prominent and numerous in plankton sample when spawning occurs. Discard content of beaker if no eggs are seen. Continue examining half liter samples until pail is empty.
- d. When eggs are present examine a representative sample under a stereoscope. Milkfish eggs are spherical with diameter of 1.1-1.2 mm, non-adhesive, and transparent with granulated yolk

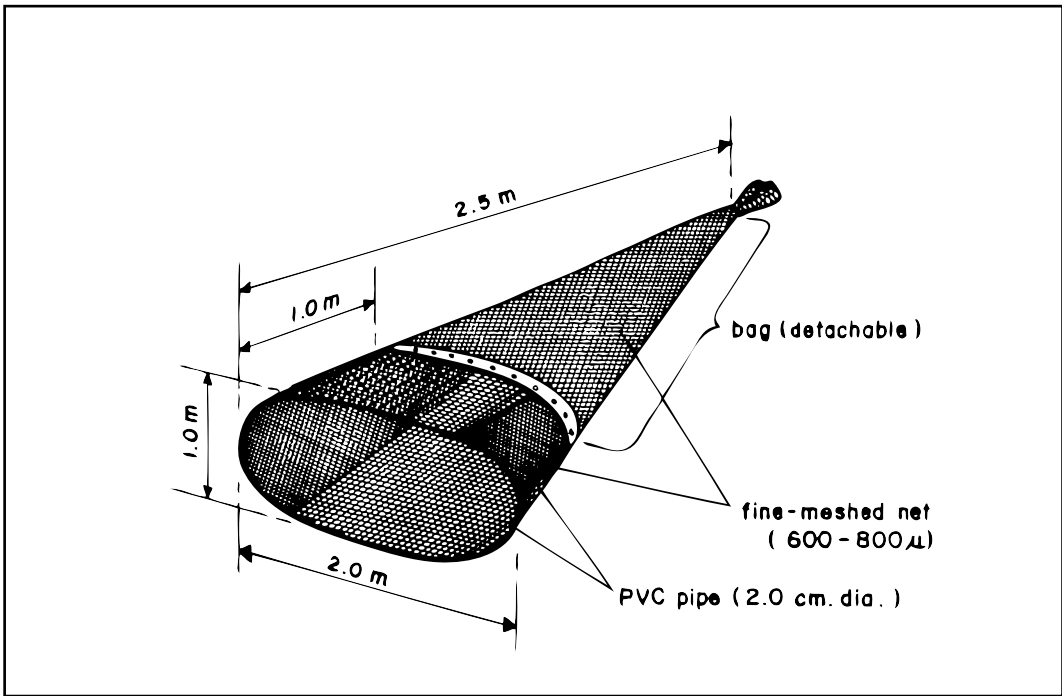


Fig. 6. A design of a milkfish egg collector.

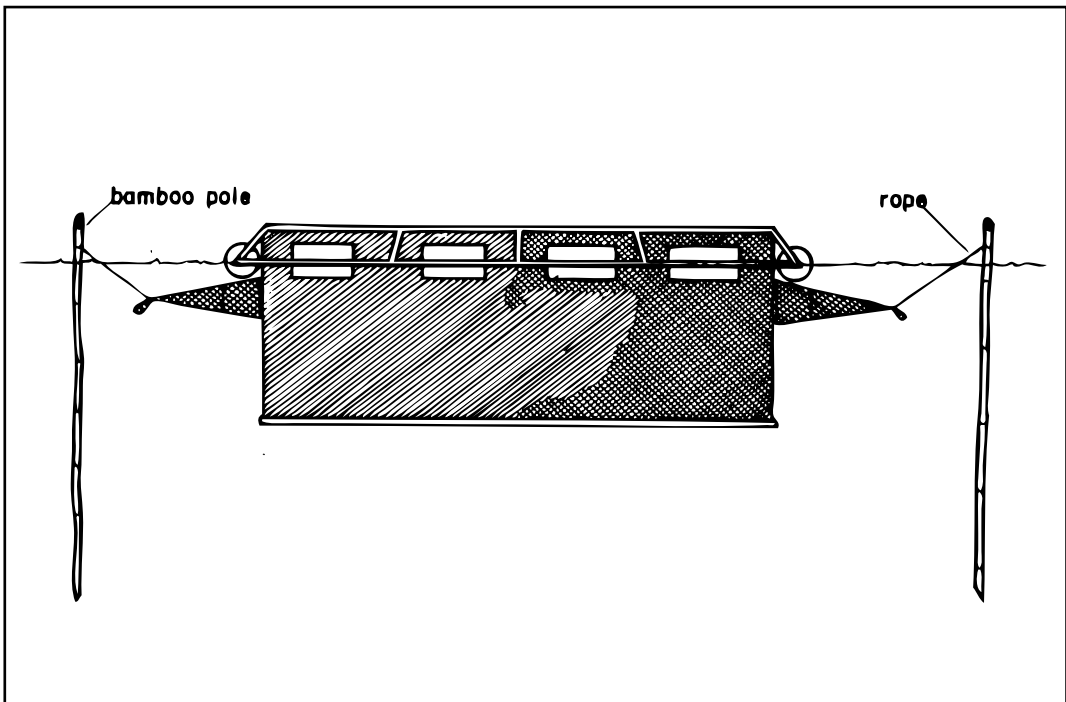


Fig. 7. Position of egg collectors beside the maturation cage.

giving it a slight yellow tinge. There are no oil globules. Under high magnification, the egg shell has prominent marking patterns of mosaic appearance (Fig. 8a).

- e. Fix a 50-100 ml subsample in 5% buffered Formalin and set aside. This will be used for estimating fertilization rate.

6.3 Sorting Eggs

- a. Shut-off aeration in pail for 5-10 minutes. Transfer live fertilized milkfish eggs to a pail of filtered seawater by carefully scooping or decanting. Live fertilized eggs remain suspended or afloat when aeration is stopped. Unfertilized or dead eggs sink.
- b. Calculate the total number of live fertilized eggs from 100 ml subsample. For reliable estimates, at least three 100 ml subsamples are counted. Refer to Annex 12 D for calculating the total number of fertilized eggs.
- c. Transfer the rest of live fertilized eggs in oxygenated plastic bags and transport immediately to hatchery for larval rearing (Sec. 7).

6.4 Determination of Fertilization Rate

Count fertilized and unfertilized eggs from the 100 ml preserved subsample (Sec. 6.2.e). Fertilized eggs have perivitellin space and blastodisc (Fig. 8b). Unfertilized eggs lack these structures and appear relatively more opaque (Fig. 8c). Fertilized eggs may be at any stage of embryonic development depending on time of fixation.

6.5. Determination of Hatching Rate

- a. In the hatchery, live eggs are transferred to 600-liter fiberglass incubation tanks containing filtered seawater and provided good aeration. Hatching occurs about 20-24 hours from the time of collection.
- b. Determine hatching rate by taking 100-ml subsample from incubation tanks and counting hatched larvae. For reliable estimates, take at least 5 subsamples from different parts of incubation tank. Since volume of water in tank is known and total number of fertilized eggs has been estimated, hatching rate can be calculated (Annex 12).

LARVAL REARING

Larval rearing follows the method described in Juario, J.V. and Duray, M.N., "A Guide to Induced Spawning and Larval Rearing of Milkfish *Chanos chanos* (Forsskal)", ISSN 0115-4710 SEAFDEC AQD Technical Report No. 10 (1983).

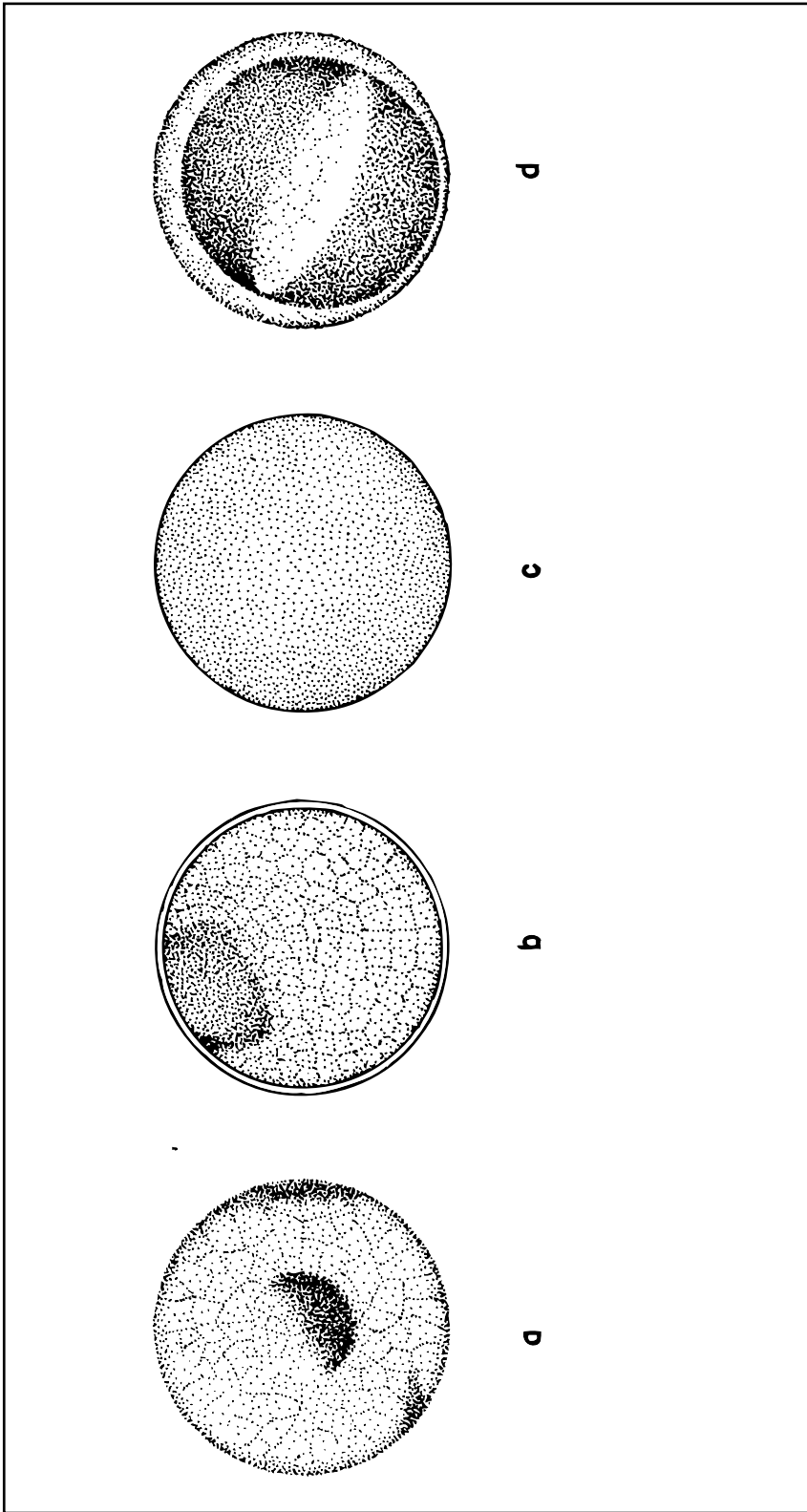


Fig. 8. a. Magnified view of the egg shell showing distinctive markings.
b. Fertilized milkfish egg showing blastodisc.
c. Unfertilized milkfish egg appearing as a relatively more opaque mass.
d. Egg stage (Late yolk invasion) at time of collection between 6-7 a.m.

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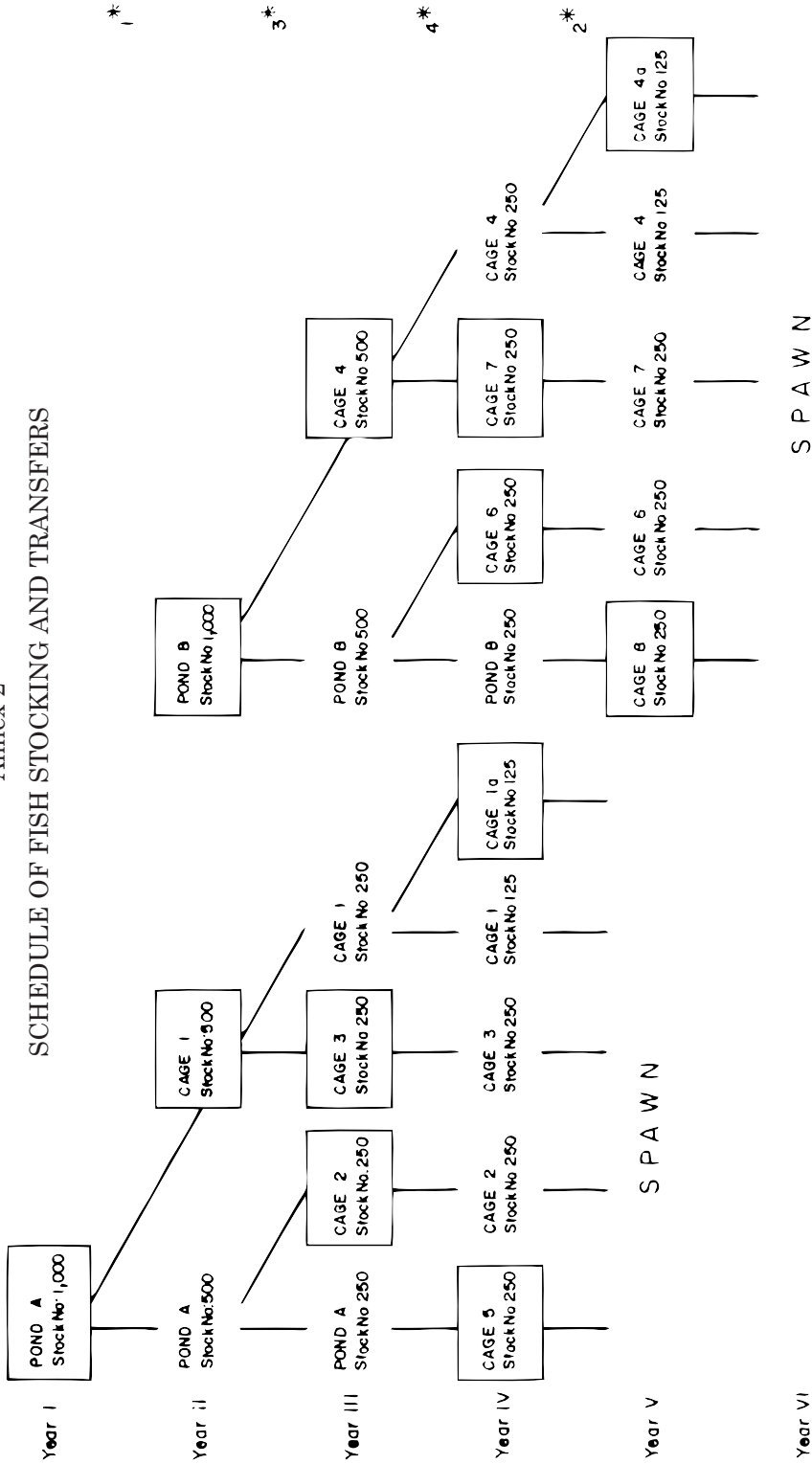
ANNEXES

Annex 1

SCHEDULE OF CONSTRUCTION AND INSTALLATION OF MILKFISH BROODSTOCK FACILITIES

	Year	Quarter	Number to be constructed/ installed	Duration (months)
Construction or repair of brackish- water pond or pen	1	1-2	1	3-6
	1	1-2	1	3-6
Construction and installation of floating net cages	1	4	1	2-3
	2	4	3	2-3
	3	4	4	2-3
	4	4	2	2-3

Annex 2
SCHEDULE OF FISH STOCKING AND TRANSFERS



* Number of cages to be constructed

Annex 3

DAILY ACTIVITY LOGBOOK
(Sample Entry)

Date	Activities Done
6 March 1981	<ol style="list-style-type: none"><li data-bbox="548 413 931 438">1. Treated fishpond with lime<li data-bbox="548 479 814 504">2. Flooding at 3 AM<li data-bbox="548 542 878 568">3. Repaired tertiary dikes<li data-bbox="548 606 642 631">4. Etc.
7 March 1981	<ol style="list-style-type: none"><li data-bbox="548 672 1013 697">1. Continued repairing tertiary dikes<li data-bbox="548 736 642 761">2. Etc.
8 March 1981	<ol style="list-style-type: none"><li data-bbox="548 799 1130 863">1. Stocked 1,000 milkfish fingerlings from Moreno's pond<li data-bbox="548 896 1130 960">2. Sampled 100 milkfish and recorded length, weight, and body weight<li data-bbox="548 993 1063 1018">3. Three fish found dead during transfer<li data-bbox="548 1056 642 1081">4. Etc.

Annex 4

STOCKING LOGBOOK

Cage No. _____

Stock No.	Ave. Wt.	Action Taken	Source	Desti- nation	Stock Record No.	Remarks
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SAMPLING AND MORTALITY LOGBOOK

Date Died Sampled	Sex	Fish Code	Holding Structure Number	Feed	Gonad Weight (gm)	Body Weight (kg)	GSI* %	Remarks	TL (cm)	FL (cm)	PAL (cm)	Fecundity**
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$$* \text{ GSI} = \frac{\text{Gonad Weight (gm)}}{\text{Body Weight}} \times 100$$

** For mature female milkfish only

Annex 6

PHYSICO-CHEMICAL DATA LOGBOOK

Holding Structure _____

Date_____

Time	Temp. (°C)	Salinity (‰)	pH	Transparency (meters)	D.O. (ppm)	Remarks (e.g. sea condition, cloud cover, wind, etc.)*

* Cloud cover: Blue sky (up to ¼ covered); Partly cloudy (¼ to ¾ covered); Mainly cloudy (not less than ¾ covered); overcast (completely covered)

Sea condition: Calm (glassy); calm (ripples — 0-0.3 m approx. height); Smooth (wavelets - 0.3-0.6 m); Slight (0.6-1.2 m); Moderate (1.2-2.5 m); Rough (2.5-4 m); Very rough (4.0-6.0 m); High (6.0-9.0 m); Very high (9.0-14.0 m).

Wind scale: 0 (calm); 1 (light airs); 2 (light breeze); 3 (Gentle breeze); 4 (Moderate breeze); 5 (Fresh breeze); 6 (Strong breeze); 7 (near gale); 8 (gale); 9 (strong gale); 10 (storm); 11 (violent storm); 12 (Hurricane).

Annex 7

STOCKING RECORD SHEET

No _____

Holding Structure _____

Date _____ Time _____

Source _____

Number of Fish Stock _____

Age of Fish _____

Average Weight (kg) _____ Range of Weight (kg) _____

No. of Samples Taken _____

Cross Reference No. _____

Means of Transport _____

Duration _____

Mortality _____

Notes/Remarks:

Annex 8

LENGTH-WEIGHT SAMPLING DATA SHEET

No _____

Date	Body Weight (kg)	Fork Length (cm)	Remarks
------	---------------------	---------------------	---------

Mean =

Mean =

Annex 9

COMPUTATION OF FEED AMOUNT GIVEN TO FISH STOCK

Formula:

$$\begin{aligned} \text{FA} &= \text{TS} \times \text{BW} \times \% \text{BW} \\ &= \text{expressed in kg or gms per day} \end{aligned}$$

where,

$$\begin{aligned} \text{FA} &= \text{feeding amount (kg or gm per day)} \\ \text{TS} &= \text{total number of stock} \\ \text{BW} &= \text{average body weight} \\ \% \text{BW} &= \text{feeding level (either 1.5 or 2.0\%)} \end{aligned}$$

Example:

$$\begin{aligned} \text{Given} &: \text{TS} = 1000 \text{ milkfish juveniles} \\ &\text{BW} = 0.250 \text{ kg} \\ &\% \text{BW} = 2.0\% \\ \text{Computation} &: \text{FA} = 1000 \times 0.250 \times 0.02 \\ &= 5.0 \text{ kg per day} \end{aligned}$$

Therefore, if feeding amount (FA) is 5.0 kg per day, then 2.5 kg shall be given per feeding time.

ANAESTHESIA PROCEDURE

Anesthetic:

200 ppm, 2 -(Phenoxy)-ethanol or Ethyleneglycolmonophenyl-ether (approximately 1 ml/5.1)

Procedure:

1. Measure 6 ml of 2-(phenoxy)-ethanol in a graduated cylinder and pour in a bottle.
2. Add seawater halfway through the bottle and shake vigorously until the reagent emulsifies.
3. Place 30 liters of seawater in a large plastic bag or plastic container.
4. Pour the emulsified anaesthetic in the plastic bag or container and mix vigorously.
5. Carefully place fish into the plastic bag or container and shake gently.
6. Change anaesthetic water every fourth batch of sampled fish or until the water becomes bubbly and the fish is observed to take a longer time reacting to the effects of the anaesthetic.

Note: Fish show signs of anaesthesia within 1-2 minutes after immersion.

Annex 11

CLASSIFICATION OF THE STAGES OF
ANAESTHESIA OF FISHES

<u>Stage</u>	<u>Phase</u>	<u>Response</u>
0		The fish is swimming and respiring normally.
I		The fish is swimming erratically with the evident loss of equilibrium and orientation. Opercular movement is increased.
II		The fish further losses equilibrium. Although opercular movement is back to normal, there is a distinct effort to maintain its upright position. It also swims aimlessly and slowly.
III	1	There is a complete loss of equilibrium with an accompanying decrease in swimming and opercular movement.
	2.	Swimming movement is absent and opercular activity is rapid and shallow. There is no response to external stimulation. THIS IS THE SURGICAL PHASE OF ANAESTHESIA WHERE FISH CAN BE SAFELY HANDLED.
	3.	Opercular movement stops. The fish may be revived by returning to untreated seawater.
IV		There is a marked spasmodic overdilatation of the opercula. Cardiac failure follows within a few minutes.

Note: The stages occur within a few seconds, hence, complete care should be observed during the process.

Annex 12
CALCULATIONS

A. Length of netting for 30% hanging ratio:

$$LN = \frac{LR}{1-0.3}$$

Where,

LN — length of stretched netting

LR — length of rope or net framing

Example:

Given: LR = 100 meters

$$LN = \frac{100}{1-0.3}$$

= 143 meters. This is the length of stretched netting required to get a 30% hanging ratio for a 100 meter rope.

Note: The hanging ratio can be calculated from the following formula:

$$H = \frac{LN-LR}{LN} \times 100$$

B. Gonadosomatic Index (GSI):

$$GSI (\%) = \frac{\text{Gonad weight (grams)}}{\text{Body weight (grams)}} \times 100$$

C. Fecundity:

$$\text{Fecundity} = \frac{\text{Number of eggs in 1 gram samples} \times \text{Gonad weight}}{\text{Gonad weight}}$$

Fecundity may also be expressed as number of eggs per unit body weight. To obtain this divide total number of eggs by body weight

D. Total number of fertilized eggs (FE)

$$FE = \frac{\text{Average counts from 100 ml subsamples} \times \text{Total volume of water (ml)}}{100}$$

E. Hatching rate (HR)

$$HR = \frac{\left[\frac{\text{Average counts of hatched larvae in subsamples} \times \text{Total volume of water}}{\text{Volume of subsample}} \right]}{\text{Total number of fertilized eggs}} \times 100$$

PREPARATION OF FIXATIVES AND PRESERVATIVES

A. 10% Buffered Formalin Solution

Reagents:

Formalin, technical grade

Sodium acid phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)

Anhydrous disodium phosphate (Na_2HPO_4)

Procedure:

1. Prepare 1000 ml of 10% Formalin solution by mixing 100 ml technical grade formalin and 900 ml distilled water.
2. Add the following:

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ — 4.0 grams

Na_2HPO_4 — 6.5 grams

If these are not available, they may be substituted with

$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ — 4.52 grams

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ — 8.15 grams

B. 5% Buffered Formalin Solution

The procedure for preparing 10% buffered formalin solution is followed but use 50 ml technical grade formalin and 950 ml distilled water.

C. Bouin's Solution

Reagents:

Saturated aqueous picric acid

Formalin, technical grade

Glacial acetic acid

Procedure:

1. Prepare saturated aqueous picric acid by mixing 75.0 ml distilled water and 1.05 grams picric acid or by constantly adding with stirring, picric acid to 75.0 ml distilled water until the acid fails to dissolve.
2. Mix the following:

saturated aqueous picric acid	75.0 ml
technical grade formalin	25.0 ml
glacial acetic acid	5.0 ml

D. Gilson's Fixative

This is recommended for fixation of gonad samples to be used for fecundity estimation. Eggs can be easily teased out of the sample using this fixative.

Reagents:

Nitric acid
 Glacial acetic acid
 Mercuric chloride
 Ethyl alcohol — 60%
 Distilled water.

Procedure:

1. Prepare 100 ml of 60% ethyl alcohol by mixing 60 ml ethyl alcohol (100%) and 40 ml distilled water.
2. Mix the following:

Nitric acid	15.0 ml
Glacial acetic acid	4.0 ml
Mercuric chloride	20.0 g
60% Ethyl alcohol	100.0 ml
Distilled water	880.0 ml

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Cover: Floating cages for milkfish broodstock in Igang