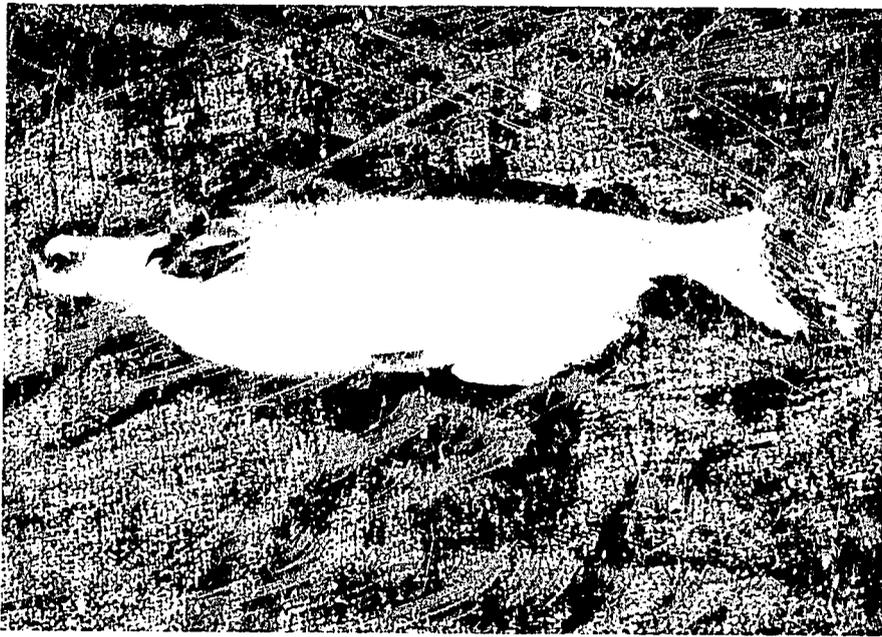


RU 817-817 65
100-46975

USAID/ARD 497-0286

AID. 497-0286-S-00-4062-00

**INDUCED SPAWNING ON
Pangasius pangasius (Hamilton)
CARRIED OUT IN SOUTH SUMATRA, INDONESIA**



By :

Samruay Meenakarn
Fish Breeding Advisor

The Directorate General of Fisheries, Indonesia
in collaboration with
The United States Agency for International Development
in the Cage Culture and Seed Production sub-project,
of the Small Scale Fisheries Development Project
Jakarta, July 1986



CONTENTS

	Page
PREFACE	ii
LIST OF ILLUSTRATIONS	iii
1. INTRODUCTION	1
2. BIOLOGY	2
3. PREPARATION OF BROODSTOCKS	2
3.1. Collection of broodstocks	2
3.2. Rearing of broodstocks	3
3.3. Selection of mature brooders	4
4. PREPARATION OF HORMONE	5
4.1. Hormone	5
4.2. Source of hormones	6
4.3. Administration of hormone	7
4.4. Preparation of hormone solution	7
5. INJECTION TECHNIQUE	8
6. SPAWNING AND FERTILIZATION	9
7. INCUBATION METHOD	10
8. LARVAL REARING	11
8.1. Type of container	11
8.2. Food and Feeding	11
8.3. Water supply	11
8.4. Hatchery management	12
REFERENCES	13
ACKNOWLEDGEMENTS	13

PREFACE

This manual has been prepared within the framework of Small Scale Fisheries Development Project, implemented by the Directorate General of Fisheries 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 spawning, specifically of Pangasius pangasius (Hamilton) which has successfully been the firstfruit of the author's trials carried out in South Sumatra province, Indonesia. It outlines 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.

Sanruay Meenakam
Fish Breeding Advisor

Jakarta, July 1986.

LIST OF ILLUSTRATIONS

Figure	Page
1. <u>Pangasius pangasius</u> (Hamilton)	1
2. Collection of broodstocks	3
3. Floating cages for rearing broodstocks	4
4. Selection of a mature brooder	4
5. Pituitary gland	5
6. Intramuscular injection	8
7. Stripping eggs or sperm (dry method)	9
8. Fertilization process takes place while the eggs being rinsed off	10
9. Hatching of eggs (Hapa)	10
10. Larval rearing activity	12

INDUCED SPAWNING ON
Pangasius pangasius (Hamilton)
CARRIED OUT IN SOUTH SUMATRA, INDONESIA

1. INTRODUCTION.

Pangasius pangasius one of quite popular freshwater fish species belongs to medium-size riverine catfish. The species is distributed over areas of open-water in Indonesia with great potential in South Sumatra and Kalimantan. The fish is familiarized with local name of "Ikan Patin". A great deal of catch comprise marketable-size and only a small part of that consists of fingerlings which are then usually reared and stocked in floating cages or earthen ponds.

Pangasius pangasius being an omnivore, is characterized by its ability to grow very fast in captivity with any kind of food. In Palembang area, the species reared in the floating cages or earthen ponds is extensively fed with locally available food stuff like small trash fish or food remnants such as frog's leg, chicken gut, soy bean curd even with all kinds of food wastes disposed from the kitchen or market. Considering that the area is quite rich with other better-quality food ingredients such as fish meal, soy bean meal, rice bran, broken rice etc, recommendation should therefore be made to intensify the fish culture by utilizing the locally available diet to improve the feeding practices.

Moreover, one of other constraints that hinder the effort in intensifying the culture of Pangasius pangasius is caused merely by lack of fingerlings. This is one of the reasons that the Directorate General of Fisheries, in collaboration with the United States Agency for International Development implement applied research on induced breeding trial and subsequently in increasing seed production.

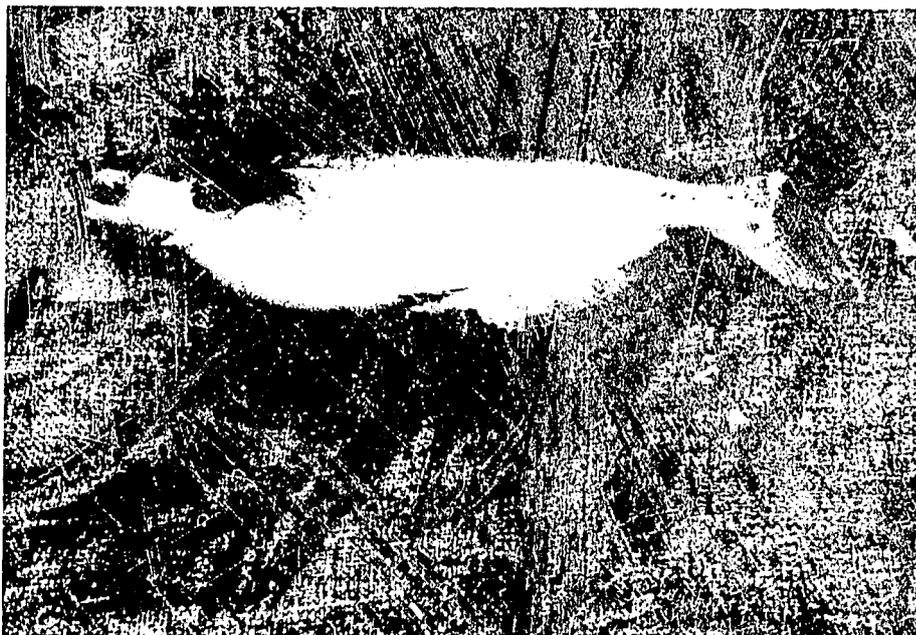


Figure 1. Pangasius pangasius (Hamilton).

2. BIOLOGY.

Pangasius pangasius originally is riverine fresh-water species, potentially found in Kalimantan, South Sumatra and also Thailand. This species may be recognized by a combination of structural characteristics and unique colour feature. The vomerine teeth were in 2 separate quadrate patches as broad as the eye, flanked by 2 narrow lenticular palatine patches as long as the eye. The maxillary barbels barely reached the base of the pectoral fins. The anal rays were iii, 28 or 29. The exil of the pectoral fins had 4 distinct pores. Back light grey-green, top of head light green. sides pearly white, belly dazzling white, sides of head and front jaws pure creamy white: dorsal fin hyaline-pink, caudal mostly pink, with dorsal part of upper lobe grey, anal hyaline distally, pink at base, ventrals and pectorals hyaline, adipose fin green-grey with a broad white posterior margin.

The largest fish attains nearly one metre and weighing 15–15 kg. They are omnivorous, subsist on any kind of food material, but during the begining of life until attaining fingerling size, they rather be more carnivorous. As riverine fishes, they spawn in the river during rainy season. The brooder produces adhesive eggs with fecundity about 100,000 – 130,000/1 kg of female weight. The incubation period is about 40 – 44 hours at water temperature of 27–29°C. Larvae and fingerlings subsist on zooplanton and water insects.

3. PREPARATION OF BROODSTOCKS.

3.1. Collection of broodstocks.

As previously mentioned that all-sizes of fish are captured by the fishfarmers from the natural habitat. The larger-size fishes, are supposedly quite appropriate for prospective broodstock, but since the size is too big the fishermen find it difficult to handle and transfer the adult fishes from the fishing ground to the floating cages, because they can easily get injured themselves and will cause high mortality during transportation.

Fingerlings seem to be more conveniently collected for prospective broodstock, although they have to be reared for a further year to obtain the sexually mature brooders. The adult fish will be effectively spawned during their 3 to 7 year of age.

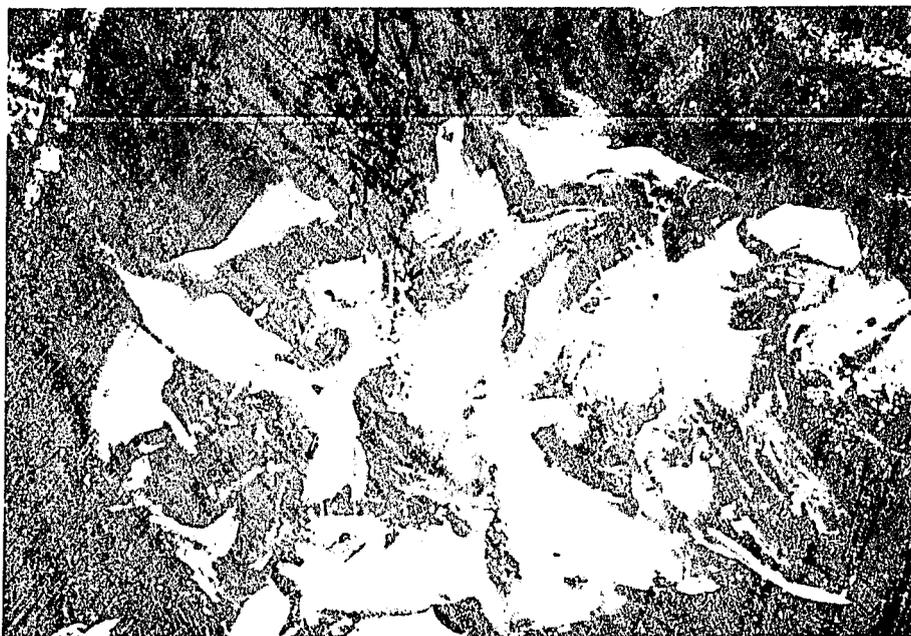


Figure 2. Collection of broodstocks.

3.2. Rearing of broodstocks.

At Gandus (Musi river) where the station of trial was located, the brooders were reared in the floating cages measuring $2 \times 3 \times 1,5 \text{ m}^3$ in which males and females were stocked together with the stocking density for 10 fishes per square metre. Cooked food with protein content of about 30% was prepared for the feeding and was given at the rate of 4% (dry weight) of body weight. Trash fishes or small fishes at about 10% of body weight were supplemented twice a week. The cooked food was formulated as follows.

Fish meal	40%
Rice bran	40%
Broken rice	18%
Vitamin	2%

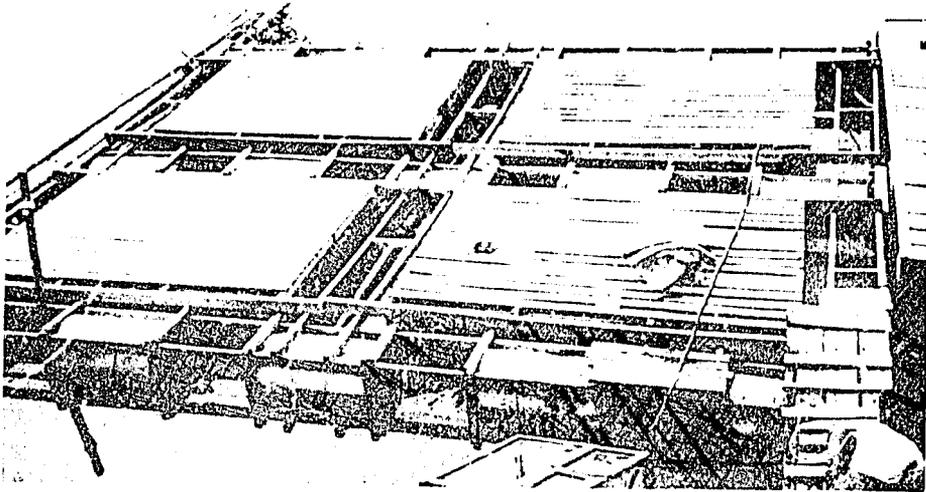


Figure 3. Floating cage for rearing broodstocks.

3.3. Selection of mature brooders.

Pangasius pangasius 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 prerequisite for any kind of induced spawning procedure, therefore, mature female and male brooders must be selected with care and exact.



Figure 4. Selection of mature brooder.

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 10–15% 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, vent reddish and swollen, whereas in males, the genital opening becomes reddish and the milt will be in oozing condition when the abdomen being stripped.

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. The hormone is secreted to control reproductive events. In nature, the reception of environmental stimuli such as, day length (photo period), temperature, and the amount of rainfall is mediated by 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 messengers 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 hormone is termed a gonadotropin. In young animals the hormone is not well developed until they reach adult stage in which the pituitary gland produces sex hormone to facilitate reproduction.



Figure 5. Pituitary gland.

The pituitary gland is located underneath the mid brain 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 before natural spawning takes place.

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

4.2. Source of hormone.

The pituitary gland being employed to induce the female 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 Pangasius pangasius, the pituitary gland of common carp is, in point of fact, more effective than that of Pangasius pangasius itself. Therefore common carp is referred to as "universal donor".

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

Following are a number of hormone-extracts that commonly administered for induced breeding practices in Thailand.

Those are :

Name	Unit
HCG (Human Chorionic Gonadotropin)	IU *)
Pregnyl	IU
Physex	IU
Chorion	IU
Synahorin	RU **)
Pare hormone	RU

*) International Unit

**) Rodent Unit

4.3. Administration of hormone.

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

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 absolute alcohol before being dried up in dessicator. The dried tissue is then accurately weight up.

The dosing method is preferable recently, particularly in Thailand because it is easier to measure body weight of both recipients and donors 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.

5. INJECTION TECHNIQUE.

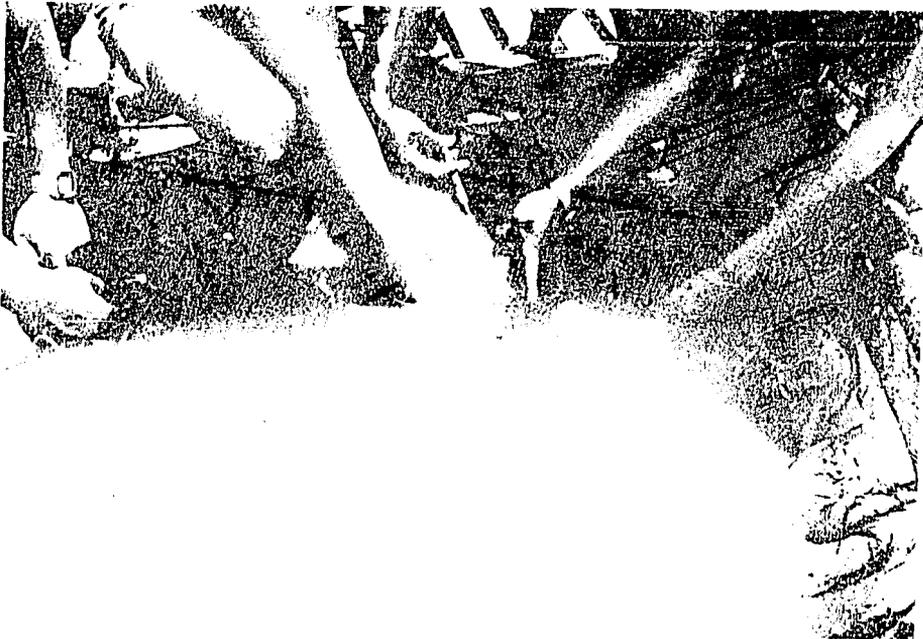


Figure 6. Intramuscular Injection.

The solution is injected intramuscularly to the female recipient on any part of area such as pelvic girdles, the side part between lateral line and dorsal fin, on pelvic girdles and dorsal of fins etc. The *Pangasius pangasius* is very conveniently injected at the back part of dorsal fin, because the brooders can be kept in the water while the injection takes place.

Two instalments of injection are given to the *Pangasius pangasius* brooders; the introductory or preparatory dose of 1.0 plus 200 IU followed by final or decisive dose of 3.0 plus 500 IU herring-extract. Between the preparatory and decisive dose there is a time-lapse of 10 to 12 hours.

The detail of dosage is as follows.

Sample	Weight (kg)	1st instalment of injection		2nd instalment of injection		Time-lapse between instalment		Ovulation after 1st injection (hours)	Hatched out (%)
		PG (dose)	HCG (IU)	PG (dose)	HCG (IU)	1st instalment	2nd instalment		
1	3.5	1/3.5	200	3/10.5	500	11	18	80	
2	3.0	1/3.0	200	3/9.0	500	11	18	80	
3	2.5	1/2.5	200	3/7.5	500	11	22	50	

Donor : Using Common carp

6. SPAWNING AND FERTILIZATION.

Immediately, after the final injection the brooder 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 Pangasius pangasius, stripping is a convenient way to enhance fertilization process. When the eggs begin to flow freely, in which case the event can occur between 18–22 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 stripping off and the sperm(milt) is then put into the same 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 transferred into an incubator or hatching devices.



Figure 7. Stripping eggs or sperm (Dry method)



Figure 8. Fertilization process takes place while the eggs being rinsed off.

7. INCUBATION METHOD.

The eggs of Pangasius pangasius is adhesive type. Hatching kakaban or hapa system immersed in stagnant water is used for hatching this type of eggs. Hatching funnel or jar system can also be operated, provided that the fertilized eggs must be rinsed with muddy water to release the mucous before hatching.

Water quality is a critical factor during the hatching process. The water must be fresh, clean and clear. Temperature should be maintained between 27–30°C for hatching this adhesive type of eggs. The hatching period of Pangasius pangasius eggs takes about 40–44 hours at water temperature of 27–30°C.

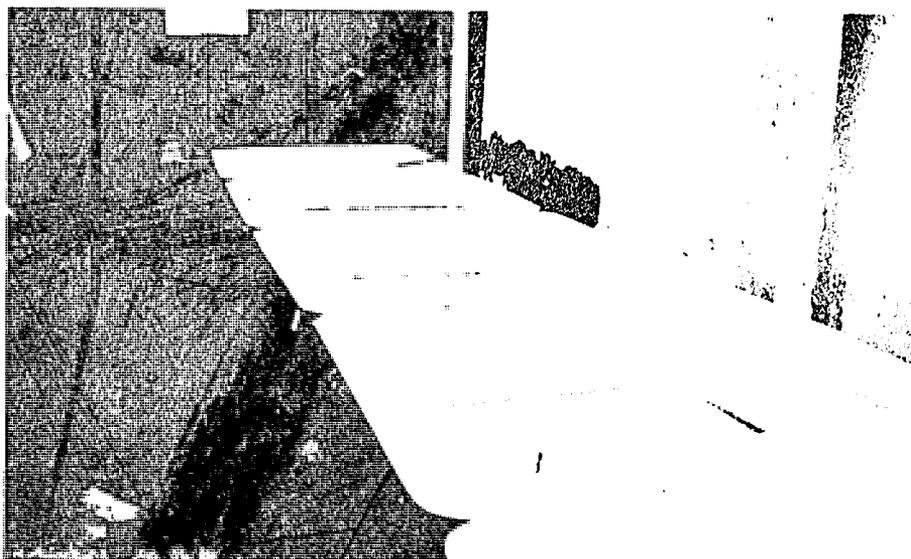


Figure 9. Hatching of eggs (hapa).

8. LARVAL REARING.

Larval rearing is one of the activities should be carefully prepared because the production of fingerlings will particularly depend on the way the larvae being treated during their critical period, although every step of breeding process like 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 Pangasius pangasius larval rearing :

8.1. Type of container

After the fertilized eggs are hatched out, the newly larvae are suitably reared in the aquarium or 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 live food such as zooplankton, tubifex, brine shrimp or artemia etc. and supplementary food like egg-yolk, rice bran, fish meal etc. Moina is particularly good for larvae rearing.

Within 2–3 days after the eggs hatched out, when the yolk sac begins to disappear, the Pangasius pangasius 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.

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 where 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 feeding rate, feeding time, preparation of water supply, control of temperature, aeration system, prevention of diseases and predators should be carefully prepared.

Diagram of Larval Rearing for Pangasius pangasius.

Age	Activity
Fertilization	– Stripped eggs, sperm, dry method.
Hatching out	– In hapa, indoor hatchery, during 40–44 hours at water temperature 27–29°C.
Newly Larvae	– Moved to aquariums or fibreglass tank. – Put antibiotic about 250 mg/aquarium. – Put some salt (Sodium chloride).
2 – 15 days	– Feeding with live moina twice/day. – Clean the bottom of aquarium by syphoning technique. – Change the water about a half of aquarium. – Put some salt (Sodium chloride) about 0,1%.
15 – 20 days	– Feeding with tubifex, red worm twice/day. – Change water, clean the bottom of aquarium, antibiotic, and salt still being applied.
30-----days	– Size about 1.5 inches. – Moved to the nursery cages (in the river) or earthen pond. – Fed with supplementary food (cooked food) or minced trash fish.
Remark	Temperature should be maintained about 28–30°C.

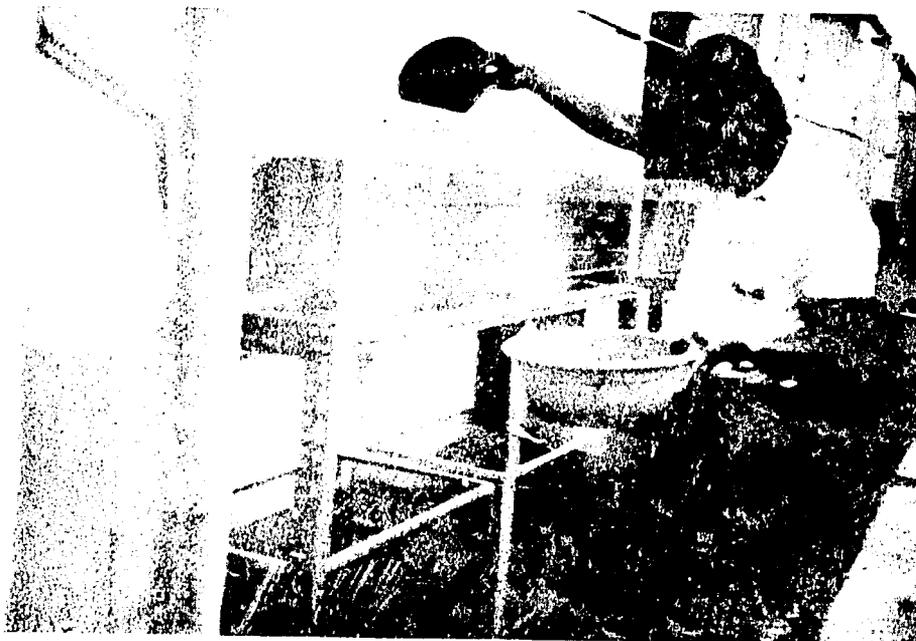


Figure 10. Larval rearing activity.

REFERENCES

1. Bardach, John E., Ryther, John and McLarney, W.O. (1972) Aquaculture – the farming and husbandry of fresh-water and marine organism.
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. 111
3. Hicklings, C.F. (1962). Fish Culture., Faber and Faber, London.
4. Ling, S.W. (1957) On the development of inland fisheries in Thailand.
FAO/ETAP Report to the Government 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.

ACKNOWLEDGEMENTS

I am most grateful to the Freshwater Fisheries Research Institute (BBPAT) personnel, namely Messrs Atmaja Harjamulia, Ondara and Zainal Arifin for their provision and care of the Pangasius pangasius brooders for the project, without those the outcome of induced breeding practices would not have become a reality.

My sincere appreciation goes to Dr. Josephine Wiryanti for her assistance in preparing this manual.

----- ooooOoooo -----