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INDUCED SPAWNING ON *Leptobarbus hovenii* (Bleeker) CARRIED OUT IN JAMBI, INDONESIA

by
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of the Small Scale Fisheries Development Project.
Jakarta, July 1986

PREFACE

This manual has been prepared within the framework of Small Scale Fisheries Development Project, implemented by the Directorate General of Fisheries, Indonesia, in collaboration with the United States Agency for International Development. It is primarily meant to disseminate technical and practical knowledge to extension workers and to other personnel engaged in fish breeding activities.

The contents deal with the induced breeding, specifically of Leptobarbus hovenii (Bleeker) which has successfully been the firstfruit of the author's trials carried out in Jambi province of Indonesia. It highligts the general biological aspects and presents the artificial breeding technique of this species. Preparation of broodstocks, spawning and fertilization, incubation method and subsequently, larval rearing technique are discussed. The preparation of hormone, collection of pituitary glands, injection technique are also dealt with. Finally, to make the manual more comprehensible, steps of activities are also profusely illustrated.

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1. INTRODUCTION

Leptobarbus havenii, originally is reverine freshwater fish which belongs to a medium size carp. This species is extensivelly distributed over a large areas of open–water in Indonesia, with enormous potential in Jambi Province anda Kalimantan. This species is populer with local name of 'Ikan Jelawat' or 'Ikan Kelemak'.

People of Jambi area have, over fifty years, practised how to collect fingerlings of this species from the natural bahibat before being stocked and reared in the floating cages or earthen ponds.

Leptobarbus hovenii being a herbivore, is characterized by its ability to grow very fast in captivity with feeding regime consists of herbage or vegetables but may also with other available food stuff or wastes disposed from kitchen or market. Additionally, the fish is not anly of good taste and flavour but it also is of good market.

Because of the above reasons, in the areas like Jambi, Palembang and Kalimantan, people are practising Leptobarbus hovenii culture with great interest. However, one of the main impediments in developing the effort is the lack of seed supplies. Fingerlings that collected from the nature are insufficient to meet huge demands.
Besides, the production of seeds which very much dependent on natural conditions is not steadily obtained.

To overcome the problem, the Directorate General of Fisheries in collaboration with the United States Agency of International Development have established an applied research dealing with the induced breeding of Leptobarbus hovenii, implemented in Jambi province, in their effort to increase the production of fingerlings.

2. BIOLOGY

As mentioned above, Leptobarbus hovenii is not only found in Sumatra and Kalimantan but the species is also distributed over Malaysia and Thai-

land. The fish is recognizable from its board head, four well depeloped barbels (rostral and maxillary), moderate-size mouth with maxillary reaching vertically from the front of the eyes, spoon–shaped pharynged teeth in three rows (5, 3, 2), medium size scales, continuous lateral line running in lower part of caudal peduncle and short dorsal and anal line with seven and five branched rays, respectively.

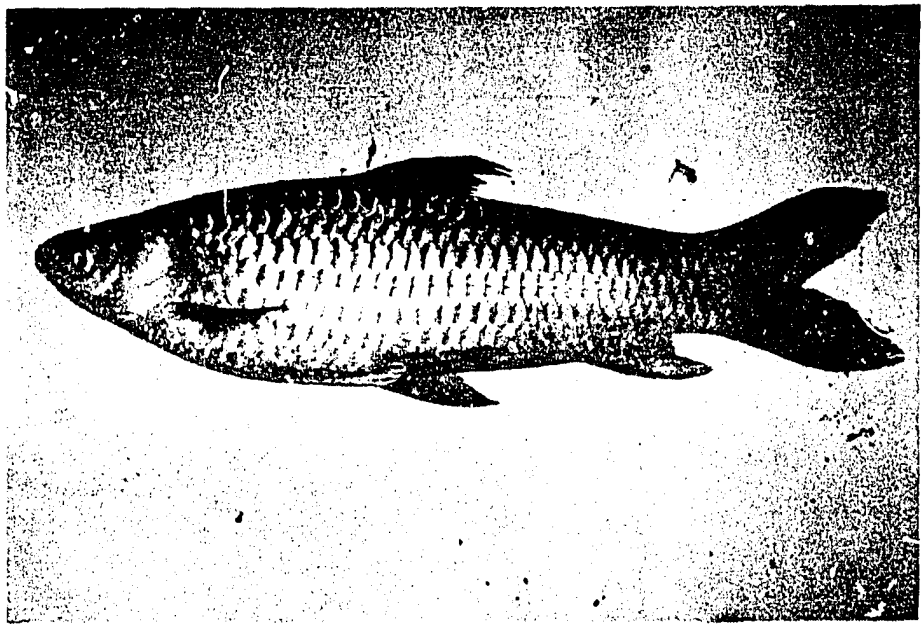

Figure 1. Leptobarbus hovenii (Bleeker)

The largest size can attain 50 cm of length and weighing about 5-6 kg. Every size of fish looks beautiful, in small or medium size for.example; there is a sharply defined broad black lateral band from head to base of caudal fin. This band, intensified in the young, becomes obscure or altogether disappears in the large fish. Scales of back and sides light green with dark green centres, belly white, top of head rich grass green with rosy reflections, opercles lustrous golden or blassy yellow, a black blotch

behind opercles, iris yellow, dorsal fin hyaline green, ventrals and anal blood red, caudal red distally and dull green at base with the interventing part light grey and pectorals hyaline.

Leptobarbus hovenii is herbivorous which subsists on aquatic plants, fruits, leaves, vegetables, etc. They are riverine fishes, spawn in the upstream during rainy season, semi-buoyant type of eggs, fecundity about 120,000 – 150,000 for 1 kg female weight. The incubation period takes about 15–18 hours at water temperature of 25 – 28 ^{o}C. Larvae and fingerlings subsist on phytoplankton, zooplankton, water insects and algae.

3. PREPARATION OF BROODSTOCKS

3.1. Collection of broodstocks

Figure 2. Collection of broodstocks

Large size of prospective brooders, in which case weight and age should be strictly selected; captured by fish-farmers can be held in captivity either in the floating cages or in the earthen ponds for the eventual broodstocks. These adult fishes will be fully mature after 1 year old and be effectively spawned during their 3 to 7 year of age. The brooders weighing 1 kg or over, which usually over 1 year old, should therefore be readily prepared in order to enable the building-up and selection of healthy brooders. In any case, fingerlings can also be collected, provided that the period of rearing should be extended for another year.

3.2. Rearing of broodstocks

At Mudung lake where the station of trial was located, the mature brooders were reared in the floating cages measuring 4 x 8 x 1,5 m^3 in

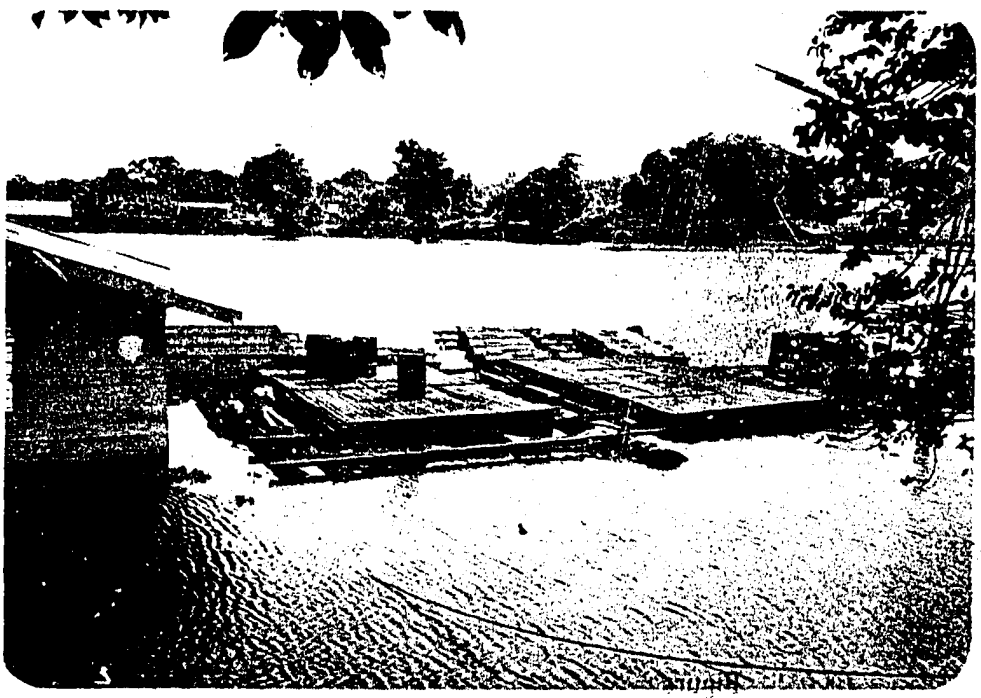

Figure 3. Floating cages for rearing broodstocks

which males and females were stocked togehter with stocking density for 2 fishes per square metre.

Pellet diet with protein content of about 30% was given at the rate of 3% of body weight per day. Vegetables at about 10% of body weight were supplemented twice a week. The pellet diet was formulated as follows:

Fish meal	35%
Soy bean meal	25%
Rice bran	20%
Copra cake	10%
Wheat flour	6%
Vitamin premixture	1%
Mineral	3%

Additionally, it was reported that in Thailand, **Leptobarbus hovenii** has been successfully reared since 1973. By applying cooked food regime with a formula consisted of 30% fish meal, 20% soy bean meal, 20% rice bran, 20% broken rice and 10% ipomea, it was indicated that the brooders attained their readiness for induced spawning (Varikul and Meenakarn, 1973).

3.3. Selection of mature brooders.

Leptobarbus hovenii will normally be able to ovulate and ready for spawning during the rainy season between October to March. Since sexually mature and healthy brooders are pererequisite for any kind of induced spawning procedure, therefore, mature female and male brooders must be selected with care and exact.

In mature females, the ripe eggs should attain the fourth stage of development process. The eggs will be rounded shape and greenish grey, the ovaries weighing about 15–20% of body weight. There are physical indications of male and female brooders that are in ready–to–spawn condition.

The females have big, well – rounded and soft belly, scales along abdomen separated and swollen vent whereas in males the pectoral jins become rough and the milt will be in oozing condition when the abdomen being stripped.

Figure 4. Selection of mature brooders

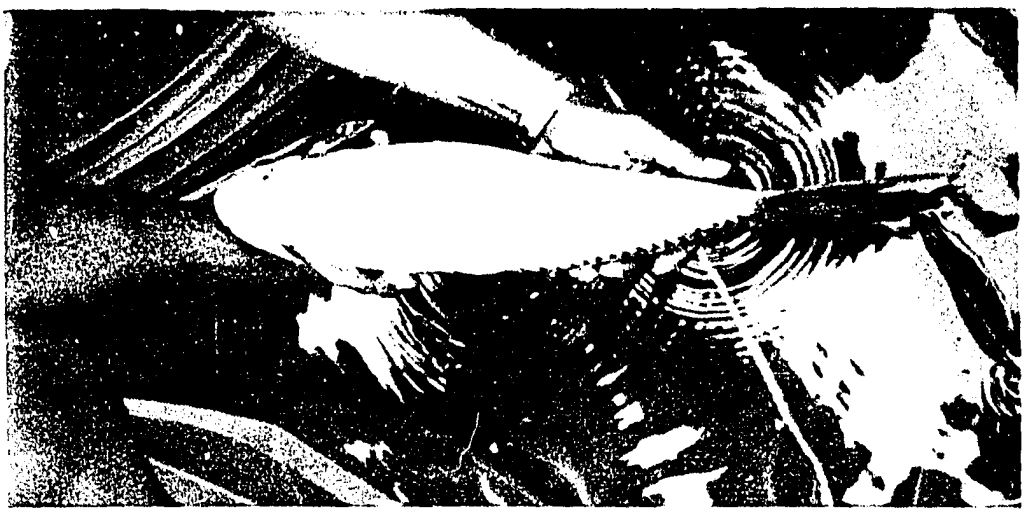

Figure 5. Mature female brooder

4. PREPARATION OF HORMONE

4.1. Hormone

Hormone normally administered for induced breeding is made up of biological compound produced in the pituitary gland of endocrine system. This hormone is secreted to control reproductive events. In nature, the reception of environnental stimuli such as day length (photo period), temperature and the amount of rainfall is mediated by the nervous system and involves the passage of information from sensory receptors to brain. This neural information, upon reaching the hypothalamus, determines the activity of the pituitary gland through chemical messengres termed releasing hormones. These in turn stimulate the pituitary gland to release, into the general circulation, a hormone whose target organ is the gonad. This hormane is termed a gonadotropin. In young animals the hormone is not well developed until they reach adult stage in which pituitary gland produces sex hormone to facilitate reproduction.

Figure 6. Pituitary gland

The pituitary gland is located underneath the midbrain of animals. Environmental stimuli such as rainfall, optimum temperature and water quality conditions, facilitate the spawning of fish. This is one of the reasons that almost all riverine species require favourable conditions in bringing about the pituitary gland to secrete the hormone in enhancing maturation of eggs and sperm prior to natural spawning takes place.

There are two hormones considered to be effective for the induced spawning process, i.e. Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FHS). The pituitary gland of adult fish usually contains both FH and FSH which are important factors for induced breeding.

4. 2. Source of Homone

The pituitary gland being employed to induce the female brooder to release eggs, is usually collected from the same species or termed 'homoplastic pituitary extract'. However, after several experimentations, it has been indicated that the pituitary gland of common carp can be effectively manipulated to induce every other species, particularly in induced spawning of **Leptobarbus hovenii**, the pituitary gland of common carp is, in point of fact, more effective than that of **Leptobarbus hovenii** itself. Therefore, common carp is referable to 'universal donor'. In Thailand the induced spawning on **Leptobarbus hovenii** has been successful by manipulating the pituitary gland of Chinese carp (Varikul and Meenakarn, 1973).

In several species, i.e. **Pangasius pangasius**, **Pangasius sutchi**, **Pangasianodon gigas** and **Leptobarbus hovenii**, ready-made hormone-extract is currently being employed, because this pure hormone contains about 90% LH and 10% FHS. Therefore, in induced breeding process the hormone–extract used should be complemented with the ectraction of homogenized pituitary gland to supplement the FHS content which is lacking in the hormone–extrac. Without the addition of crude extract of pituitary gland, the process of spawning will not effectively take place.

There are a number of hormone-ectracts that commonly administered for the induced breeding practices in Thailand. Those are:

CORRECTION

Page	Item	Correction
Contents	illustrationg HORMANE	illustrations HORMONE
1	reverine extensivelly, anda populer bahibat United States Agency of	riverine extensively and popular habitat United States Agency for
5	pererequisite well - rounded jins	prerequisite well-rounded fins
7	environnental messengres hormane	environmental messengers hormone
8	ectraction hormon-extrac hormon-ectracts	extraction hormon-extract hormon-extracts
13	(milk)	(milt)
17	a though	although
19	2-3 inches or	2-3 inches or over
21	Wish tanks would have not	wish thanks would not have

Name	Unit
HCG (Human Chorionic Gonadotropin)	IU [1]
Pregnyl	IU
Physex	IU
Chorion	IU
Synahorin	RU
Pare hormone	RU [2]

1) International Unit
2) Rodent Unit

4.3. Administration of Hormone

In induced breeding procedure, the pituitary glands are collected from donors. The dosage of pituitary glands and hormone mixture that injected to the brooders should be in optimum ration, otherwise the process of spawning will not be successful.

There are two methods of calculating the amount of pituitary gland required for induced spawning by applying weight (mg) of gland and dosage. The first method is considered rather laborious, for the glands should be preserved in pure acetone or in absolute alcohol before being dried up in dessicator. The dried tissue is then accurately weighed up.

The dosing method is preferable recently, particularly in Thailand because it is easier to measure body weight of both recipient and donor in adjusting the optimum dosing requirement.

$$\text{dose} = \frac{\text{donor}}{\text{recipient}}$$

$$1 \text{ dose} = \frac{1 \text{ unit donor}}{1 \text{ unit recipient}}$$

4.4. Preparation of hormone solution

The pituitary gland located underneath the brain is removed, using a pair of forceps, by chopping off and opening up the upper part of donor's head. The gland should then be finely ground in a homogenizer and added with either distilled water or 0,7% sodium chloride solution. The whole solution is used to inject the brooders.

The volume of solution is adjusted between 1–2 mls, depending on the size of the brooders. Normally 1 ml of solution is administered to 1 kg body weight of recipient.

Figure 7. Preparation of hormone solution

5. INJECTION TECHNIQUE.

The solution is injected intramuscularly to the female recipient on any part of area such as pectoral girdles, the side part between lateral line and dorsal fin, on pelvic girdle and dorsal girdle etc. The **Leptobarbus hovenii** is very conveniently injected at the back part of dorsal fin, because the brooders can be kept in the water while being injected.

Two instalments of injection are given to the **Leptobarbus hovenii** brooders; the introductory or preparatory dose of 0.5 followed by final or decisive dose of 2.5 plus 100 IU hormone-extract. Between the preparatory and decisive dose there is a time - lapse of 6 to 8 hours. The detail of dosage is as follows:

Sample	Weight (kg)	1st Instalment of Injection		2nd Instalment of Injection		Time-Lapse between 1st-2nd Instalment (hours)	Ovulation after 1st Injection (hours)	Hatch - ed out (%)
		PG (dose)/ donor's*) weight (kg)	HCG (IU)	PG (dose) donor's*) weight (kg)	HCG (IU)			
1	2.2	0.5/ 1.1	–	2.5./ 5.5	100	6	10	50
2	2.0	0.5/ 1.0		2.5/ 5.0	100	6	10	50
3	1.7	0.5/ 0.85		2.5/ 4.8	100	6	10.5	80

Common carp was used for donor.
PG : pituitary gland.

Figure 8. Intramuscular injection

6. SPAWNING AND FERTILIZATION

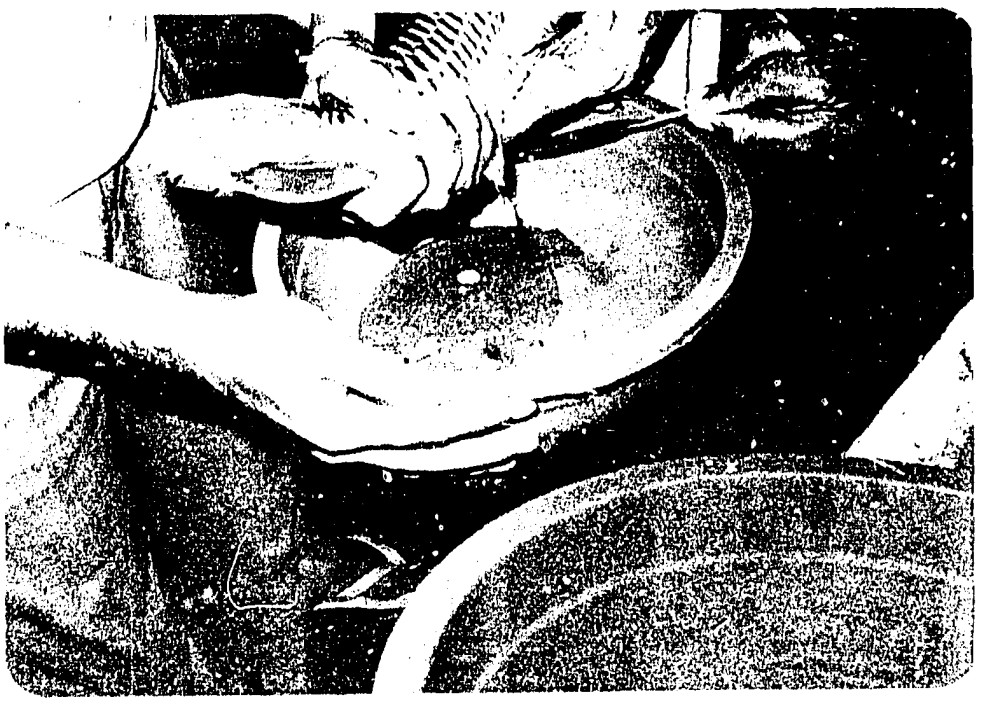

Figure 9. Stripping eggs or sperm (Dry method)

Immediately, after the final injection the brooders should be returned to the hapa which has been suspended in the tank or cage. The brooders must be carefully watched for indications that the eggs and sperm are ready to be stripped. In **Leptobarbus hovenii**, stripping is a convenient way to enhance fertilization process.

When the eggs begin to flow freely, in which case the event can occur between 10–12 hours after the first injection, the females to be netted gently for being stripped first during their ovulation period and the eggs are then placed in a dry container (Plastic bowl). Immediately, the males are also stripped off and the sperm (milk) is then put into the same

Figure 10. Fertilization process takes place while the eggs being rinsed off.

container. The eggs and sperm are mixed together very gently using feather for half of a minute before adding water to cover the eggs and stirred for 1–2 minutes to rinse and release mucous. In this dry method, the fertilization takes place immediately after the water is added. In wet method, the eggs and sperm are mixed together with water in a container. Following this process which completely takes about 1 hour, the eggs will be transfered into an incubator or hatching devices.

7. INCUBATION METHOD

The eggs of **Leptobarbus hovenii** is semi-bouyant type. Hatching funnel or jar immersed in aerated water and equipped with water installation is suitable for hatching this type of eggs, however, the hapa system suspended in stagnant water can also be operated, provided that number of eggs must be in optimum density.

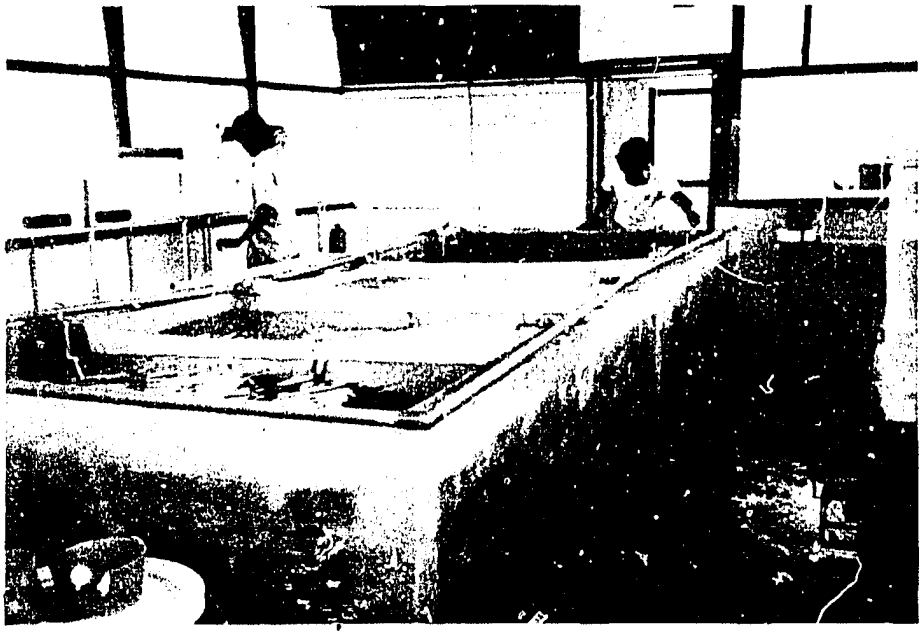

Figure 11. Hatching of eggs (Hapa)

Water quality is a critical factor during the hatching process. The water must be fresh, clean and clear. Temperature should be maintained between 25 – 28°C for hatching this semi-bouyant type of eggs. Higher water temperature (29°C or over) will speed up the development process

of eggs too early and that will cause high mortality.
Hatching period of the **Leptobarbus hovenii's** eggs takes about 15 – 18 hours at the water temperature of 25 – 28°C.

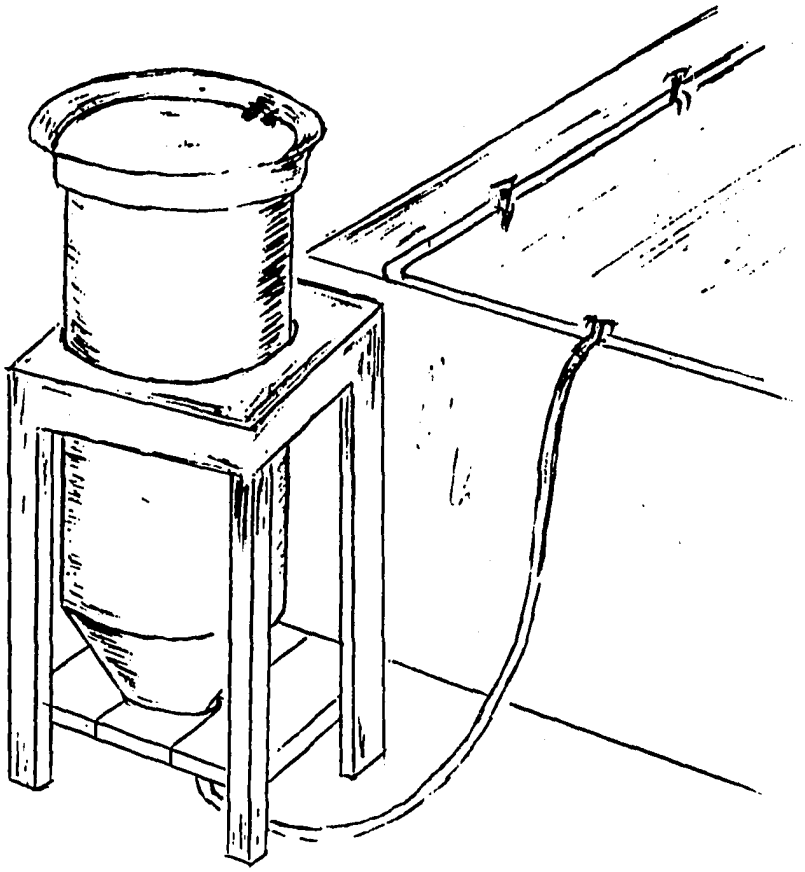

Figure 12. Incubator used for semi-bouyant type of eggs.

LARVAL REARING

Larval rearing is one of the main activities should be carefully prepared because the production of fingerlings will particularly depend on the way the larvae being treated during their critical period, a though every step of breeding process i.e. selection of brooders, injection technique, fertilization and hatching should be carried out thoroughly.

There are several crucial points should be taken into account in the process of larval rearing:

8.1. Type of container

After the fertilized eggs are hatched out, newly larvae are suitably reared in the aquariums or in the concrete tank during their first 15 - day of life, in order to make the cleaning of container easier. After 15 – day of age the larvae can then be transferred into the nursery earthen pond or floating net with small size meshes.

8.2. Food and Feeding

Two different types of food are given to fish fry in a form of life-food such as phytoplankton, zooplankton, tubifex, brine shrimp or artemia etc. and supplementary food like egg--yolk, rice bran, fish meal etc.

Within 2 3 days after the eggs hatched out when the yolk sac begins to disappear, the **Leptobarbus hovenii** larvae will be able to eat immediately.

Live-food is crucially required for feeding fry during 15–day of life, because the food will retain longer in every part of water and will not pollute the container. Supplementary food is given to the fry after 15–days old when, the fry being reared in the earthen ponds. Live-food is still required during the period of life in the nursery which can be provided by way of fertilizing the pond.

The feeding rate can not be calculated from the percentage of body weight, but the amount of food should be estimated according to the density of fry and the size of container.

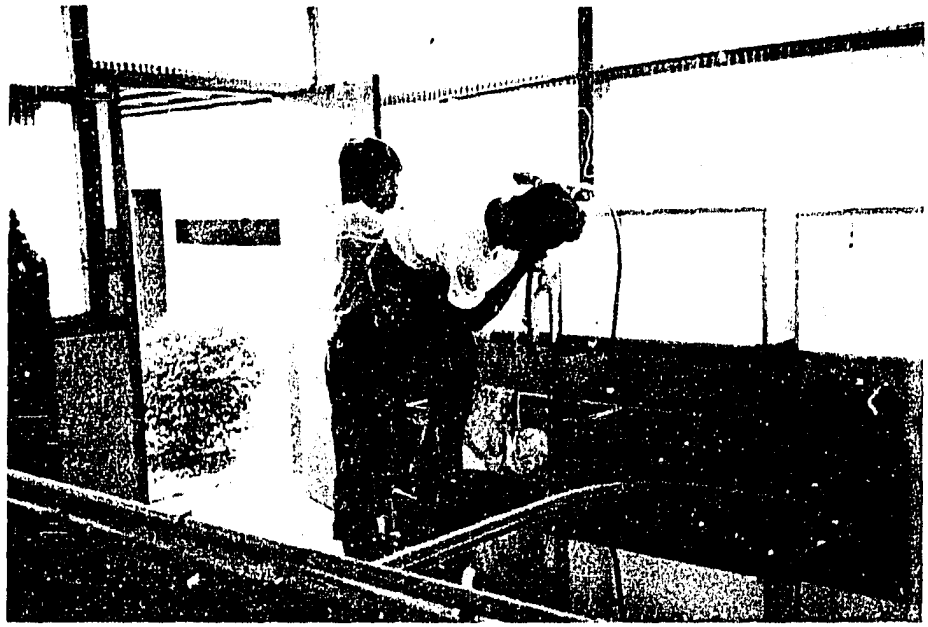

Figure 13. Larval rearing activity.

8.3. Water supply

The water, during the first 15-day of life, supplied for the young fry should be clean and should contain no harmful substances. During this period the container should be cleaned and the water should regularly be changed every day for about one third of the volume. After 15 days of their life when the fry are nursed in the earthen pond, fertilizer should be applied to provide live food.

8.4. Hatchery Management

Hatchery management is the most important aspect to be undertaken

Preparation of food, optimum feedingrate, feeding time, preparation of water supply, control of temperature, aeration system, prevention of diseases and predators should be carefully performed.

DIAGRAM OF LARVAL REARING FOR **LEPTOBARBUS HOVENII**

Age	Activities
Fertilization	– Stripped eggs, sperm, dry method
Hatching out	– In hapa, aquarium, during 15 – 18 hours period at water temperature of 26 – 28° C
1 – 2 days	– Rearing in the hatching containers.
3 days	– Transfer from hatching containers to aquarium, fibreglass tank or concrete pond. – Start feeding with boiled egg–yolk every 6 hours interval.
4 days	– Adding some water about 2–3 inches or.
5 – 15 days	– Feeding with live moina twice a day – Clean the bottom of containers by syphoning method
15 – 60 days	– Feeding with supplementary food with ration for fish meal 5 : soy bean meal 2 ; rice bran 2; wheat flour 1; (by weight).
Remark	Every step of activities should be equipped with aeration system.

REFERENCES

1. Bardach, John E. Ryther, John and Mc Larney, W.O (1972).
   Aquaculture– the farming and husbandry of fresh-water and marine organisms.
   Wiley – Interscience, John Wiley and Sons, Inc. New York.

2. Harvey, B.J. and Hoar, W.S. (1979).
   Theory and practice of induced breeding in fish. Ottawa, Ont. IDRC 48, p.lll.

3. Hicklings, C.F. (1962).
   Fish Culture, Faber and Faber, London.

4. Liang, S.W. (1957).
   On the development of inland fisheries in Thailand.
   FAO/ETAP Report to the Goverment of Thailand FAO Rep. 653, FAO/57/6/4117, 50. pp.

5. Smith, H.M. (1945).
   The fresh–water fishes of Siam or Thailand.
   Bull. U.S. Nah. Mus. 188.

6. Varikul, V and Meenakarn, S. (1973).
   Report on induced breeding of **Leptobarbus hovenii** in Thailand. (unpublished).

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