AQUACULTURE EXTENSION MANUAL NO. 18
APRIL 1998

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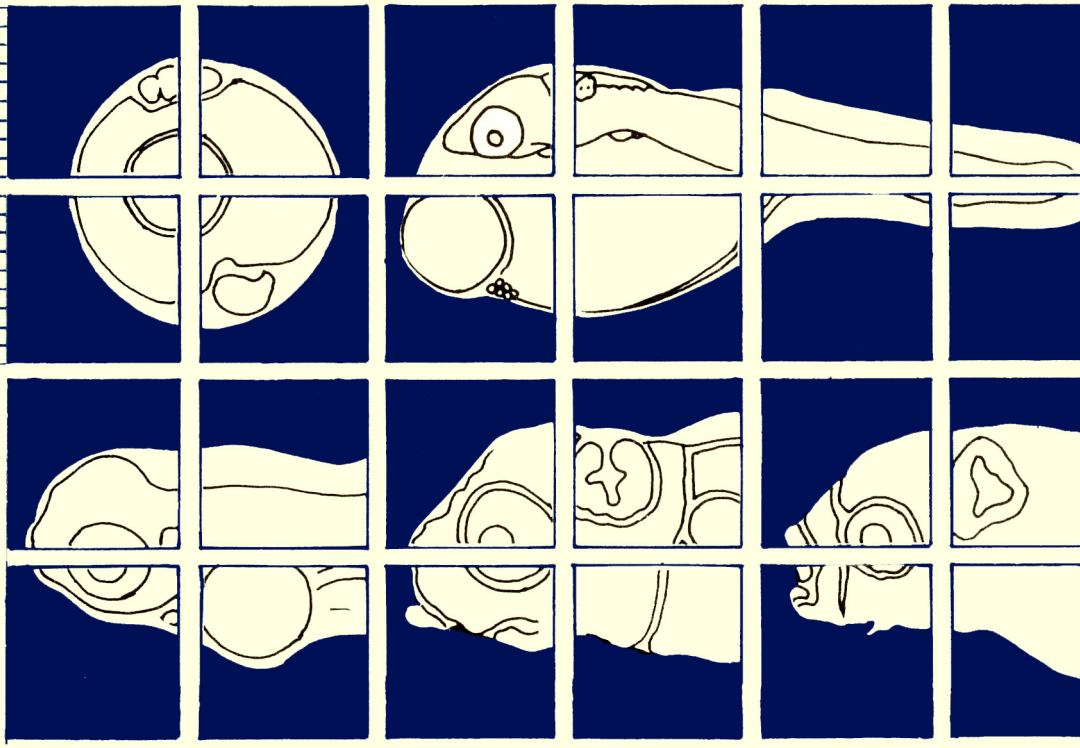

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PREFACE

Sea bass remains a popular food fish in Southeast Asia. It is not however well-known among many Filipinos. Sea bass is sold in very few quantities in wet markets, perhaps a reflection of depleted fisheries in many parts of the country or a lack of awareness of producing sea bass through aquaculture. This manual addresses the latter concern.

Aquaculture Extension Manual No. 18 entitled "Sea Bass Hatchery Operations" was first published in July 1990 in response to the growing need to promote the culture of other food fishes aside from milkfish and tiger shrimp. Technical information in this manual were derived mainly from published information. But, since 1990, developments in the local aquaculture industry have rapidly changed. Notably, many shrimp hatcheries have been abandoned as seed demand for shrimp grow-out culture have all but diminished. Sea bass seed production therefore offers a viable alternative. Recent developments on sea bass seed production have been further validated through research at the SEAFDEC Aquaculture Department. The breeding techniques in sea bass have basically remained the same. A new development on larviculture is described in this revised edition, particularly the use of an alternative live food for sea bass larvae.

Hopefully, sea bass culturists may be able to easily follow the contents of the manual. In adopting this manual, an awareness of promoting the sustainability of this particular technology is a final advice that the authors wish sea bass culturists to seriously consider.

Suggestions and comments for the next edition of the manual will be welcomed.

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Tigbauan, Iloilo, Philippines
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INTRODUCTION

Sea bass (*Lates calcarifer* Bloch) contributes to the capture fishery and aquaculture production of many countries in the Indo-West Pacific region. In Australia and Papua New Guinea, the sea bass, locally known as *barramundi,* is an important resource, forming the basis of commercial and recreational fishery. In Asia, sea bass aquaculture is already established particularly in Thailand, Hong Kong, Singapore, and Malaysia. Its profitability in Thailand is based on four factors: (1) availability of fry in commercial quantities; (2) low labor costs; (3) high market price for table-sized fish; and (4) sufficient supply of cheap trash fish.

In the Philippines, only recently has sea bass culture received much attention. It will remain limited unless a suitable artificial diet and a technique for mass fry production are made available.

This manual addresses fry production in the hatchery. It describes the principles and practical procedures for rearing sea bass - from eggs until metamorphosis - as practiced by the Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC/AQD) in Tigbauan, Iloilo, Philippines.

This manual is intended for easy reading and hopes to serve as background material for many practitioners in the aquaculture industry who may be interested in sea bass culture. SEAFDEC/AQD looks forward to receiving comments from users.

Terminologies

Hatchery. "Hatchery" is popularly associated with "artificial propagation" or "seed production," the process of obtaining young fish from captive or migrating parents. Strictly, a hatchery is a place for hatching eggs. However, distinction is made here between hatchery and nursery operation. The latter implies the rearing period from the hatching of eggs to the stage of free-swimming and feeding fry, whereas the former suggests procedures of collecting eggs and sperms to egg hatching. This manual takes "hatchery" in its broadest context to include physical facility, broodstock, larval rearing procedures, natural food culture, and disposal of products.

Broodstock. In husbandry, it means the captive parent stock is allowed to attain sexual maturity and spawning readiness to make eggs and sperm available.

Ovarian eggs. This is the female reproductive cell in the ovary.

Larva. This is the young fish emerging from the egg and which is fundamentally unlike its parents and must metamorphose before assuming adult characters.

Fry. This is an arbitrary term for the young metamorphosed fish.

Induced spawning. It is the deliberate use of active chemical agents, e.g., hormones, to manipulate sexual maturation and final release of sexual products.

Spontaneous spawning. It is the natural release of eggs or sperm from the body of the fish.

Incubation. This means to simulate conditions favorable to development and hatching of fertilized eggs.

Larval rearing. This is simulating conditions conducive to growth and survival by giving adequate care, proper food, and good quality water to the larvae in captivity.

GENERAL PRINCIPLES

Operation of a sea bass hatchery is aimed at maximizing economic return. For this reason, the economic as well as the biological factors involved in it must be understood. There are various ways of evaluating these factors but for practical purposes, it is enough to keep in mind the following points:

• The market for which the product (fry) is intended must be identified and carefully assessed.

• The cost of production under local economic conditions should be thoroughly appraised so that resources can be used efficiently.

Since these considerations make use of biological phenomena as basis for production, it is necessary to understand the biology of sea bass and the environmental processes occurring in the hatchery. Profit or loss largely depends on being able to ensure the maintenance of conditions conducive for larval development, growth, and survival. In practice, bear in mind the generalizations listed below:

• Developing eggs and larvae are the most vulnerable stages in the life history of the fish and therefore require proper care, good quality water, and sufficient supply of suitable food.

• "Good quality" water is a relative term; it refers to water that can support and maintain environmental requirements and sanitary standards necessary to sustain larval growth. Quality refers to suitable temperature and salinity, sufficient supply of oxygen, and minimal concentrations of ammonia, other toxic substances, and pathogens.

- *Temperature.* Its effect on larvae is largely biochemical and is reflected in growth performance. Extremely high or low temperature cause death. Sudden temperature changes cause stress and stressed larvae give unsatisfactory return.

- *Salinity*. Changes in salinity influence the water balance of the larvae. In waters with high salt content, water is drawn out from the larva; in waters with low salt, the larva draws in water - both causing stress and eventual death.

- *Oxygen.* Necessary for almost all organisms to sustain life, oxygen must be available at all times especially when rearing densities in the hatchery are high.

- *Ammonia.* The end-product of protein breakdown among organisms, ammonia retards growth and causes stress and eventual death. Therefore, its content in the water must be minimized.

- *Toxic substances.* These occur in the form of metabolites produced by the growing and feeding larvae, inorganic and organic wastes from sewage disposal systems inadvertently taken in with hatchery water, chemicals accidentally dumped in water, and others. Such substances have adverse effects on the larvae, hence water must be free from these substances.

- *Pathogens.* These organisms occur anywhere in nature, hence cannot be totally eliminated. The water used must not contain high numbers of pathogens. As the adage goes, an ounce of prevention is always better than a pound of cure.

BIOLOGY

Sea bass is a percoid fish belonging to the family Centropomidae, Order Perciformes. As shown in Fig. 1, the eggs are spawned and fertilized in the sea and the larvae enter brackishwater swamps and mangrove areas where they thrive on the abundant food found in these nursery grounds. Sea bass also inhabits estuaries, rivers and lakes, and returns to marine waters to spawn, thus completing a life cycle spent in both freshwater and seawater. In fact, sea bass is euryhaline.

Wild stocks of sea bass juveniles in northern Australia mature initially as males after 3-4 years but invert to females on the sixth year. However, not all males become females because primary females do occur. Seabass, therefore, is a protandrous hermaphrodite. In captivity, sea bass can be spawned quite readily with or without the use of spawning agents.

Although there are no documented evidence in the Philippines of sea bass undergoing gonadal maturation and spawning in the wild, the onset of their natural breeding season is indicated by the appearance of sea bass fry in milkfish fry collections. The breeding season coincides with the monsoon months from late June until late October. Because sea

Fig. 1. Life cycle of sea bass including migration pattern from the open sea to estuaries, to freshwater areas, and back

bass is an incidental species in most fry collections, its collection from natural fry grounds is not reliable, largely inefficient, and tedious.

In general, sea bass is an opportunistic predator throughout its life cycle. Fish less than 4 cm feed on "microscrustacea" almost exclusively; 30-cm fish have diets of "macrocrustacea" and fish; larger individuals predominantly prey on fishes. In captivity, sea bass accepts pelleted rations.

Sea bass growth rate varies depending on culture conditions but is generally high. Sea bass fed trash fish in cages grow from an average of 22 g to 573 g in 7 months, and in ponds, from 7.8 g to 369 g after 7.5 months.

SELECTING A SUITABLE SITE

The major criterion in selecting a site for the construction of a sea bass hatchery is the ecological requirement for spawning and egg and larval development. A suitable site, therefore, should be an area where access to a sufficient supply of pollution-free seawater is possible. The salinity should range from 25 to 32 ppt. For practical reasons, the hatchery should also be near the source of eggs; however, a hatchery may have its own broodstock facilities.

The site should be spacious, gently sloping, and protected from strong winds and wave action. A gently sloping area gives the advantage of using gravity to distribute water from an elevated storage facility, and affords protection from water inundation or flooding during the rainy season. The area should also be located where natural protection from adverse weather conditions (e.g., typhoons) is possible.

The seawater source should have minimal siltation problems and should be far from mouths of rivers as freshwater runoffs will cause high turbidity and drastic fluctuations in water salinity. The site should also have an ample supply of freshwater. Freshwater is necessary when low salinity seawater is required. It is also necessary for cleaning and washing hatchery equipment and facilities.

The site should also have access to a reliable source of electric power and to transportation and communication facilities.

DESIGN

Once a suitable site has been selected, the design of a hatchery must be considered. It is important to have in mind the production target and the available financial resources as these two factors determine the size of the hatchery to be constructed.

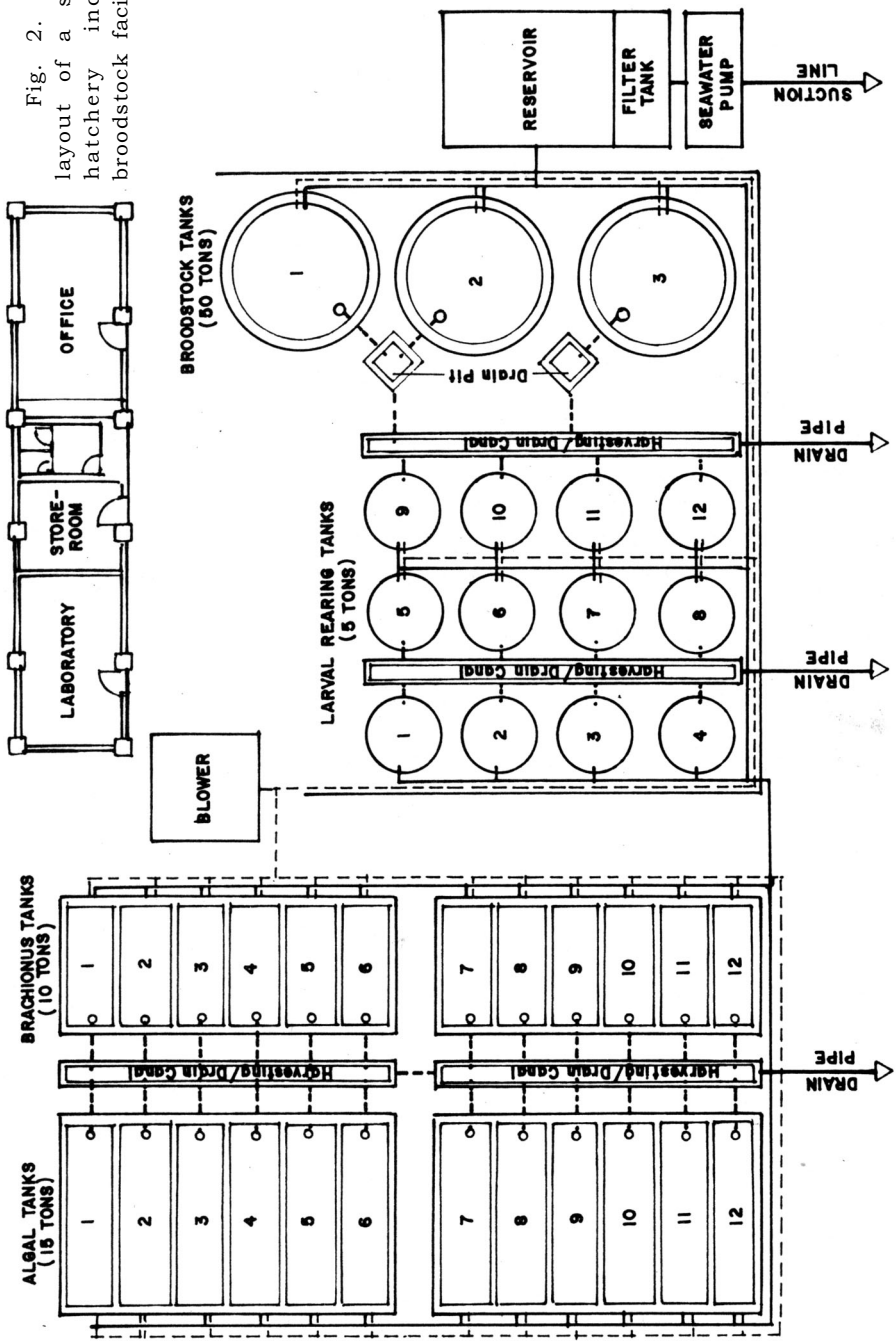

Fig. 2. Sample layout of a seabass hatchery including broodstock facilities

Tank Systems

Three major kinds of tank systems are needed in a sea bass hatchery, namely, broodstock, larval rearing, and natural food tanks. Figure 2 shows a sample layout of a sea bass hatchery with broodstock facilities. The design may be modified according to available capitalization and the physical characteristics of the site. With certain modifications, most prawn hatcheries may be converted into sea bass hatcheries.

Broodstock Holding Structures. Sea bass breeders or spawners are maintained in these structures which are installed either on land or in open waters. Land-based structures include circular or rectangular concrete tanks. The tanks are usually big and have water capacities of more than 50 t. At SEAFDEC/AQD, breeders are kept in rectangular cages measuring 4 x 4 x 3 m (Fig. 3).

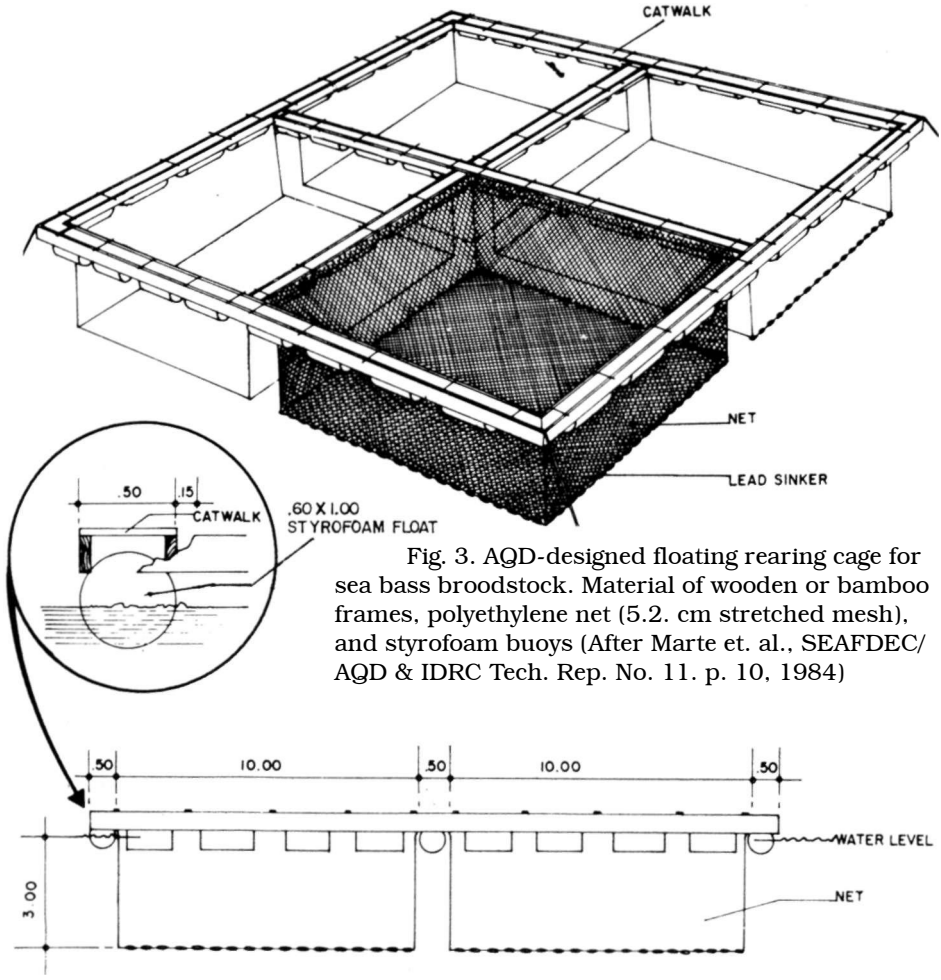

Fig. 3. AQD-designed floating rearing cage for sea bass broodstock. Material of wooden or bamboo frames, polyethylene net (5.2. cm stretched mesh), and styrofoam buoys (After Marte et. al., SEAFDEC/AQD & IDRC Tech. Rep. No. 11. p. 10, 1984)

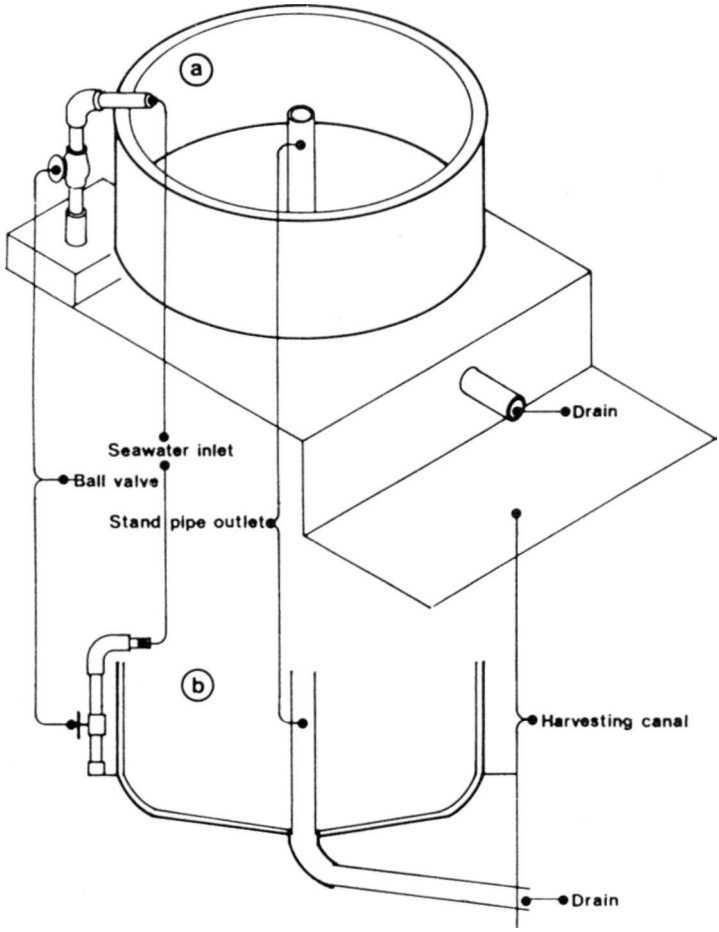

Fig. 4. Circular tank: (a) isometric view, (b) cross-sectional view

Larval Rearing Tanks. Sea bass larvae are reared in concrete tanks, such as those used by SEAFDEC, or in circular canvas tanks, such as those used for prawns by private operators. These tanks are provided with roofings to protect the larvae from direct sunlight. Circular tanks have conical bottom and a centrally located drainage pipe connected to the canal (Fig. 4). This design facilitates cleaning and harvesting.

Rectangular concrete tanks may also be used. These have a flat bottom and the drainage is usually located opposite the inlet pipe (Fig. 5). There is no functional difference between circular and rectangular tanks but the latter has practical disadvantages which include difficulty of siphoning dirt that accumulates in tank corners and formation of "dead corners" or portions of the water column in the tank where there is no water circulation.

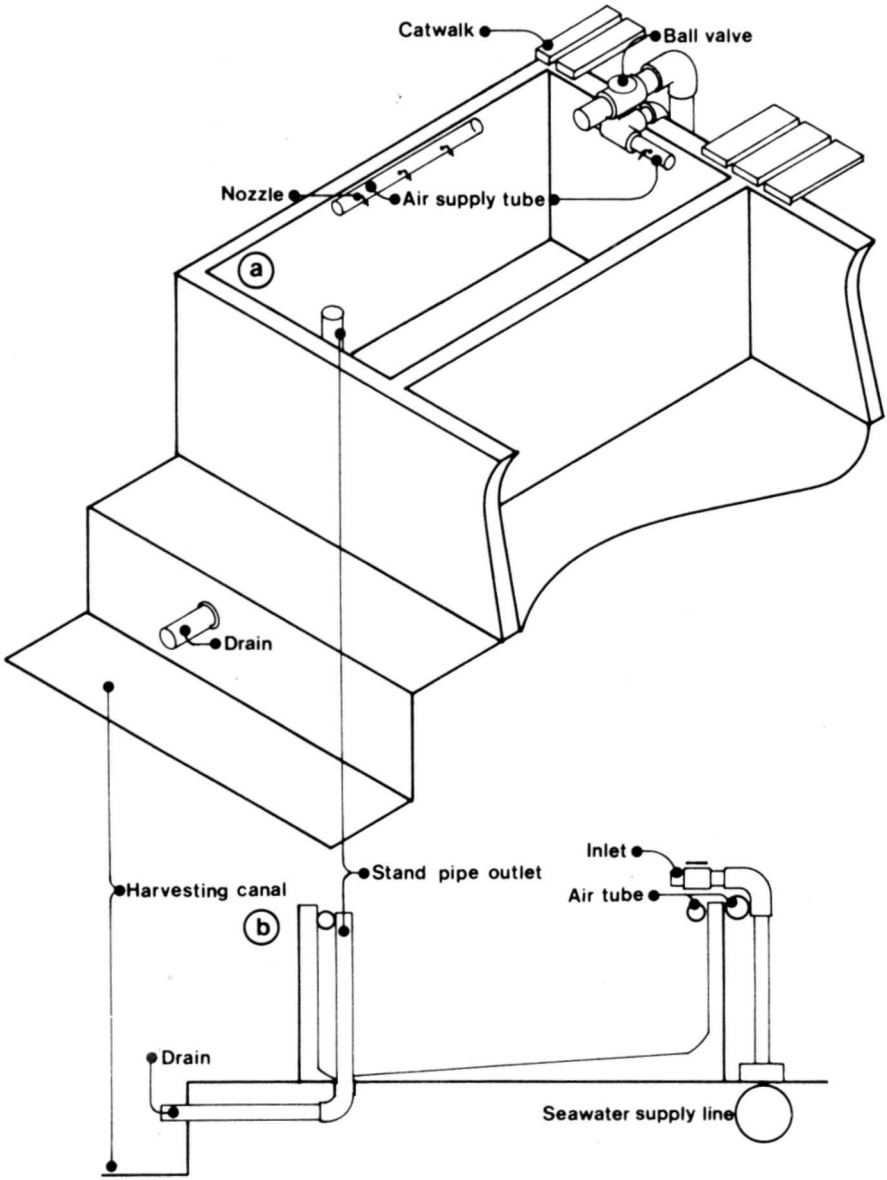

Fig. 5. Rectangular tank: (a) isometric view: (b) cross-sectional view

Canvas tanks can also be used for larval rearing. These may be cheaper to construct but do not last as long as concrete tanks. In the long run, concrete tanks will turn out cheaper

Natural Food Tanks. Tanks are required for the culture of live food organisms, e.g., *Chlorella, Brachionus.* The tonnage ratio of *Chlorella* to *Brachionus* tanks is about 1.5:1, that is, for every ton of *Brachionus* tank, 1.5 t of *Chlorella* is needed. The ratio of *Brachionus* to larval rearing tanks is 1:0.5.

Circular canvas or rectangular concrete tanks with capacities of more than 10 t can be used for the culture of algal food. These tanks should be situated outdoors to enhance the propagation of *Chlorella* which needs sunlight.

Seawater Supply system

The seawater supply should be clean and free from pollutants. If the water is relatively clear, it can be pumped directly to an elevated filter tank, stored in the reservoir, and then distributed by gravity to the different culture tanks. During heavy rains when the seawater may become turbid, water may be pumped into a sedimentation tank to allow suspended solids to settle. Only the upper layer of clear water is pumped into the filter tank.

The pumping capacity of the marine pump needed in the hatchery will depend on the water volume requirement, pumping time, and total head. Total head is the difference in elevation between the surface of the source of water and the point of discharge. The daily water volume requirement can be calculated from the total volume of the tanks and the rate of water exchange.

Freshwater Supply System

In some instances, salinity lower than what is normal of seawater (35 ppt) is necessary, in which case freshwater is added to seawater to reduce the salinity to the desired level. Also, routine hatchery activities like cleaning and washing of tanks, basins, filter bags and other hatchery equipment require freshwater. The hatchery therefore should have ample supply of freshwater.

Aeration System

A roots blower is commonly used for this purpose. Water depth, number of aeration outlets, and cross-sectional area of the outlets have to be considered in determining the required blower capacity.

Other Facilities

In addition to the basic facilities and life-support systems, the hatchery should also have a small laboratory, where a microscope and other laboratory equipment may be kept, and a store room as well.

BROODSTOCK

An important aspect in the production of sea bass fry in a hatchery is the availability of eggs. A fish farmer desires to produce sea bass eggs on demand. To overcome difficulties in obtaining eggs or fry from wild or natural sources, SEAFDEC/AQD has recently developed techniques to control the reproductive cycle of sea bass under captive conditions in the hatchery.

Source of Breeders

Wild or hatchery fry may be reared in captivity until they become fully ripe. Nonetheless, adult sea bass may be obtained from natural spawning grounds or from a broodstock farm. Wild spawners weighing 2-8 kg each are caught by gill nets, hook-and-line and fish traps, often near river mouths from June to October. They must be wound- and disease-free, with no missing body parts, and strong and active upon capture. Ripeness of the reproductive organs (i.e., gonads) can be checked as follows:

1. Transfer fish into a shallow tank filled with seawater containing 250 ppm (0.25 ml per liter of seawater) of anaesthetic. Ethyleneglycol monophenylether (or 2-phenoxyethanol available from Merck, P.O.B. 4119, Darmstadt, Federal Republic of Germany)* is a common fish anaesthetic.

2. Turn over anaesthetized fish on its back.

3. Gently massage the abdomen following a head-to-tail direction. A milky white substance of medium-thick consistency extruded out of the urogenital opening indicates the presence of milt among sexually ripe male spawners.

4. When no milt is extruded after repeated massage of the abdomen, the tapered end of a polyethylene cannula (Clay Adams PE 100, inner diameter = 0.86 mm, outer diameter = 1.52 mm, available from Becton, Dickinson & Company, Parsippany, New Jersey 07054, USA), is gently inserted 10 cm into the urogenital opening of the fish (Fig. 6). The other end of the cannula is then gently aspirated by mouth as the inserted end is carefully withdrawn from the fish.

5. Inspect the contents of the cannula. A milky substance indicates milt, whereas tiny spherical bodies are eggs and the spawner is female.

6. Blow out eggs into a small vial containing 5% buffered formalin solution as fixative (Appendix 1).

*Mention of a company or product in this manual does not mean endorsement by the authors or SEAFDEC/AQD.

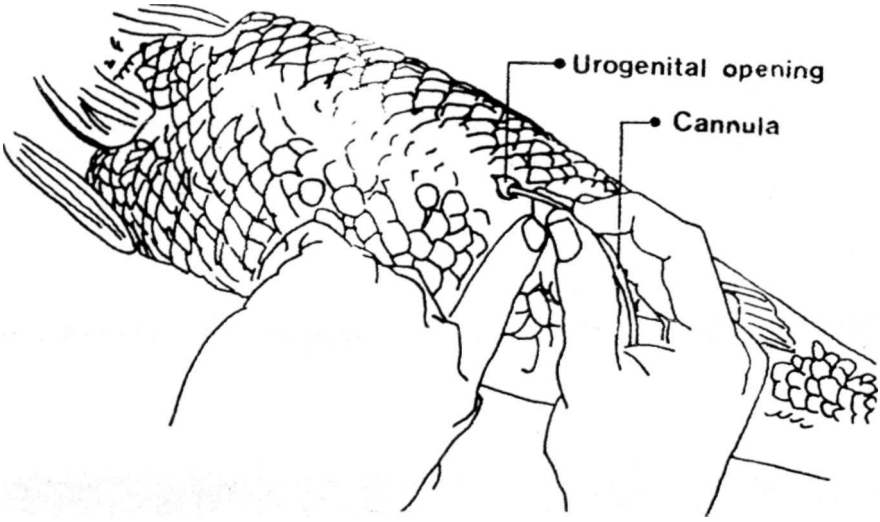

Fig. 6. Sampling for sex determination. A cannula is inserted into the urogenital opening to obtain egg or sperm sample

7. Transfer a few eggs onto a glass slide and measure the diameter of 30 eggs with a calibrated microscope (Appendix 1). Calculate the average egg diameter. A female having an average egg diameter of at least 0.40 mm is sexually ripe.

Wild and sexually immature adults can be reared in captivity until the gonads ripen. Breeders may also be obtained after 3-5 years of rearing fry and juveniles in sea cages, tanks, or earthen ponds.

Natural Spawning

Wild and sexually immature adults can be reared in captivity until the gonads ripen. Under appropriate rearing conditions, sea bass fry held in tanks or in floating net cages mature and spawn naturally after 2.5-3 years in captivity. Likewise, sexually immature adults become readily mature after several months in captivity. Being a protandrous hermaphrodite, sea bass first becomes sexually mature as males on the third year of its life history, although only a few may initially mature as females. Many of these males change to females in succeeding years.

Mature sea bass spawn naturally in tanks and in floating net cages from late June until late October, peaking in June and July. Spawning is highly predictable and to occur within 3-4 days before or after the quarter moon phase, usually between 7 and 11 pm during low tide. A

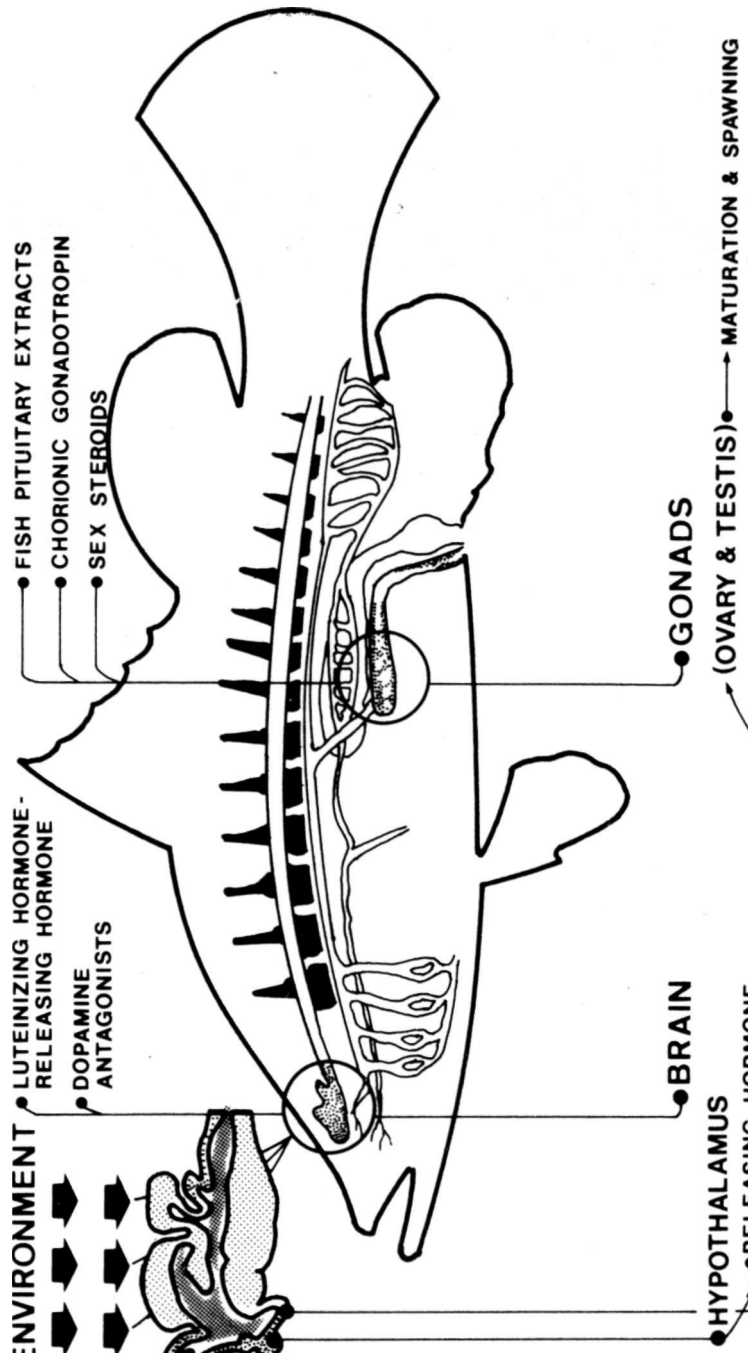

Fig. 7. Levels of exogenous intervention in the hypothalamus-pituitary-gonad axis leading to gonadal maturation and spawning

mature male or female may spawn more than once during the breeding season.

In anticipation of natural spawnings, preparations are therefore undertaken for the collection of eggs in tanks or in floating net cages. Refer to the section on "Egg Collection, Transport, and Hatching."

Hormone-Induced Breeding Techniques

Reproduction in all fishes is controlled by the hypothalamo-pituitary-gonad axis. As shown in Fig. 7, the hypothalamus is a discrete region of the brain whereas the pituitary is a small gland located at the base of the brain. In response to various environmental stimuli, the chemical substances (hormones) secreted by the axis directly influence the onset of sexual maturation resulting in the shedding of ripe eggs and sperm. The sequential mechanism by which these hormones act on the gonads makes it possible to intervene in the process of sexual maturation and spawning. This is commonly done by the exogenous application of substances which mimic the action(s) of endogenously secreted hormones on the axis. Depending on the type of hormone applied to the fish, it acts mainly at the level of the hypothalamus, pituitary, or gonad.

Successful attempts to manipulate the reproductive cycle in sea bass have employed three hormones, namely: human chorionic gonadotropin (HCG), luteinizing hormone-releasing hormone analogue (LHRHa) and 17 α-methyltestosterone (MT). These hormones induce the gonads to mature earlier than is normal during the natural breeding season or trigger sexually mature fish to spawn. The following technique focuses on the use of these hormones.

Hormonal Induction of Sexual Maturation. This technique involves implantation of two hormones, LHRHa and MT, incorporated and pelleted in a matrix of cholesterol powder. Starting in late February, monthly implantation of these hormones at a dose of 0.1 mg/kg body weight results in gonadal maturation of male and female sea bass in floating net cages in May or two months earlier than the known peak breeding period in the wild. The procedure for hormone implantation is as follows:

1. Condition healthy adult broodstock (more than 4 years old) in floating net cages for at least 2-3 months.
2. Weigh each fish several days before implantation and prepare hormone pellets as described in Appendix 2.
3. Lift the stocking cage and gently scoop out fish one at a time.
4. Transfer fish into a shallow tank with 300 ppm of anaesthetic.
5. Turn anaesthetized fish on its back and with a pair of tweezers, pull out several scales at a point 7-8 cm from the anus to expose the flesh of the fish.

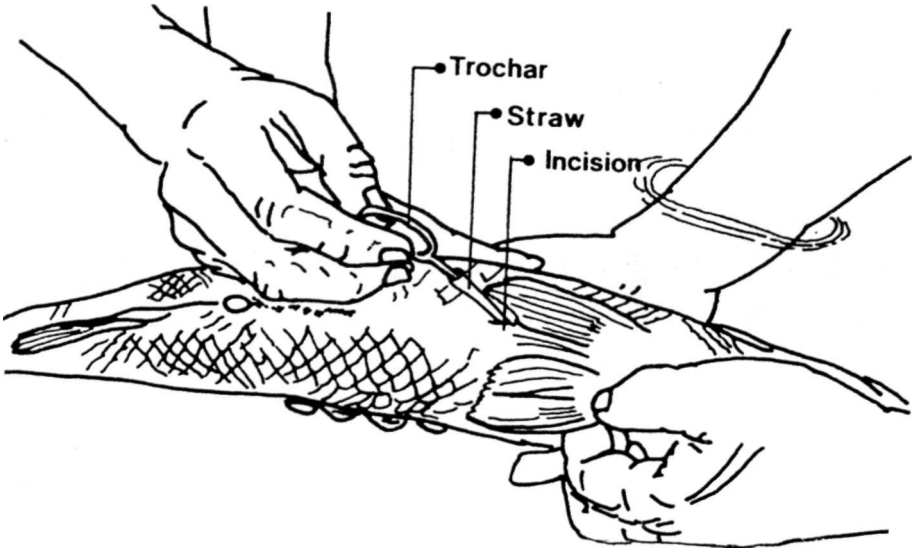

Fig. 8. Implantation of hormone pellet using a metal trochar and straw guide

6. Carefully make a short (0.5 cm long) but deep incision on the exposed flesh until the tissue lining the body cavity is punctured. No internal organ must be injured during the incision procedure.

7. Fit a metal trochar into an 8-cm long plastic drinking straw and carefully insert both into the incision. The trochar implanter and plastic straw guide will loosen when they reach the hollow body cavity.

8. Withdraw the metal trochar implanter leaving about 3 cm of the plastic straw guide protruding from the incised wound.

9. Drop the hormone pellets into the plastic straw guide and replace the trochar implanter to push the pellets into the body cavity of the fish (Fig. 8).

10. Pull out both the implanter and the plastic straw guide from the incision.

11. Apply to the wound a small amount of oxytetracycline ointment (Terramycin ointment, from Pfizer, Inc., Metro Manila).

12. Let the fish recover in a tank of fresh seawater before placing it back into the cage.

13. Implant fish monthly from February to May.

14. Check monthly for the presence of milt or yolky eggs by following the cannulation technique previously described (see p. 11).

Hormonal Induction of Spawning. Although HCG and LHRHa are two common spawning agents in sea bass, LHRHa is more cost-effective than HCG. Hence, the following steps involve only the use of LHRHa to spawn mature sea bass in floating net cages:

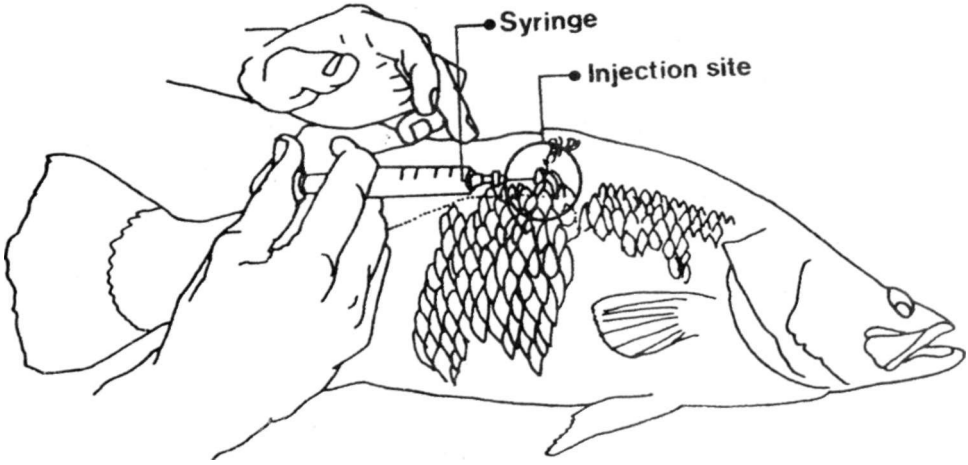

Fig. 9. Injection of hormone into fish muscle.

1. Spawning cages are prepared, i.e., lined with fine mesh *hapa net* (0.6-0.8 mm or "skin" cloth). Alternatively, spawning tanks may be used.

2. Take out sea bass from its stocking cage or tank and anaesthetize it.

3. Weigh fish.

4. Check the initial egg diameter of ripe females (Fig. 6). Also check the presence of freely-flowing milt among mature males.

5. There are two effective methods of introducing LHRHa to mature fish: injection or pelleted hormone implantation.

- ***Injection***

a) Prepare a fresh solution of LHRHa (Appendix 2).

b) An LHRHa dose of 20-100 µg of the hormone/kg body weight is recommended. An injection volume of 0.1 -0.5 ml/kg body weight may be followed.

c) Draw out enough of the hormone solution with a 1-ml capacity tuberculin syringe.

d) Carefully lift a scale with the hypodermic needle and inject the hormone at a point 5-10 scales below the dorsal fin of the anaesthetized fish (Fig. 9). Prevent unnecessary spillage during injection by gently pressing on the point of injection as the hypodermic needle is withdrawn. Mature male fish may receive at least 40 *µg* hormone/kg body weight. Inject hormone during daytime.

e) After injection, let fish recover before returning it to the spawning cage or tank. Maintain a 1:2 (female:male) sex ratio in the spawning cage.

f) Wait for fish to spawn two nights after the hormone injection. Mature fish injected with higher than 20 ng LHRHa/kg will spawn for three consecutive nights.

• ***Pelleted Hormone Implantation***

a) Prepare fish for implantation: weigh; anaesthetize; measure egg diameter.

b) Prepare hormone pellets (Appendix 2) at a dose of 5-75 µg of the hormone/kg body weight.

c) Implant pellets to ripe male and female fish in daytime.

d) Stock implanted fish in the spawning cage or tank at a 1:2 (female:male) sex ratio. Fish will spawn two nights after implantation of the pelleted hormone. Mature female fish implanted with 5-10 µg LHRHa/kg will spawn once; 20-40 µg/kg, from two to three consecutive nights; 40-70 µg/kg, up to four consecutive nights.

EGG COLLECTION, TRANSPORT, AND HATCHING

Egg Collection

Eggs are collected early in the morning (5 to 7 am). The procedure for collecting spawned eggs from tanks or cages is as follows:

Tanks

1. Check for the presence of spawned eggs in the tank by examining water samples collected with a glass container. Fertilized sea bass eggs float and are normally transparent.

2. If eggs are present, collect the eggs with a soft and fine-mesh seine net (mesh size of about 0.4-0.5 mm). Concentrate the eggs in the middle of the seine net and gently scoop out eggs with a small bowl.

3. To collect the remaining eggs, securely place at the outlet canal a wooden box with a fine-mesh screen bottom.

4. While the tank is gradually being drained, gently scoop out eggs from the wooden box and transfer them to a 15-l pail containing aerated fresh seawater. This procedure minimizes impact-related stress and mortalities during draining and collection of eggs.

5. Screen out any debris mixed with the collected eggs.

6. Rapidly but gently transfer eggs to a fiber glass tank containing 250 1 of aerated fresh seawater.

7. Spawners are returned to the spawning tank after refilling with seawater.

Floating Net Cages

To facilitate egg collection, cages are lined with a fine mesh *hapa* net installed a few days before the anticipated day of spawning.

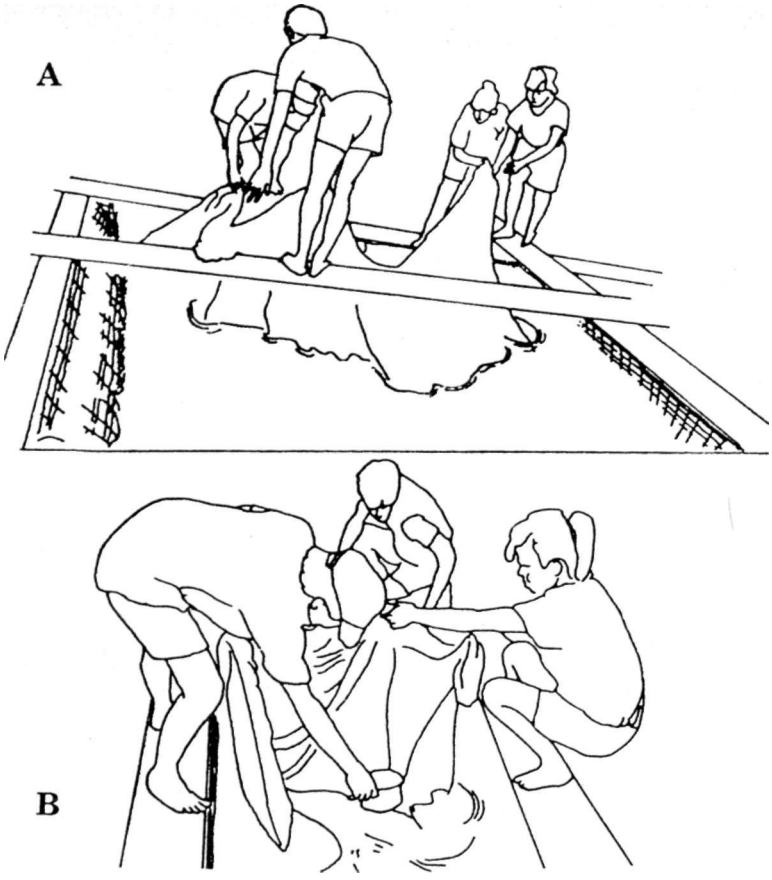

Fig. 10. Harvesting spawned eggs: (A) the *hapa* net is lifted to concentrate the eggs: (B) washing the sides of the net to remove adhering eggs

1. Check for the presence of spawned eggs in the cage by examining water samples in a glass container.

2. If eggs are present, transfer spawners to a spare cage or a large fiberglass tank.

3. Slowly lift the fine *hapa* net. At the same time, splash the sides of the net to wash out adhering eggs. Continue lifting and splashing the *hapa* net until eggs are fully concentrated to one side (Fig. 10).

4. Gently scoop out eggs from the *hapa* net and transfer to a pail of seawater.

5. Rinse the *hapa* net before installing it back to the stocking cage. Transfer spawners back to this cage.

6. Remove any extraneous debris mixed with collected eggs.

7. Rapidly but gently transfer collected eggs to a fiberglass tank containing 250 l of aerated seawater.

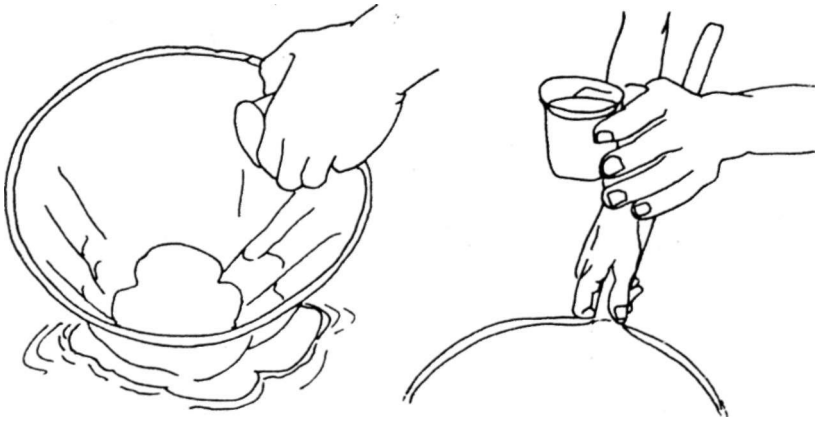

Fig. 11. Drained eggs ready for transfer to transport bags

Egg Transport

If hatchery facilities are located some distance away from spawning tanks and cages, these steps are followed during egg transport done early in the morning (5-7 am).

1. Remove aeration. Vigorously swirl seawater in the fiberglass tank containing collected eggs. Let dead eggs (white and opaque) settle down; good ones float.

2. Carefully siphon out dead eggs from the tank bottom.

3. Scoop out good eggs with a fine-mesh net and transfer to a graduated glass beaker (Fig. 11).

4. Quickly transfer (100 ml of eggs) to a double-lined plastic bag containing 15 1 of fresh aerated seawater.

5. Inflate with oxygen until the air space occupies 2/3 of the total volume of the plastic bag.

6. Tie plastic bag with several rubber bands and place into a *pandan* bag or *bayong*.

7. Keep loaded bags in a cool, dry place. Never expose eggs to heat in such areas like near a running motor (in pumpboats or land vehicles) or in open decks receiving direct sunlight.

Egg Incubation and Hatching

Incubate fertilized eggs (diameter = 0.80 mm) in 500-1 fiberglass tanks at a density of 1,200 eggs/1 or less. Provide gentle aeration to keep the eggs suspended in the water column. Hatching will occur approximately 14 h after fertilization at 28°C and 32-33 ppt.

LARVAL REARING

The procedure of rearing seabass larvae is schematically presented in Figure 12.

Stocking Density

Upon hatching, transfer larvae from the incubation tank to the larval rearing tank. Compute the volume of water containing the larvae to attain the desired stocking density in the larval rearing tank (refer to Appendix 3).

Stock larvae at an initial density of 30 ind/l. Higher densities, e.g., more than 90 ind/1, may be adopted based on production targets (in terms of fry size and number of individuals harvested) and level of capitalization. Reduce the density to 15 ind/l when larvae reach 10 days old and further to 6 ind/1 when 20 days old.

Feed Types and Feeding Management

Sea bass begin to feed 50 h after hatching at 28°C. However, it is advisable to introduce the larval feed at an earlier time. If larvae are unable to feed 60 h after hatching, irreversible starvation will occur and at least 50% of them will die.

Larvae should be weaned gradually to each new food type. This is done by increasing daily the proportion of the new food type while gradually reducing that of the preceding food. This is necessary to train the fish to recognize and accept the new feed particle. If weaning is done properly, feed wastage and fish mortality due to starvation are minimized.

Live Food. Larval rearing of sea bass is largely dependent on the use of live food organisms, e.g., rotifer (*Brachionus plicatilis*), *Artemia* (newly hatched nauplii, enriched nauplii, subadult or adult biomass), and the freshwater cladoceran *Moina.* The method for estimating the required volume of larval food is shown in Appendix 4.

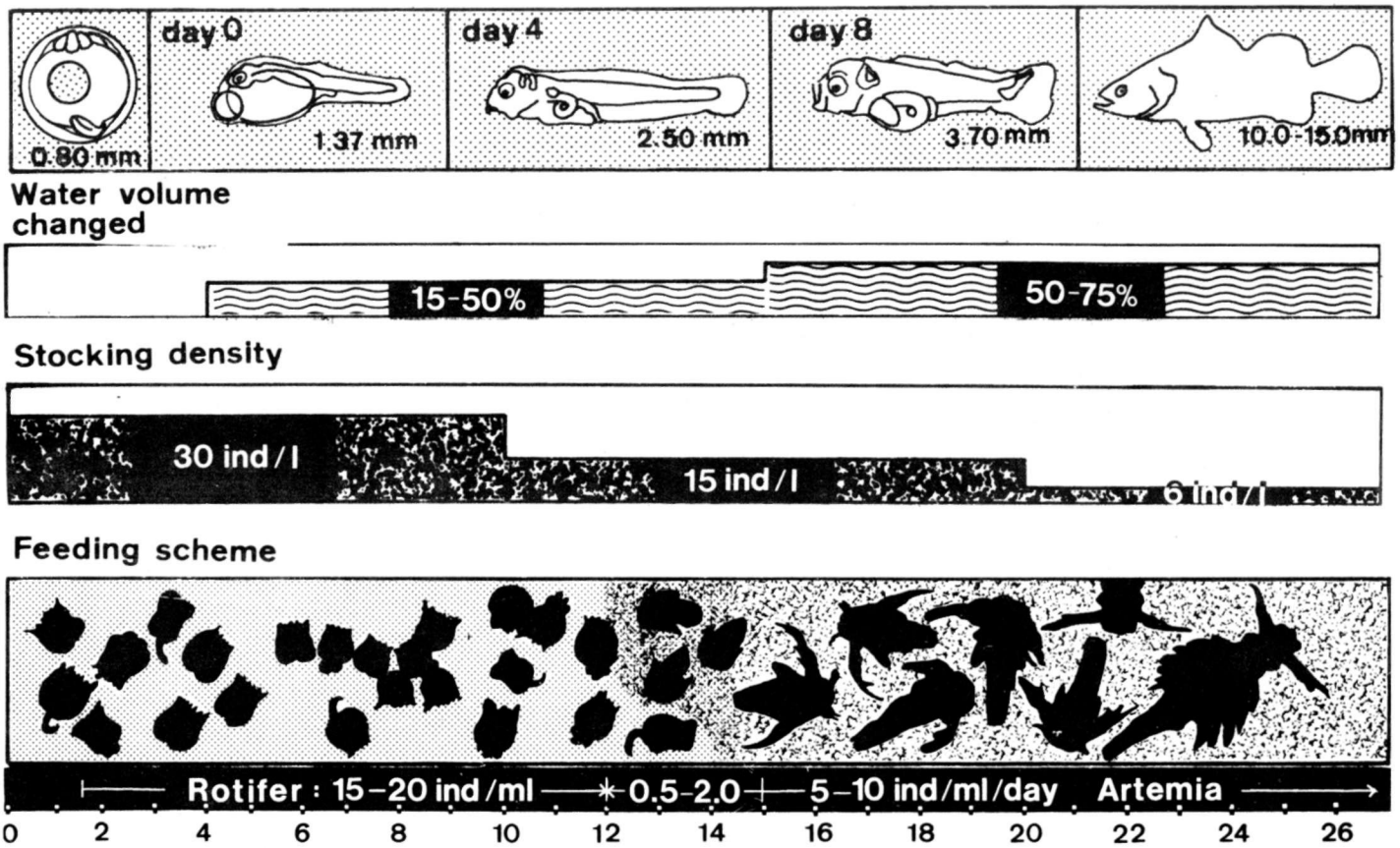

Fig. 12. Schematic presentation of sea bass larval rearing based on age of larvae

Approximately 36 h after hatching of eggs, add rotifers to the rearing tanks at 15-20 ind/ml until larvae reach day 12. Maintain this density by daily addition of rotifers. Also add 60 liters of *Chlorella* in peak bloom to attain a density of 1-3 x 10^5 cells/ml. *Chlorella* is added to maintain water quality and serve as food for rotifers. The method for determining algal cell density is shown in Appendix 5. From day 12 to 15, gradually reduce rotifer density until it is totally removed by day 15.

Wean larvae to newly hatched *Artemia* nauplii over a 3-day period (day 12 to 14), although weaning may be done as early as day 8 or later depending on food availability and production target. During weaning, provide *Artemia* at increasing densities of 0.5 to 2.0 ind/ml/day. On days 15 to 17, increase *Artemia* density to 5-10 ind/ml/day.

Feed larval fish with enriched *Artemia* nauplii from day 18 to 23 at a density of 5-10 ind/ml/day. This is important because sea bass exhibits higher survival rate during metamorphosis when fed enriched *Artemia.*

As the larvae grow bigger, they ingest larger feed particles. They are fed subadult or adult *Artemia* biomass at 1 ind/ml or higher.

As a partial or a complete replacement of *Artemia*, the freshwater cladoceran (*Moina macrocopa*) may be fed to sea bass larvae. Fed partially or as a complete replacement of *Artemia, Moina* can be fed to 17- to 20-day old larvae at a feeding rate of not less than 1 ind/ml. *Moina* are first sieved to obtain small adults and neonates for feeding to 17-day old larvae. Unsieved *Moina* can be fed directly to 20-day old larvae.

When feeding with *Moina,* gradually lower the salinity of rearing water to about 10 ppt within a 24-h period. Larvae are then fed at least 4 times daily.

The brackishwater cladoceran, *Diaphanosoma celebensis*, is also a potential live food for sea bass larvae. It can be fed once daily to 15-day old and older larvae at 2 ind/ml at 32-35 ppt.

None-live Feeds. Some non-live feeds used include frozen *Artemia* biomass, frozen *Moina,* fish-by-catch, and artificial diets. These feeds can, in excess, cause rapid deterioration in the quality of rearing water. Thus, food ration and feeding frequency must be controlled to avoid water fouling.

Prepare frozen *Artemia* biomass by freezing freshly harvested subadult or adult *Artemia* in plastic bags, each to contain the amount needed for one day feeding. During feeding, break the frozen biomass into small pieces and distribute in the tank. As it thaws, the *Artemia* particles will slowly separate allowing the sea bass larvae to swallow each piece whole.

Newly-harvested *Moina* adults and neonates are cleaned of debris in running freshwater. After draining excess water, pack *Moina* in small plastic sachets to contain amounts needed for one feeding. During feeding, remove frozen *Moina* from the container and let float on the rearing water. Fish feed on Moina as it gradually thaws.

Only fresh trash fish should be used. Remove head, entrails, and bones, then chop to fine bits the remaining flesh. During feeding, give trash fish slowly to allow fish ample time to feed before the trash fish particles sink to the bottom. Feed at least 3 times a day.

Artificial diets hold promise for sea bass fry production. However, feeding management has not yet been standardized and the economics is still unknown.

Water Management

Starting on the 4th until the 14th day of culture, drain and replace daily 15-50% of the water volume. On the 15th day, when feeding level with newly hatched *Artemia* nauplii is maximum, change daily 50-75% of water. Once feeding with non-live feed begins, change 100% of water daily.

During water exchange, drain water in the rearing tank using a siphon with its inlet covered with plankton net. This is to prevent larvae from being drained. Refill tanks to its original volume with new seawater. In addition, siphon off feces and other debris found at the tank bottom every morning from the 3rd day until harvest.

Size-grading of Larvae

Cannibalism among sea bass reduces fry survival. Hence, grade stocks often to remove "shooters" and reduce the chances for cannibalism. Usually, cannibalistic fish swallow their prey whole. Since the maximum size of prey that a cannibal may ingest is approximately 60-67% or 2/3 of its length, separate fish with length differences of 33% or more.

During size-grading, use a wide hose (inner diameter=5 cm) to siphon off fish into sorter boxes. Arrange the boxes serially so that the net with the biggest mesh size is innermost while the finest mesh net is outermost (Fig. 13). When siphoning is completed, gently lift and lower the innermost box repeatedly until fish that remain inside are too big to pass through the net. Scoop out these "shooters" and rear them separately. Do the same for the next sorter box. Fish that remain in the second box should constitute the average-sized population. Fish that

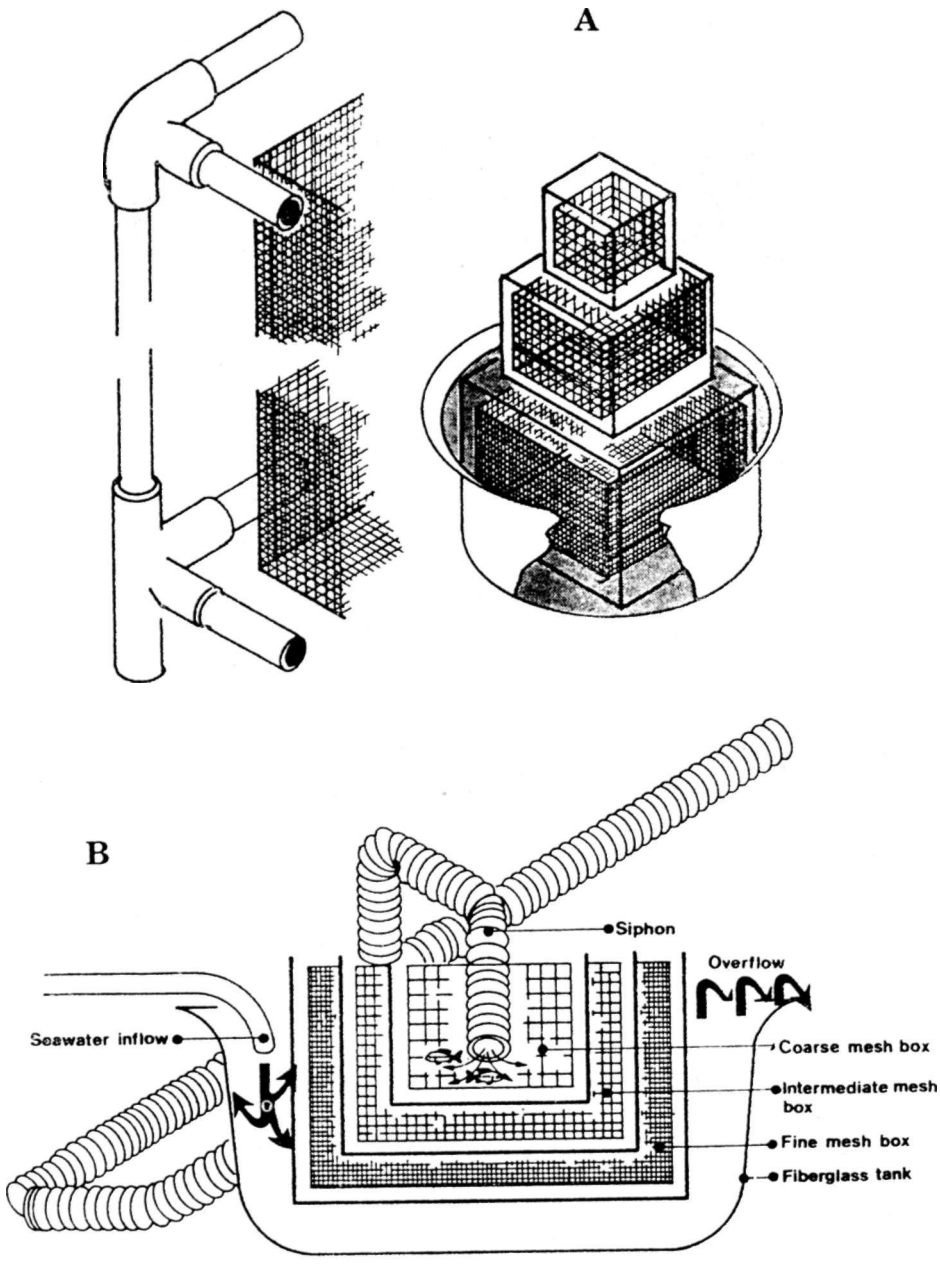

Fig. 13. Sorter boxes for grading sea bass fry; (A) details and arrangement of boxes: (B) cross-sectional view

pass through the first two boxes are retained in the outermost box and are the smallest individuals.

The nets may be changed accordingly to obtain different mesh sizes. Siphoning is possible only if the rearing tank is at a higher elevation than the sorter boxes. If this is not the case, simply drain water in the rearing tank and use a scoop net to gather and transfer fish to the sorter boxes.

Usually, shooters begin to develop several days after *Artemia* feeding is started. Therefore, the first grading should be done at this time. Grade subsequently whenever shooters are evident.

HARVEST AND TRANSPORT OF FRY

Twenty one day-old sea bass fry, approximately 1 cm total length, can be harvested from the hatchery for rearing in nursery systems. Transporting them to the farm site is easy since the fry are quite hardy.

Harvest the fry by partially draining the water in the tank. Using a fine-mesh scoop net or small basin, scoop the fry and transfer them to containers, preferably big white basins. To estimate the number of

Fig. 14. Counting harvested sea bass fry

harvested fry, put a known number of fry in a basin with a known volume of water (Fig. 14). Use this as model for visual estimation of the number of fry in similar basins.

Pack the fish in double-lined plastic bags filled with fresh seawater. Inflate the bags with oxygen at a water-to-air ratio of 1:2, seal with rubber bands, then put bags inside *buri* bags or styrofoam boxes. The density normally used during transport of 1-cm fry is 500 fry/1 or 5,000 fry/10 l of transport water. However, as much as 1,600 of 1-cm fry/1 can be packed for 8 h of transport at 28°C. For practical purposes, always adjust the loading density, based on the duration of transport and size of fry to be transported. Do not feed fry at least 24 h before transport so as to reduce production of metabolic wastes. It is best to transport fry during cooler periods of the day, i.e., early morning or late in the afternoon.

PROPAGATION OF LARVAL FOOD

The larval food types utilized for sea bass fry production include green algae (*Chlorella, Tetraselmis*), rotifer (*Brachionus*), brine shrimp (*Artemia*) and *Moina.* Mass propagation of green algae and rotifer is normally done in big canvas tanks, whereas *Artemia* and *Moina* production is done in 250 1 hatching vessels and 1-2 t fiberglass tanks.

At SEAFDEC/AQD, pure culture strains of *Brachionus, Chlorella Tetraselmis* and other Phytoplankton species are maintained in a well-equipped laboratory to ensure constant availability of starter culture. For the fish farmer, however, operating a live food laboratory may be expensive and impractical. An alternative is to contact government agencies or research institutions, e.g., SEAFDEC/AQD, which can provide starter cultures of the different larval food types.

Chlorella

For *Chlorella* propagation in 1 t fiberglass tanks, use 850 l filtered seawater and 150 l algal starter. Add a fertilizer mix (100 g of 21-0-0 + 20g of 16-20-0 + 20 g of 46-0-0 for every ton of seawater). Aerate well to keep algal cells from clumping. When cell density has increased, use this as starter for *Chlorella* culture in canvas tanks of 10-30 t capacity.

To propagate Chlorella in big tanks, use 4/5 filtered seawater and 1/5 *Chlorella* starter volume for an initial cell density of 2-5 x 10^6 cells/ml. Add a fertilizer mix (same as above) and aerate well. Peak bloom of

Chlorella may occur after 4 days. At this time, transfer a portion of *Chlorella* water into other canvas tanks to serve as starter, then add rotifers.

Rotifer (*Brachionus*)

When *Chlorella* culture reaches peak bloom, add rotifers at about 15 ind/ml. Allow 4 days for rotifer density to increase. Harvest and concentrate using a hose and a 48-**µm** plankton net bag. Reserve some of the harvested stock as starter for other tanks, then use the rest as feed for the larvae. After each harvest, thoroughly wash and clean the canvas tanks using freshwater and liquid bleach solution in preparation for the next culture cycle.

In instances where rotifer culture is contaminated with diatoms, stop aeration for a few hours, allow diatoms to settle, then harvest the rotifers using 63-µm plankton net bag. It is advisable to discard cultures with heavy diatom contamination.

Whenever *Chlorella* is insufficient, rotifers may also be reared on baker's or marine yeast (approximately 1 g yeast/million *Brachionus*/day). It is necessary, however, to improve the quality of rotifers grown on yeast by feeding them *Chlorella* for at least 12 h before feeding to larvae.

Culture cycles for algae and rotifer must be scheduled to ensure daily harvest of rotifer.

When rotifer and *Chlorella* tanks are situated in the same area, the *Chlorella* tanks must be positioned such that water foam and spray from the rotifer tanks are not windswept into the *Chlorella* tanks. Otherwise, rotifer contamination may occur causing collapse of *Chlorella* cultures.

Artemia

Newly hatched nauplii. Incubate *Artemia* at a density of 5 g cyst/1 or less in plastic hatching vessels filled with clean filtered seawater and provided with vigorous aeration (Fig. 15a). The cysts will hatch normally within 24 h. To harvest *Artemia*, stop aeration and cover the upper portion of the tank with black cloth to keep out light but leaving a small portion at the bottom exposed to light. After a few minutes, the positively Phototactic *Artemia* nauplii will concentrate in the exposed area, while cysts will float to the surface. Drain slowly through an outlet at the bottom of the incubator tank, and use a filter box fitted with 150-µm plankton net to retain the nauplii (Fig. 15b). Immerse the filter box in water using a basin to keep *Artemia* in water.

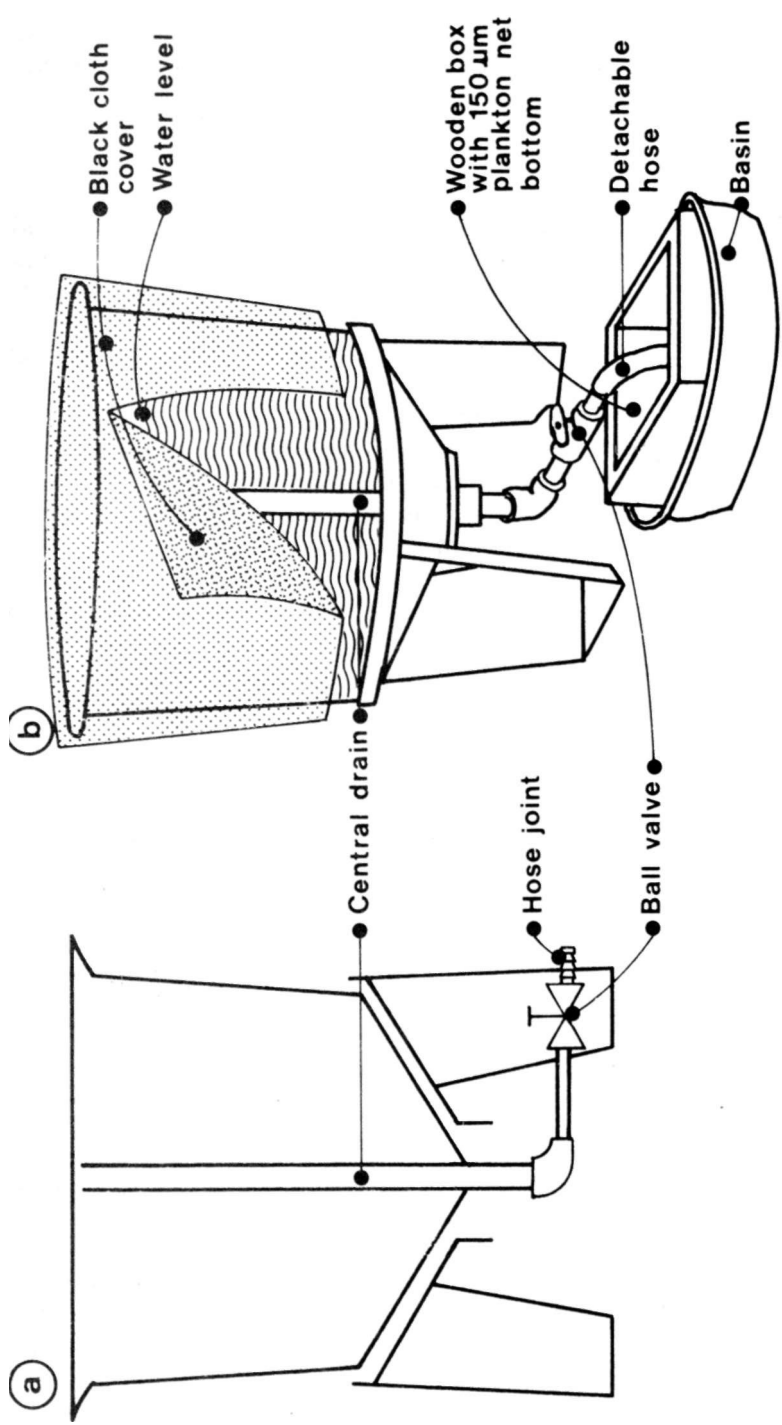

Fig. 15. *Artemia* incubation tank used at SEAFDEC/AQD: (a) cross-sectional view; (b) side view

Artemia cysts may be decapsulated using a hypochlorite solution prior to incubation. If decapsulation is done, harvest procedure is simplified since cyst particles are no longer present. *Artemia* cysts may be decapsulated as follows:

1. Hydrate *Artemia* cysts in a glass container at 1 g cyst/4 ml seawater. Aerate well to keep cysts in suspension.

2. After about 1-1/2 hours, check under a microscope if cysts have become hydrated. Fully hydrated cysts appear round instead of concave.

3. Add 0.33 ml of 40% sodium hydroxide solution per gram of cyst to raise pH.

4. Add hypochlorite solution at 0.3 gm active chlorine (obtained from commercially available liquid bleach) per gram of cyst. Keep water temperature low by immersing container in ice at least 30 minutes prior to and upon addition of hypochlorite solution.

5. After 5-6 minutes, check if 90% of the cysts have turned orange. If so, drain the decapsulated *Artemia* into a plankton net and wash with seawater. Continue washing until chlorine smell disappears.

6. Incubate decapsulated *Artemia* at the same density and duration as *Artemia* cysts (see p. 27). Keep decapsulated *Artemia* away from direct sunlight.

Enriched nauplii. After hatching, separate nauplii from broken cysts and other debris. Rinse well in clean seawater. Transfer *Artemia* into another tank at a density of 200,000 nauplii/1 seawater, then add an enrichment solution (e.g., Selco) composed of vitamins and highly unsaturated fatty acids. Aerate vigorously or dissolved oxygen level may fall sharply causing *Artemia* mortality. Add enrichment solution every 12 h for at least 24 h prior to feeding *Artemia* to the larvae.

Biomass. Culture *Artemia* on rice bran extract for several days to produce biomass (for details, see Sorgeloos et al., 1986). The length of culture period biomass production will depend on the desired size of fry to be fed to sea bass fry.

Moina

Obtain *Moina* starter from stagnant freshwater bodies (canals, ponds) or from a pet shop. For continuous propagation and harvest, prepare a series of 8-10 culture tanks of 1-2 t capacity, of any shape, and made of cement, marine plywood, or fiberglass. Fill each tank with freshwater up to 1 m in depth, then suspend 2-3 kg of chicken manure placed inside small nylon screen bags. Add *Moina* starter the following day. Water color will change from pale to deep brown, then greenish due to Phytoplankton growth. Begin harvesting on the 7th day or when

original density has increased three-fold. Harvest *Moina* in the morning as they are found mostly on the water surface. Insect larvae and other organisms which prey on *Moina* must be scooped out. Use a fine net (0.3-0.5 mm mesh) to harvest. Spare gravid individuals for subsequent propagations. Gravid *Moina* appear to have a distended abdomen which may contain tiny offsprings. When water turns clear, indicating low fertility, drain half the volume and replace with new water and chicken manure.

Diaphanosoma

Propagate the green algae, *Tetraselmis tetrahele,* in 6-8 units of 1-2 t fiberglass tanks by following the same culture methods for *Chlorella* propagation.

Schedule *Tetraselmis* culture on a daily basis. When *Tetraselmis* reaches peak bloom in 4-days' time, reduce the salinity from 32 ppt to 20-25 ppt and then add *Diaphanosoma* at about 100 ind/1. However, should the *Tetraselmis* density becomes insufficient, add rice bran extract (0.3 g/l) for the *Diaphanosoma* to feed on. Allow 5 days for the density of *Diaphanosoma* to increase. When rearing water turns polluted, drain 50% of the water volume and replace with an equal volume of freshwater and *Tetraselmis* or, when the tank bottom is filled with feces, the entire cladoceran culture is replaced.

Harvest and concentrate *Diaphanosoma* with a hose and a harvesting box lined with a 324μm mesh size net.

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APPENDICES

APPENDIX 1. Fixation and Measurement of Sea Bass Egg

Materials

Formalin
Sodium acid phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$)
Disodium phosphate, anhydrous (Na_2HPO_4)
Distilled water
Filter paper
Microscope
Glass slide
Ocular micrometer (100 divisions)

Stage micrometer (100 divisions = 1mm)
Sharp point probe

Procedure

1. Prepare a 5% buffered formalin solution.
   a. Filter technical grade formalin with a filter paper.
   b. Mix 50 ml of filtered formalin and 950 ml of distilled water.
   c. Add and dissolve in this solution 4.14 g of sodium acid phosphate monohydrate and 5.068 g of disodium phosphate anhydrous.
   d. Stir well.
2. Store eggs in formalin.
3. Measure sea bass egg diameter.
   a. Calibrate the microscope
      - Insert ocular micrometer into the microscope eyepiece.
      - Focus into view the 100 divisions (equivalent to 1 mm) of the stage micrometer
      - Focus and align together the "O" marks of the ocular and stage micrometers.
      - Count and record the total number of divisions in the ocular micrometer coinciding with the 100th mark of the stage micrometer.
      - Calculate the calibration factor, K

$$K = \frac{\text{100 stage micrometer divisions}}{\text{Number of ocular micrometer divisions}}$$

- Take out the stage micrometer. Do not change objectives once the microscope has been calibrated.

b. Measure the diameter of at least 30 preserved sea bass eggs.
- Spread out preserved eggs on a clean, dry glass slide.
- Focus into view several eggs.
- Count the number of ocular micrometer divisions corresponding to the diameter of the egg.
- In some cases, the preserved egg loses its perfectly spherical configuration; hence, measure the diameter (in ocular micrometer divisions) from the egg's longest and shortest axes. Calculate the mean of these two readings and multiply this value by K. Convert the product to millimeter by multiplying it by 0.01.
- Calculate and record the mean (in mm) of the 30 eggs.

Example:

1. Calibration of microscope at 100x magnification (low power objective).

$$K = \frac{100 \text{ stage micrometer divisions}}{97 \text{ ocular micrometer divisions}} = 1.03$$

2. Egg diameter measurement.

One preserved egg had 43 and 41 ocular micrometer divisions recorded for the longest and the shortest egg axes, respectively. Therefore, mean diameter (in number of divisions) of this egg is

$$\frac{43 + 41}{2} = 42 \text{ ocular divisions}$$

42 divisions x 1.03 x 0.01 mm/division = 0.433 mm.

APPENDIX 2. Hormone Preparation

Materials

LHRH analogue (luteinizing hormone-releasing hormone analogue)*
17 α-methyltestosterone (MT)**
Cholesterol powder
80% ethanol
Weighing scale (accuracy of 0.01 mg)

*Available from Lam Hua Dragon (H.K.) Company, Ltd., 271 Hennessy Road, Wanchai, Hong Kong.

**Available from Sigma Chemical Company, P.O.Box 14508, St. Louis, Missouri 63178, USA.

Petri dish
Metal spatula
Pellet maker
Pipette (0.1 ml graduation)
Distilled water
Sodium chloride reagent

Hormone Pellets

Procedure

1. Pelleted LHRHa and MT
   a. Calculate the total amount of LHRHa and MT required when a dose of 0.1 mg of each hormone/kg body weight is used.
   b. Initially dissolve 1 mg MT with 0.2 ml of 80% ethanol. Set aside.
   c. Break open enough of LHRHa ampoules and empty the content in a clean, dry petri dish.
   d. Combine dissolved MT with LHRHa in the petri dish.
   e. Add 0.5 ml of 80% ethanol to the hormone mixture. Mix well with a metal spatula.
   f. Weigh cholesterol powder following a ratio of 0.2 g of cholesterol per mg of hormone.
   g. Combine cholesterol powder with dissolved hormones in petri dish.
   h. Mix well with a metal spatula until the powdered mixture is completely dry.
   i. Calculate and weigh the total amount of hormone contained in the powdered mixture for each fish to be implanted.
   j. Compact the weighed, powdered mixture into cylindrical pellets (3 mm diameter, 3-5 mm height).
   k. Store hormone pellet in separate vials for each fish.
   l. Each milligram of the powdered mixture contains 0.005 mg of each hormone.

Example:

Hormone dose = 0.1 mg LHRHa/kg body weight and 0.1 mg MT/kg body weight
Total number of fish to be implanted = 20 (at 4 kg each)
Total body weight of 20 fish = 80 kg
Total amount of LHRHa = Dose of LHRHa x Total body weight
= 0.1 mg LHRHa/kg x 80 kg
= 8 mg
Total amount of MT = Dose of MT x Total body weight
= 0.1 mg MT/kg x 80 kg
= 8 mg

Volume of 80% ethanol required = 1.6 ml
Amount of cholesterol powder required = 1.6 g

Therefore, a fish weighing 3.5 kg requires 70 mg of the powder mixture which contains 0.35 mg each of LHRHa and MT.

2. Pelleted LHRHa
   a. Determine the dose of LHRHa to be implanted to fish.
   b. Calculate the total amount of LHRHa required based on the dose level determined.
   c. Break open the ampoules containing LHRHa and empty the content in a clean, dry petri dish.
   d. Dissolve each milligram of the hormone in 0.2 ml of 80% ethanol.
   e. Weigh out cholesterol powder following a ratio of 0.2 g of cholesterol per mg of hormone.
   f. Combine the cholesterol powder and dissolved hormone in the petri dish and mix well with a clean metal spatula until completely dry.
   g. Calculate the total amount of hormone required for each fish.
   h. Weigh the total amount of powdered mixture containing the hormone required for each fish.
   i. Compact the weighed powdered mixture into cylindrical pellets. Minimize unnecessary loss during preparation.
   j. Store hormone pellets individually in capped vials. Hormone pellets remain bioactive after storage for 120 days at room temperature (28-30 °C).

   k. Each mg of the powdered mixture contains 0.005 mg of LHRHa.

Example:

Hormone dose = 0.05 mg LHRHa/kg body weight
Total number of fish to be implanted = 5 (at 4kg each)
Total body weight of 5 fish = 20 kg
Total amount of LHRHa = Hormone dose x Total body weight
= 0.05 mg LHRHa/kg x 20 kg
= 1 mg
Volume of 80% ethanol required = 0.2 g
Amount of cholesterol powder required = 0.2 g

Therefore, a fish weighing 4 kg requires 40 mg of the powdered mixture which contains 0.2 mg of LHRHa.

Hormone Solution

Procedure

a. Determine the LHRHa dose level and the injection volume per kg body weight of fish.
b. Calculate the concentration of the hormone solution required based on the dose level and the injection volume.
c. Calculate the total amount of LHRHa required based on the hormone dose level determined.
d. Break open enough ampoules containing 0.2 mg of LHRHa.
e. Prepare a 0.9% salt solution by dissolving 0.9 g of sodium chloride in 100 ml of distilled water.
f. Measure with a pipette the volume of 0.9% salt solution needed to dissolve LHRHa in order to arrive at the desired concentration of the hormone solution. Swirl the ampoules gently to completely dissolve the hormone.
g. Dissolved hormone may be stored at room temperature (28-30°C) for as long as 90 days. However, excess LHRHa solution are preferably kept in a refrigerator (4- 10°C) for 90 days or in a freezer (-4°C) for 50 days at the most. Re-using such solutions beyond these storage periods may not be effective in spawning breeders.

Example:

Hormone dose = 0.05 mg LHRHa/kg body weight
Injection volume = 0.1 ml/kg body weight

$$\text{Concentration of hormone} = \frac{\text{Hormone dose}}{\text{Injection volume}}$$

$$= \frac{0.05 \text{ mg LHRHa/kg}}{0.01 \text{ ml/kg}}$$

= 5 mg LHRHa/ml of 0.9% salt solution

Total number of fish to be injected = 5
Total body weight of 5 fish = 20kg (at 4kg each)
Total LHRHa needed
= Hormone dose x Total body weight
= 0.05 mg LHRHa/kg x 20 kg
= 1 mg

Since each ampoule contains 0.2 mg of LHRHa, a total of 5 ampoules is needed.

The volume of 0.9% salt solution required to dissolve 0.2 mg LHRHa is 0.4 ml so that the hormone solution will have a concentration of 0.5 mg LHRHa/ml.

Therefore, a fish weighing 5 kg will require 0.25 mg LHRHa dissolved in 0.5 ml of salt solution.

APPENDIX 3. Estimation of water volume for stocking of larvae

Materials

PVC pipe
Pail
Beaker (100 ml)
Stereomicroscope
Petri dish

Procedure

1. Collect five water samples from different areas of the 500-1 fiberglass tank (1 at center, 4 at periphery) using a PVC pipe (1.5 in diameter and 5 ft in length) and drain into a pail.
2. Mix the samples thoroughly, then get five 100-ml subsamples.
3. Pass each subsample through a net and transfer the larvae in a petri dish.
4. Count the number of larvae in each subsample under a stereomicroscope and determine the average larval density.
5. Compute the volume of water needed for transfer from the incubation to the rearing tank using the formula:

$$\text{Water volume to transfer} = \frac{\text{Desired larval density (ind/l) x Total volume of rearing tank (l)}}{\text{Larval density in incubation tank (ind/l)}}$$

Example:

Desired larval density in rearing tank = 30 larvae/l
Volume of rearing tank =3 t (3000 l)

Average larval density in incubation tank = 125, 150, 132, 120, 152/100 ml

$$\text{or } \frac{125+150+132+120+152}{5} = 135.8 \text{ larvae/100 ml}$$

or 1358 larvae/l

$$\text{Therefore, water volume to transfer} = \frac{30 \text{ larvae/l} \times 3000 \text{ l}}{1358 \text{ larvae/l}}$$
$$= 66.3 \text{ l}$$

APPENDIX 4. Estimation of the required volume of larval food (rotifer)*

Materials

Fiberglass tank	Counting chamber
Pipette	Lugol's solution
Graduated cylinder (10 ml)	Stereomicroscope

Procedure

1. Keep the harvested stock of rotifer in a small aerated fiberglass tank.
2. Thoroughly mix stock, then use a pipette to transfer a 1-ml sample into a 10-ml graduated cylinder.
3. Dilute the sample ten times by adding 9 ml of filtered seawater**
4. Mix well, then take three 1-ml subsamples and place in a counting chamber.
5. Add a few drops of Lugol's solution*** to stain and immobilize the rotifers.
6. Count under a stereomicroscope the number of rotifers in each 1-ml subsample. Compute the average number of rotifers/ml of sample.
7. Multiply the number obtained in Step 6 by 10 (if diluted 10 x, and 100 if diluted 100x) to determine the actual density of rotifers in the stock.
8. Compute the volume of rotifer stock required for feeding using the formula:

$$\text{Volume of rotifer stock required (ml)} = \frac{\text{Desired rotifer density in the tank (ind/ml)} \times \text{Volume of rearing tank (ml)}}{\text{Density of rotifer in the stock (ind/ml)}}$$

Example:

Desired rotifer density in rearing tank =20 rotifers/ml
Volume of rearing tank = 3 t (3,000,000 ml)

*This procedure may also be adapted for *Artemia.*

**If rotifer density is still too thick, dilute the sample hundred times instead; follow the same procedure above.

***Lugol's solution: dissolve 2 g potassium iodine and 1 giodine crystals in 100 ml water. Keep solution in a dark bottle.

Density of rotifer in the stock harvest = 863, 920, 902 rotifers/ml

$$\text{or } \frac{863+920+902}{3} = 895 \text{ rotifers/ml}$$

Therefore,

$$\textbf{Volume of rotifer stock required} = \frac{20 \text{ rotifers/ml} \times 3{,}000{,}000 \text{ ml}}{895 \text{ rotifers/ml}}$$

$$67039 \text{ ml} = 67.04 \text{ l}$$

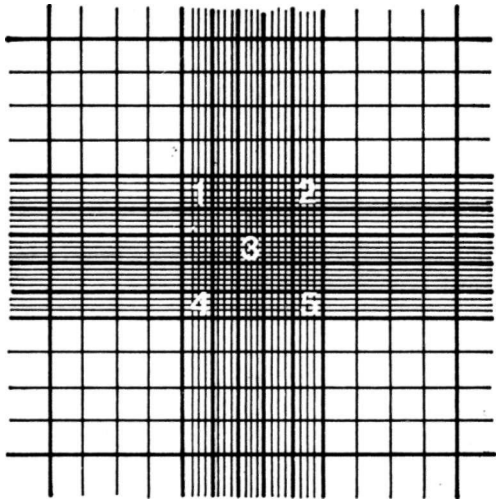
APPENDIX 5. *Estimation of Chlorella density*

Materials

Pipette
Graduated cylinder (10 ml)
Micropipette
Haemacytometer
Microscope

Procedure

1. Obtain a 1-ml sample of *Chlorella* from the culture tank.
2. Dilute the sample 10 times using filtered sea water in a 10 ml graduated cylinder. Mix the sample thoroughly.
3. Pippete a small amount into a haemacytometer using a micropippete. Be sure that only enough sample is introduced into the counting chamber to prevent overflow or the creation of bubbles which cause distortion in algal cell distribution.
4. Wait 1-2 minutes to allow algal cells to settle. Check under low power magnification if cell distribution is homogenous. If so, shift to a higher magnification and follow the next step. If not, discard the sample, clean the haemacytometer, and repeat steps 3 and 4.
5. Count all algal cells in each of 5 blocks of the counting chamber as shown below. Compute the average number of algal cells/ block.



Haemacytometer counting chamber
(For details, see Martinez et al. 1975.)

6. Estimate *Chlorella* density using the formula:

Chlorella density (cells/ml) = Average number of algal cells x 10^6 cells/ml x 10 (dilution factor)

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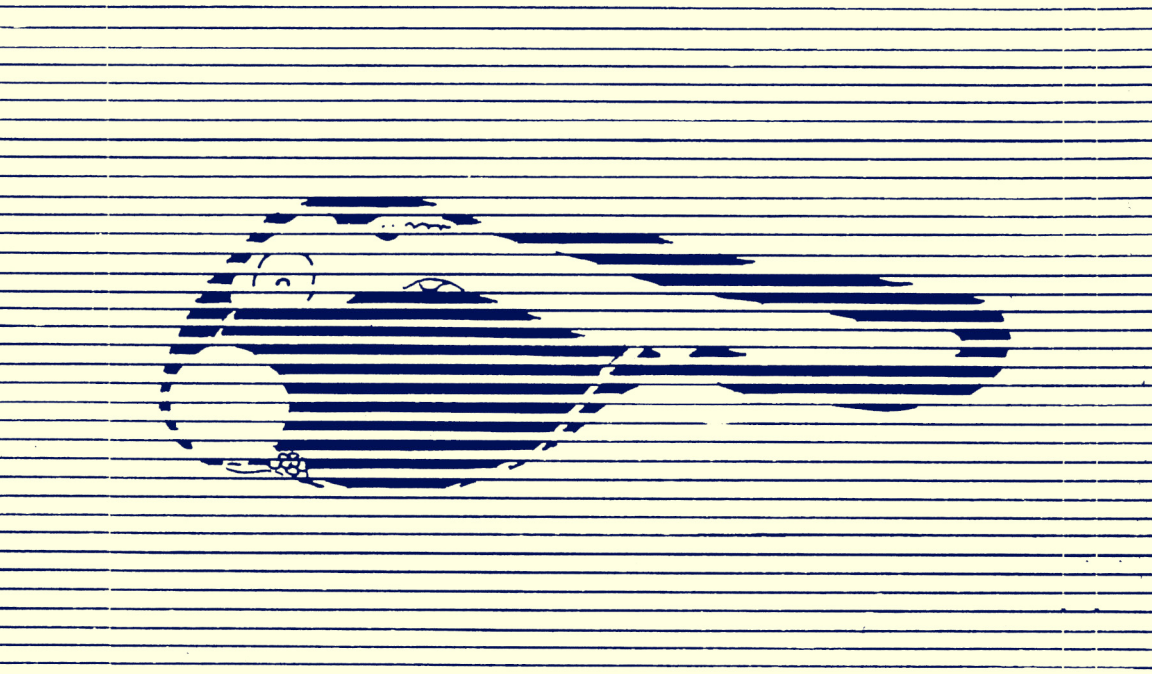

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