Technical Manual For Artificial Propagation Of The Indonesian Catfish, *Pangasius djambal*

Edited by:

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Technical Manual For Artificial Propagation Of The Indonesian Catfish, *Pangasius djambal*

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ABSTRACT

Although 14 pangasiid species have been recognised in the Indonesian ichtyofauna, *Pangasianodon hypophthalmus* initially introduced from Thailand was until recently the only pangasiid cultivated in Indonesia. Aiming to utilize local biological diversity, the potentialities of Indonesian *Pangasius* species for fish culture have been investigated since 1996 throughout the «Catfish Asia» research program supported by European Union. Among these species, *Pangasius djambal* Bleeker, 1846, was pre-selected as a new aquaculture candidate for its large size (more than 20 kg individual body weight), for its broad geographic distribution and for its popularity among consumers from Sumatra or other Indonesian islands. Zootechnical evaluation of this species has demonstrated its valuable characteristics for aquaculture. Artificial breeding was firstly achieved in 1997.

Based on six years of working experience, this technical manual attempts to provide fish farmers and scientists with elements on the biology of *P. djambal* and all technical guidelines allowing to succeed in its artificial propagation. After providing an identification key to *P. djambal*, practical aspects related to fish transportation, broodstock management, induced spawning, artificial fertilization, egg incubation, larval rearing and fish health management are developed.

AKNOWLEDGEMENTS

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Contents

Catfishes, in particular the Pangasiidae and the Clariidae, are important economic resources in South-East Asia with more than 250 000 tonnes in 2001. In Indonesia, two allochthonous species – *Pangasianodon hypophthalmus* introduced from Thailand and *Clarias gariepinus* from Africa – are the main cultivated catfishes. For some years now, Indonesia has been trying to improve and diversify its aquaculture, notably with autochthonous species, better appreciated by Indonesian consumers, and with a commercial value two to three times that of introduced species.

In this context, IRD, the Institute of Research for Development from France, in partnership with the Central Research Institute for Aquaculture (CRIA) and the Directorate General for Aquaculture (DGA) launched in 1996, with the support of the European Union and of the French Ministry of Foreign Affairs, a programme entitled "Catfish Asia" which brought together basic and applied research. Using a series of studies (molecular phylogenetics involving allozymic and DNA analyses, morphometry, zoogeography) on the biological diversity of these two catfish families in Indonesian rivers, the species distribution areas were defined and identification keys were drawn up. From this work, six species of *Pangasius* new to science were discovered and described.

Knowledge acquired on the biology of Indonesian catfishes and the breeding trials conducted using specimens initially captured from the wild have brought to light several local pangasiid species, presenting a high potential for fish farming. After several years of experimentation, *Pangasius djambal*, never used for aquaculture before, appeared as a promising species for furthering the diversification of fish culture in Indonesia.

Pangasius djambal is one of the fourteen pangasiid species now recorded in Indonesia. Sampling campaigns (with more than 2000 captured specimens) have shown that it lives in the main rivers of Java, Sumatra and Kalimantan islands. However, it is becoming rarer owing to overfishing, water pollution and dam construction. This species was chosen for aquaculture trials for its high commercial value, its large maximum size (over one meter) and its broad geographical distribution. Its artificial breeding could also reduce some of the fishing pressure currently affecting wild fish stocks. Furthermore, its whitish flesh is preferred over the yellow flesh of *P. hypophthalmus*, not only in Indonesia but also in other markets, in Asia, Europe and North America which constitute potential export destinations.

Rearing began in 1997, the year when the research team for the first time achieved the induced reproduction of *P. djambal* in culture conditions. Since then, the team has developed its research in two experimental aquaculture

stations, at the Sukamandi CRIA station in Java and at the Jambi Freshwater Aquaculture Development Centre (JFADC) of DGA in Sumatra, and has found how to manage the different steps of this species' breeding cycle. In rearing ponds, fish born in captivity reached sexual maturity after three years, at an individual weight of 3 to 5 kg and their reproduction could be induced throughout the year. This is an advantage over species that have a seasonal reproductive cycle. In captivity, *P. djambal* fry, fingerlings or adults displayed a growth rate superior to that of the introduced species *P. hypophthalmus*. Based on the promising results obtained so far, *P. djambal* is recognised as a promising species for freshwater aquaculture in Indonesia.

Like most fish species, *P. djambal* does not reproduce spontaneously in artificial rearing conditions. Fry production is made possible using a hormonal treatment which triggers ovulation, followed by artificial fertilization. The aim of the present technical manual is to summarize elements on the biology of this catfish species and all useful information for farmers and scientists to correctly identify the fish and to succeed in its artificial propagation. After providing an identification key to *P. djambal*, practical aspects related to fish transportation, broodstock management, induced spawning, artificial fertilization, egg incubation, larval rearing and fish health management are developed in this manual.

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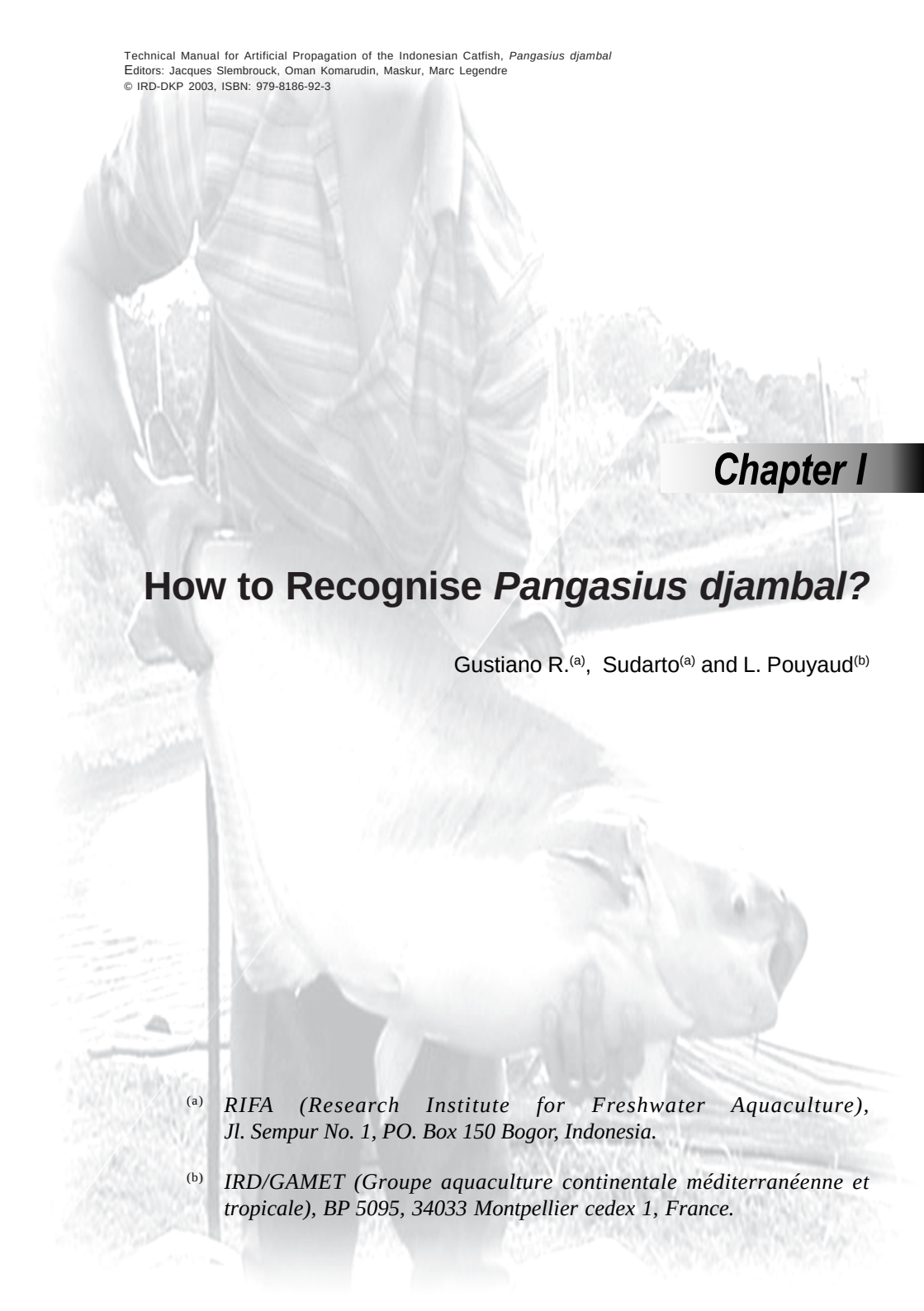

Chapter I

How to Recognise *Pangasius djambal?*

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Systematics is the study of organisms' biodiversity and relationships among the taxa (Elredge, 1992). This involves arranging the diversity into a system of species-classification and defining identification keys (Helfman *et al*., 1997). All theories and strategies for the conservation and sustainable use of the biodiversity can only be efficient when the species are correctly identified. Referring to the literature, it is clear that one of the main barrier to breed wild species and to optimise production of the reared species is the scarcity of systematic knowledge (Lazard, 1999; Legendre, 1999).

Since the taxonomic group of Pangasiidae was first recognised and established as Pangasini Bleeker, 1858 (Ferraris and de Pinna, 1999), its content and classification have been greatly changed through the endeavours of various ichthyologists. Considerable confusion arose in the systematics of this catfish group as most of the previous workers described species without consulting existing type specimens. Nearly all authors encountered problems recognising juveniles of large species, and junior synonyms were often based on small sized specimens. The case of *Pangasius djambal* perfectly illustrates this situation. Vidthayanon (1993) indicated that this species was only known from Java (Batavia, Krawang, Tjikao and Parongkarong Rivers), Kalimantan (Barito and Kapuas Rivers), and Sumatra (Musi River). Roberts and Vidthayanon (1991) reported that this species was reared in Java and in South Sumatra, and was erroneously identified as *P. pangasius* by Meenakarn (1986). Subsequent studies conducted by Legendre *et al*., (2000) and Pouyaud *et al*. (1999, 2000) demonstrated that the pangasiid species reared in Java until 1996 was *Pangasianodon hypophthalmus* (Sauvage, 1878) (ex. *P. sutchi*), and that the local species bred in Sumatra before 1996 could have been *P. djambal*, *P. kunyit* Pouyaud, Teugels and Legendre, 1999, *P. nasutus* Bleeker, 1863, or a possible mix of those species.

SPECIES DIVERSITY OF PANGASIIDAE IN INDONESIA

Following Gustiano (2003), 14 valid species of Pangasiidae are reported from Indonesia, including *P. hypophthalmus* an exotic species introduced from Thailand. These species are distributed in 4 genera, respectively *Helicophagus* Bleeker, 1858 (2 species: *H. typus* Bleeker, 1858 and *H. waandersii* Bleeker, 1858); *Pangasianodon* Chevey, 1930 (1 species: *P. hypophthalmus*); *Pteropangasius* Fowler, 1937 (1 species: *P. micronemus* (Bleeker, 1847) and *Pangasius* Valenciennes, 1840 (12 species: *P. lithostoma* Roberts, 1989; *P. humeralis* Roberts, 1989; *P. nieuwenhuisii* Popta, 1904; *P. macronema* Bleeker, 1851; *P. polyuranodon* Bleeker, 1852;

P. mahakamensis Pouyaud, Gustiano and Teugels, 2002; *P. kunyit*; *P. rheophilus* Pouyaud and Teugels, 2000; *P. nasutus*; *P. djambal* Bleeker, 1846). In Indonesia, the pangasiids inhabit most of the major river drainage systems from the central alluvial plain of Sumatra (Way Rarem, Musi, Batang Hari and Indragiri Rivers), from the eastern part of Java (Brantas and Bengawan Solo Rivers), and from Kalimantan (Kayan, Berau, Mahakam, Barito, Kahayan and Kapuas Rivers).

The species diversity of Pangasiidae is unequally distributed within each major river basin with large species diversity in Sumatra and high endemicity rates in Kalimantan (a species is considered as endemic when its natural distribution is confined to a single river system). The species composition of each major river system is given in Table I.1, which summarises the results given by Vidthayanon (1993), Pouyaud *et al*. (2000) and Gustiano (2003).

Table I.1

Natural distribution of the 13 autochthonous Pangasiidae species per river basin in Indonesia (bold face refers to endemic species).

River basin (Geographic area)	Pangasiidae species composition
Way Rarem (South Sumatra)	*Pteropangasius micronemus, Pangasius polyuranodon*
Musi (South Sumatra)	*Pangasius djambal, Pteropangasius micronemus, Pangasius nasutus, Pangasius polyuranodon, Pangasius kunyit, Helicophagus waandersii, Helicophagus typus*
Batang Hari; Indragiri (Central and North Sumatra)	*Pangasius djambal, Pteropangasius micronemus, Pangasius nasutus, Pangasius polyuranodon, Pangasius kunyit, Helicophagus waandersii, Helicophagus typus*
Brantas, Bengawan Solo (Central and East Java)	*Pangasius djambal, Pteropangasius micronemus*
Barito, Kahayan (Central Kalimantan)	*Pangasius djambal, Pteropangasius micronemus, Pangasius nasutus, Pangasius polyuranodon, Pangasius macronema, Pangasius kunyit, Helicophagus typus*
Kapuas (West Kalimantan)	*Pangasius kunyit, Pteropangasius micronemus, Pangasius nasutus, Pangasius polyuranodon,* ***Pangasius lithostoma, Pangasius humeralis,*** *Helicophagus typus*
Mahakam (East Kalimantan)	*Pangasius kunyit, Pteropangasius micronemus,* ***Pangasius nieuwenhuisii, Pangasius mahakamensis***
Kayan, Berau (East Kalimantan)	***Pangasius rheophilus***

GEOGRAPHIC DISTRIBUTION AND MONITORING OF NATURAL POPULATIONS OF *P. DJAMBAL*

Pangasius djambal is widely distributed throughout Indonesia (Table I.1). Nevertheless, since 1995 field observations and market surveys indicate that captures of this species are in decline in the Musi River (Sumatra) and in the eastern part of Java. This is probably the consequence of a lethal cocktail between strong modification of their natural habitat (dam construction), spoiling, and/or overfishing. Artificial reproduction of *P. djambal* could be a positive alternative to the deterioration of wild stocks through an expected decrease in fisheries pressure and in the promotion of restocking programs. To be successful, propagation of artificial seeds of *P. djambal* should be done according to the spatial distribution of their natural populations (Sudarto *et al*., 2001). According to these authors, *P. djambal* is composed of three natural stocks, respectively in Java, Sumatra and Kalimantan. Each stock is highly differentiated genetically from each other, and should not be genetically polluted or modified by influx of exogenous genes.

METHODOLOGY USED FOR DISTINGUISHING *P. DJAMBAL*

Biometrics is a powerful method to characterise and to distinguish *P. djambal* from all other pangasiid species present in Indonesia. This method is based on morphological measurements taken on the body and on the vomerine and palatine toothplates. Gustiano (2003) in a systematic revision of Pangasiidae used 35 point to point measurements (Plate I.1) which were taken with dial callipers.

MORPHOLOGICAL CHARACTERISTICS OF *P. DJAMBAL*

Pangasius djambal Bleeker, 1846 (Plate I.2) and its junior synonym, *Pangasius bedado* Roberts, 1999 are distinguished by a unique combination of the following characters: 6 pelvic fin rays, robust anterior part of snout width (29.3 – 36.6% of head length), long maxillary barbel (>200% of eye diameter; between 31.8 and 66.2% of head length), adipose fin well developed, vomerine toothplate with lateral extension, long predorsal length (35.5 – 41.9% standard length), great eye diameter (10.1 – 21.3% head length), long distance from the snout extremity to the isthmus (103.8 – 133.3%

snout length), robust dorsal width (5.7 – 9.5% head length), large body width (16.8 – 21.4% standard length), head length (21.8 – 27.1% standard length), head width (13.4 – 19.4% standard length), and 27 to 39 gill rakers on the first branchial arch.

DETERMINATION KEY FOR *P. DJAMBAL*

In order to be effective, this determination procedure must be used following the successive steps given below:

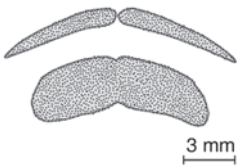
1 **a.** A fish with 8 – 9 pelvic fin rays, a long predorsal length (>37% of standard length), and a slender dorsal spine width (3.5 – 5% of head length) is *Pangasianodon hypophthalmus*, a species introduced in Indonesia from Thailand for aquaculture.
b. A fish with only 6 pelvic fin rays is a local species from Indonesia. See **2**.

2 **a.** A fish with a slender anterior part of snout (<16.5% of head length), with posterior nostril situated between anterior nostrils and orbit, is *Helicophagus waandersii* or *Helicophagus typus*.
b. A fish with a robust anterior part of snout (>16.5% of head length), with posterior nostril close behind anterior ones and above imaginary line from anterior nostrils and orbit belongs to the genera *Pangasius* or *Pteropangasius.* See **3**.

3 **a.** A fish with eye relatively large, minute maxillary barbel (<192% of eye diameter), dorsal and pectoral fins relatively thin, pectoral fin with minute and numerous serrations on the anterior and posterior edge of the fin spine, and minute adipose fin, is *Pteropangasius micronemus.*
b. A fish with relatively long maxillary barbel (>192% of eye diameter), robust dorsal and pectoral fins, and adipose fin well developed, belongs to the genus *Pangasius.* See **4**.

4 **a.** A fish with a vomerine toothplate without lateral extension (Figure I.1) is one of the endemic species from Kalimantan which include *Pangasius lithostoma* (Kapuas), *Pangasius humeralis* (Kapuas) and *Pangasius nieuwenhuisii* (Mahakam).

Figure I.1.
Vomerine toothplate without lateral extension (e.g. *Pangasius humeralis*).



b. A fish with a vomerine toothplate with lateral extension, namely palatine toothplate, (Figure I.2) is one of the following species *Pangasius djambal*, *Pangasius macronema*, *Pangasius polyuranodon*, *Pangasius mahakamensis*, *Pangasius nasutus*, *Pangasius kunyit* or *Pangasius rheophilus.* See **5**.

Figure I.2.
Vomerine toothplate with lateral extension (e.g. *Pangasius polyuranodon*).

5 **a.** A fish with very long maxillary barbel length (100.5 – 203.9% of head length), long mandibular barbel (76.8 – 176.5% of head length) is *Pangasius macronema.*
b. For a fish with maxillary barbel less than 100.5% of head length and mandibular barbel less than 76.8% of head length. See **6**.

6 **a.** A fish with a predorsal length between 25.1 and 31.2% of the standard length and with an eye diameter between 16.0 and 30.3% of the head length is *Pangasius polyuranodon.*
b. A fish with a predorsal length between 30.1 and 32.7% of the standard length and with an eye diameter between 22.8 and 29.4% of the head length is *Pangasius mahakamensis.*
c. For a fish with a predorsal length more than 31.8% of the standard length and with an eye diameter less than 22.8% of the head length, see **7**.

7 **a.** A fish with a short distance from the snout extremity to the isthmus (less than 110% of the snout length) is *Pangasius kunyit.*
b. For a fish with a long distance from the snout extremity to the isthmus (more than 110% of the snout length). See **8**.

8 **a.** A fish with a dorsal spine width between 4.7 and 6.2% of the head length, a head length between 19.6 and 23.2% of the standard length, a head width between 11.0 and 14.2% of the standard length, and a body width between 14.9 and 17.0% of the standard length is *Pangasius rheophilus.*
b. For a fish with a dorsal spine width between 5.4 and 10.4% of the head length, a head length between 21.3 and 28.8% of the standard length, a head width between 11.9 and 20.6% of the standard length, and a body width between 16.5 and 21.4% of the standard length. See **9**.

9 **a.** A fish with 16 to 24 gill rakers on the first branchial arch is *Pangasius nasutus.*

b. A fish with 27 to 39 gill rakers on the first branchial arch is *Pangasius djambal.*

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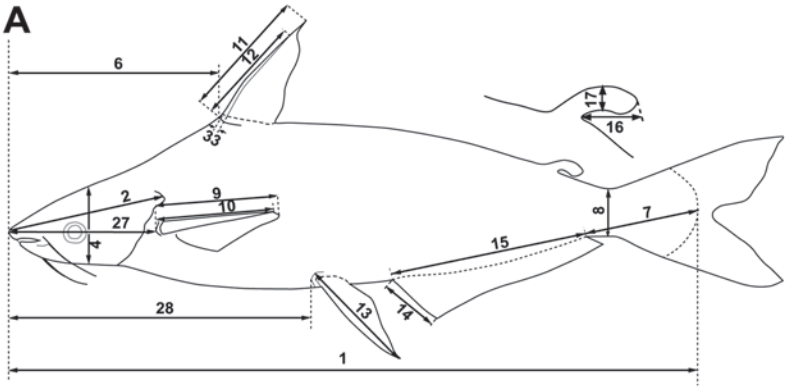
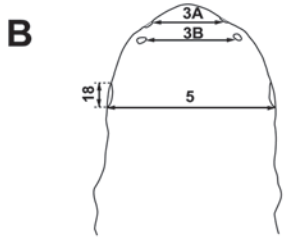
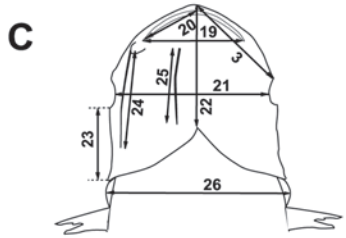
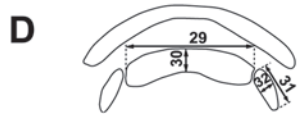




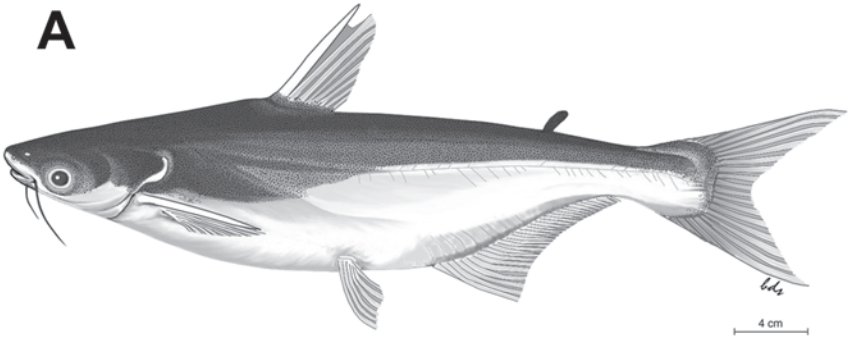


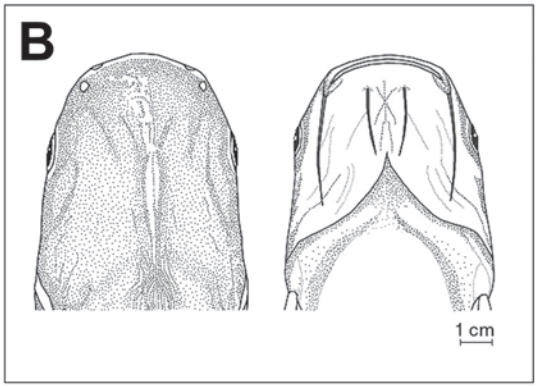


1. Standard length;
2. Head length;
3. Snout length;
3A. Anterior snout width;
3B. Posterior snout width;
4. Head depth;
5. Head width;
6. Predorsal length;
7. Caudal peduncle length;
8. Caudal peduncle depth;
9. Pectoral fin length;
10. Pectoral spine length;
11. Dorsal fin length;
12. Dorsal spine width;
13. Pelvic fin length;
14. Anal fin height;
15. Anal fin length;
16. Adipose fin height;
17. Adipose fin width;
18. Eye diameter;
19. Mouth width;
20. Lower jaw length;
21. Interorbital length;
22. Distance snout to isthmus;
23. Postocular length;
24. Maxillary barbel length;
25. Mandibulary barbel length;
26. Body width;
27. Prepectoral length;
28. Prepelvic length;
29. Vomerine toothplate width;
30. Vomerine toothplate length;
31. Palatine toothplate length;
32. Palatine toothplate width;
33. Dorsal spine width.

Plate I.1.

Measurements taken on *Pangasius* specimens (Gustiano, 2003).





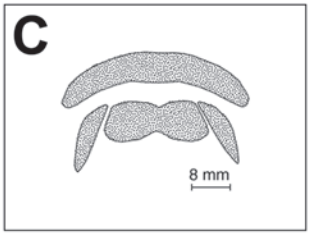


Plate I.2.

Pangasius djambal description.

A. Lateral view of the body (IRD-68, 418 mm SL); **B.** Dorsal (left) and ventral (right) view of the head; **C.** Premaxillary (above) and vomerine (below) toothplates of the same specimen.

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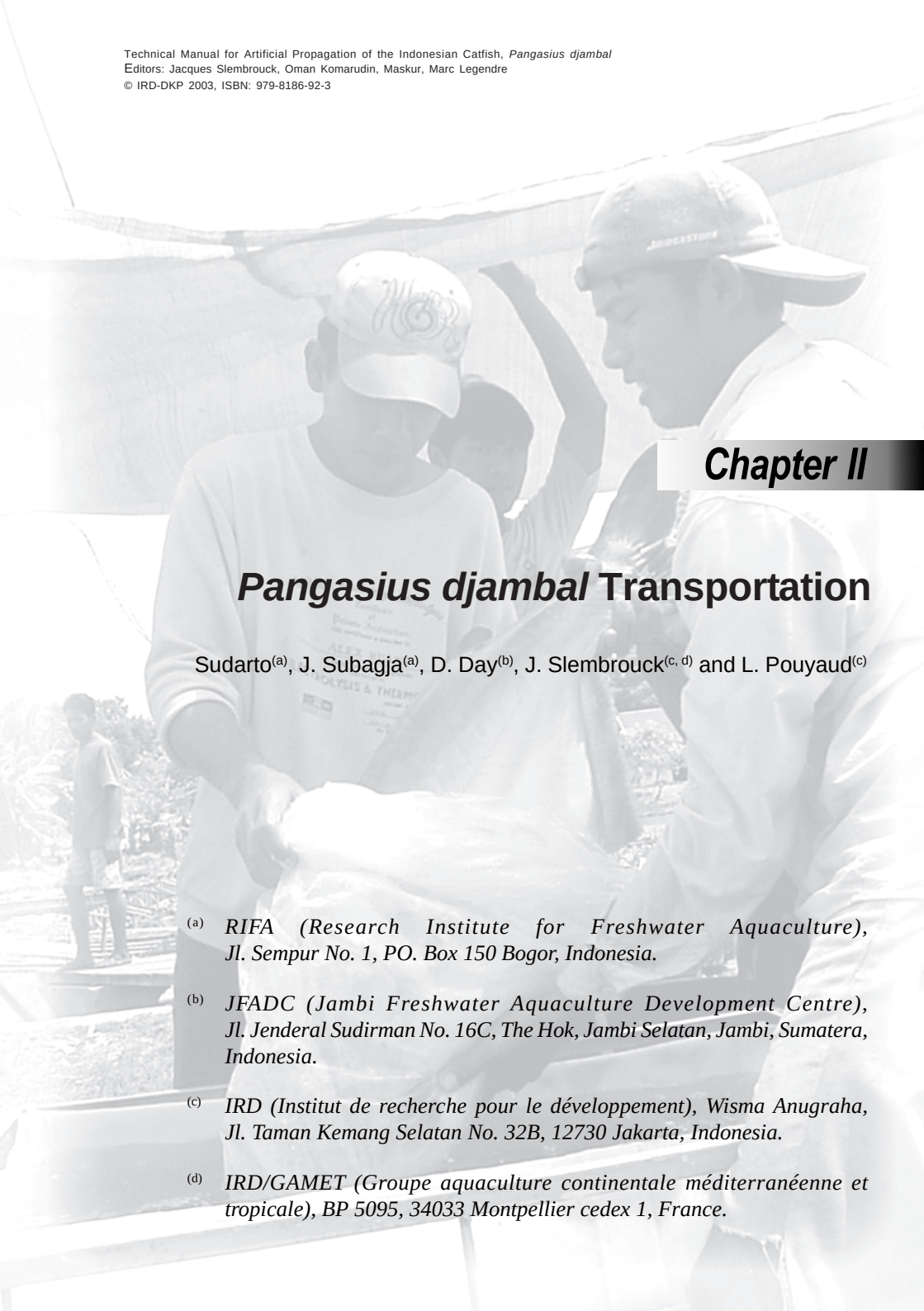

Chapter II

Pangasius djambal Transportation

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Building up a stock of sexually mature fish is a prerequisite for fingerling producers to start the process of artificial propagation. Broodfish can be caught from the wild or transported from farm to farm.

As *Pangasius djambal* is a new candidate for aquaculture, it is still difficult to find broodfish from farmers. Nowadays the two following options can be considered to obtain young *P. djambal* brooders:

- capturing fish from the wild;
- transporting young fish from JFADC (Sumatra) or RIFA (Java). These two governmental stations have bred young brooders that could be made available for farmers wanting to start culture of *P. djambal*.

In both cases, getting broodfish need preparation in order to transport fish in good condition. Recommendations for obtaining wild fish and some transportation techniques successfully used for different sized specimens of *P. djambal* are presented in this chapter.

P. DJAMBAL FROM THE WILD

Surveys and field studies should be conducted first to evaluate the possibilities of collecting *P. djambal* from the wild. The presence of fishermen and of facilities to keep fish alive near the sampling location are determinant.

The first young *P. djambal* brooders used by the "Catfish Asia Project" team at the RIFA or JFADC stations originated from the Indragiri River (Riau, Sumatra) where fish were caught using gill nets or hooks. In this river fishermen generally catch fish of 50 – 1000 g body weight, and then rear them in wooden cages using cassava as the main feed source until they reach marketable size.

After adaptation to culture conditions in wooden cages, about a hundred of these fish, weighing between 200 to 1100 g each, were bought and collected for further activities (Legendre *et al*., 2000). It was necessary to transport them for 12 hours by road and airplane from the origin to the final rearing destination. Due to appropriate methods and special care, this operation could be achieved without any fish mortality (Sudarto and Pouyaud, 2000).

PREVENTION, CONSTRAINTS AND ADVICES

General prevention

For any kind of fish transportation, the following main safety precautions

should be considered:

- fish should be in healthy condition before transportation; injured fish could die during transport leading to water spoilage and mortality of other fish;
- fish should be starved for 24 to 48-h prior to transportation in order to avoid waste that can spoil water and cause mortality;
- transport containers should be filled with clean water;
- water used for transportation should be at the same temperature as the initial rearing water;
- large variation of temperature should be avoided;
- during transportation, the water should be oxygenated.

Antibiotic use

Farmers generally use antibiotic bath during fish transportation in order to prevent development of bacteria. However, as explained in Chapter VIII, antibiotic should be used at the right dose and during a period of time long enough to ensure the total elimination of bacteria. When these rules are not followed, bacteria may become resistant to the drugs.

In the case of transportation, this rule is not respected because antibiotic treatment lasts a maximum of 48-h, corresponding to the maximum transportation time.

Therefore, it is strongly recommended to use disinfectant applied at a low dose instead of antibiotic during transportation.

P. djambal specific constraints

- this species has very sharp spines (first ray of the dorsal and two pectoral fins);
- fish are difficult to handle when their individual size exceeds 1 kg;
- they are sensitive to injury, which may cause high mortality during transportation;
- they need a supply of oxygen and a good water quality during transport.

Advice:

- the dorsal and pectoral spines of *P. djambal* of 200 g and over can easily tear plastic bags used for transportation, the risk increasing with the size of fish. To avoid this problem one of the following procedures should be considered:
    - ✓ insert a thick transparent plastic sheet between two plastic bags in order to prevent the outer one from being damaged by the sharp spines. The type of thick plastic sheet generally used in Indonesia for table cloth is very convenient for this purpose;
    - ✓ insert sharp spines in a rubber pipe (Plate II.1).

- air transportation needs special packaging adapted to the air line's regulation. It is important to note that the maximal authorized box size could differ from one company to another;
- road transportation during the night is recommended in order to avoid increasing water temperature due to sunlight exposure of fish containers.

TRANSPORTATION IN PLASTIC BAG WITH OXYGEN

Air transportation

As mentioned previously, air transport needs some specific preparation. Even if fish are transported by truck from rearing structures to the airport, air packaging must be ready from the start in order to avoid supplementary handling of fish.

The closed system packaging describe below was used for fish of less than 1 kg body weight. These fish were transported by truck and plane for 12-h, including the process of packaging. This technique resulted in a 100% survival rate after transport and no mortality was observed in the month following release of fish in a new rearing pond.

Packing

After inserting a transparent plastic sheet of 200 µm of thickness between two plastic bags, each double plastic bag is placed in a Styrofoam box and filled with 15 L of river water. Then, 1 to 5 fish for a maximum weight of 1 – 2 kg are placed in the bag, depending on the weight of the fish. Each bag is inflated with oxygen under pressure and closed tightly (Plate II.2), and each box placed again inside two layers of plastic bag to prevent oxygen leaking if inner plastic bags are perforated. This packing is finally placed in a cardboard box closed firmly then covered again with two layers of plastic bag to prevent water leaking if the Styrofoam box is broken.

Road transportation

Broodfish

Similar techniques were used to transfer sexually mature or immature *P. djambal* by land with a double plastic bag filled with water and oxygen under pressure as above. However, no thick plastic sheet was used because fish sharp spines were inserted in a rubber pipe as illustrated in Plate II.1 in order to avoid puncturing the plastic bag.

After inserting double transparent plastic in the plastic sackcloth (food bag) or in a Styrofoam box (Plates II.1 and II.2), each double plastic

bag was placed and filled with clean water up to the gills of the fish. Then 1 to 4 fish were added for a maximum of 8 kg of fish per bag. Each bag was filled with oxygen under pressure and put in a car.

This technique was used with success (100% survival rate) for several trips that ranged between 4 and 12-h duration.

Juveniles

Another method, using cool water as a tranquilizer, was applied to transfer *P. djambal* juveniles (100 g average weight) from ponds to floating cages in a lake.

Fish were caught by net (5 mm of mesh size) in ponds and transferred to concrete tanks (2 x 4 m in size, about 1200 litre of water volume) for 2-day of starvation.

In order to tranquilize fish during the transport, the water temperature was decreased in a tank from 29°C to 20°C using blocks of ice. Before packaging, fish were anesthetized in this cool water tank (water depth 15 cm) until they become unconscious.

Then, fish were packed in double plastic bags placed in a Styrofoam box. Bags were filled with 10 L of cool water (20°C) to which two blocks of ice for a total weight of 1 kg were added. Styrofoam boxes were closed tightly in order to maintain water temperature between 22 and 24°C during the transport. Each bag contained 25 fish and was filled with 50% water and 50% oxygen.

The duration of transportation was about 6-h by truck and the number of fish transported was around 2000. The survival rate was 100% after transportation then 97% after 2 days from arrival. No further mortality was observed after these 2 days.

TRANSPORTATION IN PLASTIC OR FIBER TANK WITH OXYGEN SUPPLY

To reduce risk during long duration transportation, another system has been tested using large fiber tanks placed on a truck, filled with clean water and fitted with slow diffusion of pure oxygen. This technique was used by the JFADC for transporting or moving young broodfish of *P. djambal* for a long distance corresponding to about 42-h travel. After transportation, the survival rate was 100% and no mortality was observed after releasing fish in their rearing structure.

Method and management of transport

Three 1 m^3 plastic tanks were placed on a truck and almost filled to the brim to avoid splashing during the travel. An oxygen diffuser was placed on the bottom of each tank (Plate II.3).

Two days before transportation, fish were placed in a net cage implemented in a pond for fasting. Each transportation tank contained 25 fish of 1.6 kg average weight. Water was changed each 14-h and ice blocks were added when necessary to keep temperature between 27 and 30°C.

RELEASING FISH IN NEW REARING STRUCTURE

After release, fish should not be fed during 1 or 2 days, and then the behavior has to be observed carefully, checking for some abnormality or mortality. After the stress of the transportation, fish have to adapt to their new environment and present high risk of disease development.

Before releasing fish in their new rearing structure, special care should be given as follows.

General advice

Whatever the transportation method used, a period of adaptation should be respected to avoid stress:

- before opening the inflated plastic bags, place them floating in the final water in order to slowly equilibrate temperature between the water of transport and of the new rearing structure;
- after temperature equilibration, plastic bag could be opened and water from the new rearing structure mixed slowly with transport water;
- after this short period of adaptation, fish could be released delicately while checking their behavior.

Broodfish

Before release, each broodfish should be inspected carefully in order to detect injuries. Injuries should be disinfected with a external treatment (Betadine or alcohol) or bath (formalin, see Chapter VIII).

TRANSPORTATION EQUIPMENT

Materials for air transport

1 Rubber pipes (optional).
2 Plastic bags 80 x 100 cm.

3 Thick transparent plastic sheet 80 x 40 cm, thickness 200 µm.
4 Styrofoam box 35 x 40 x 60 cm (according to the airline restriction).
5 Cardboard box fitted to styrofoam box.
6 Oxygen.
7 Rubber band and adhesive tape roll.

Materials for road transport

1 Ice blocks.
2 Plastic bag 80 X 100 cm.
3 Styrofoam box (35 x 40 x 60 cm) or plastic sackcloth (60 x 90 cm).
4 Oxygen.
5 Rubber band.

Materials for road transport in fiber tank

1 Oxygen bottle.
2 Rubber pipes.
3 Oxygen diffusers.
4 Plastic or fiber tank.
5 Ice blocks.
6 Rubber band.

REFERENCES

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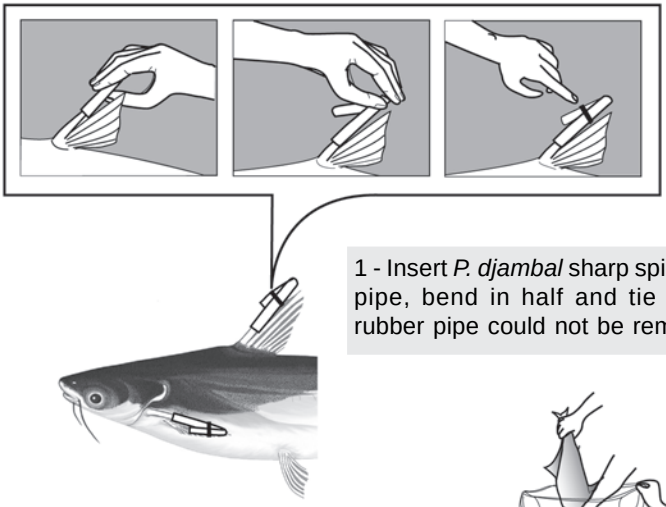
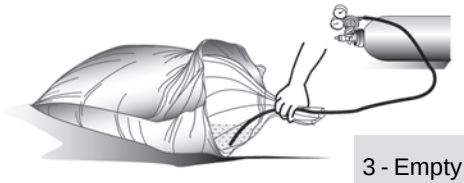
Sudarto and L. Pouyaud, 2000. Mengangkut ikan berduri, calon induk patin local yang aman. *Warta, Penel. Perik. Indonesia,* 6: 22-24.



1 - Insert *P. djambal* sharp spines in a rubber pipe, bend in half and tie to make sure rubber pipe could not be removed.

2 - Fill a double plastic bag fitted into sackcloth with clean water and place the fish delicately inside.



3 - Empty out air from plastic bag and replace it with pure oxygen. When lay bag down, fish head should be covered with water.

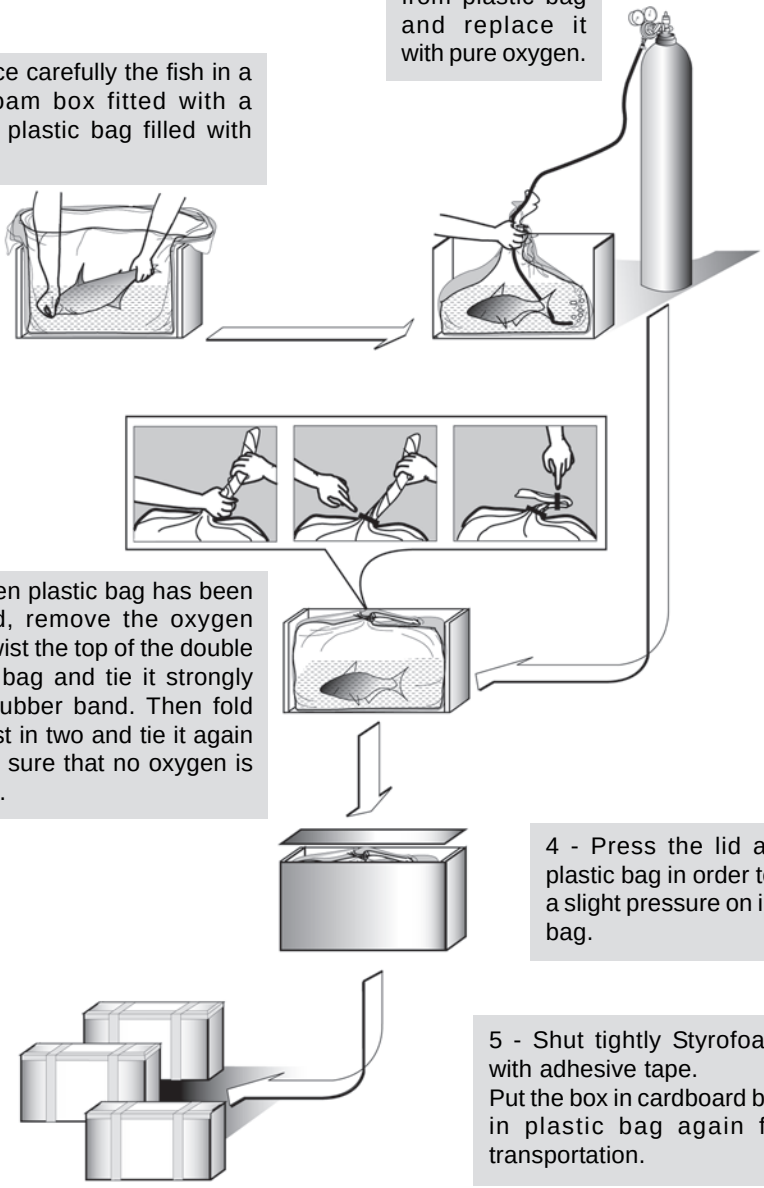
4 - Plastic bag is tied as shown in Plate II. 2.

Plate II.1.

Packing in plastic bag with oxygen.

1 - Place carefully the fish in a Styrofoam box fitted with a double plastic bag filled with water.

2 - Empty air from plastic bag and replace it with pure oxygen.



3 - When plastic bag has been inflated, remove the oxygen pipe, twist the top of the double plastic bag and tie it strongly using rubber band. Then fold the twist in two and tie it again making sure that no oxygen is leaking.

4 - Press the lid against plastic bag in order to exert a slight pressure on inflated bag.

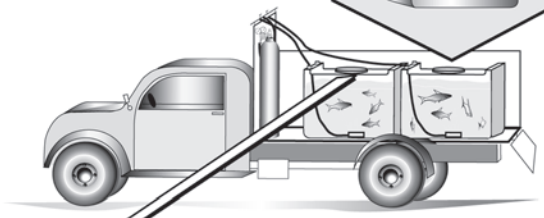
5 - Shut tightly Styrofoam box with adhesive tape.
Put the box in cardboard box and in plastic bag again for air transportation.

Plate II.2.

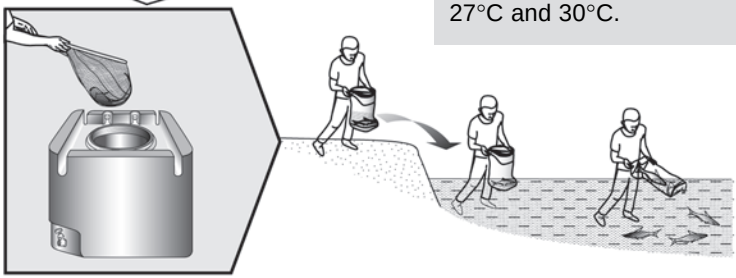
Packing in Styrofoam box with oxygen.

1 - After 24-h of fasting each fish is delicately netted and individually placed in a plastic bag filled with water.

2 - Fish are transferred to transportation tank almost full to avoid splashing during travel.



3 - Broodfish in tanks with oxygen supply. Ice blocks could be added to keep water temperature between 27°C and 30°C.



4 - After transportation, fish are delicately caught and placed in plastic bag as above.

5 - Release fish when water temperature in the bag has been equilibrated with that of the new rearing water.

Plate II.3.

Transportation in plastic or fiber tank with oxygen supply.

Technical Manual for Artificial Propagation of the Indonesian Catfish, *Pangasius djambal*
Editors: Jacques Slembrouck, Oman Komarudin, Maskur, Marc Legendre

Chapter III

Broodstock Management

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Unpredictable and variable reproductive performance is an important limitation for the successful mass production of fish juveniles (Izquierdo *et al.*, 2001). It is known that results of artificial propagation depend largely on healthy condition of the breeders. Broodstock management is clearly one of the key factors for successful seed production.

This chapter gives information on rearing conditions, zootechnical performances, methods of broodfish management and assessment of sexual maturity. These methods, used successfully by the team of the "Catfish Asia" program, were developed for *P. djambal* from juveniles caught in the wild and reared in captivity up to their full sexual maturation. As *P. djambal* was bred in culture conditions for the first time in 1997 (Legendre *et al*., 2000), the results are based on 6 years of working experience on this species.

REARING STRUCTURE, STOCKING DENSITY AND SEX RATIO

From their natural habitat (Indragiri River, Sumatra), *P. djambal* specimens were temporary stocked by anglers in floating cages then transferred to earthen ponds. Wild *P. djambal* has been rapidly and successfully adapted to these rearing structures.

After 6-year follow up, these new cultured fish showed a very high survival rate, a fast growth and reached full sexual maturity naturally in ponds.

A very good gonad development was observed also for both adults and premature fishes transferred from ponds to floating cages in a river of the central part of Sumatra. All together, these performances demonstrate a good adaptation of *P. djambal* to captivity and indicate that environmental conditions prevailing in the rearing structures meet the requirements of this species for growth and sexual maturation.

Characteristics, advantages and disadvantages of ponds and floating cages

The characteristics of rearing structures used for *P. djambal* culture and the range of variation of the main environmental factors observed during the whole rearing period are presented here.

Ponds

The ponds used were man-made with four concrete walls and an earthen bottom. They were of 200 m² with 1 m depth at the Sukamandi station (Java) and 600 m² with 1.8 m depth at the JFADC station (Sumatra).

The supply of water depended on season and water shortage was sometimes observed for 1 or 2 months during the dry season.

Parameter	Range
Stocking density	0.1 – 0.8 fish per m²
Sex-ratio	1 / 1 males to females ratio
Dissolved oxygen	0.1 – 15 mg.L^{-1}
Temperature	27 – 32°C
Conductivity	19 – 191 µS
pH	5 – 9.7

Table III.1.

Range of fish stocking densities and environmental parameters observed during rearing of *P. djambal* broodfish in earthen ponds.

Figure III.1.

Fishponds at the Sukamandi station (RIFA).

Advantages

- suitable for flat lands;
- can be isolated in case of external pollution;
- natural production increases fish yield.

Disadvantages

- possibility of water renewal depends on the season;
- strong variations of water quality (pH, dissolved oxygen, etc.) between day and night;
- need for regular clean out of silt.

Floating cages

The floating cages were (surface: 6 m², depth: 1.5 m) were built of wood according to the practice in Jambi region (Sumatra). These structures were settled in current to maximize water exchange between river and inner part of cage.

Parameter	Range
Stocking density	1.25 fish per m²
Sex-ratio	1 / 1 males to females ratio
Dissolved oxygen	5.9 – 8.1 mg.L^{-1}
Temperature	25 – 31°C
Conductivity	35 – 75 µS
pH	6 – 7

Table III.2.

Fish stocking densities and environmental parameters observed during rearing of *P. djambal* broodfish in river floating cages.

Advantages

- small volume and high fish stocking densities;
- inexpensive and simple technology;
- easy to manipulate and manage.

Disadvantages

- risk of fish escape in the river;
- risk of unexpected water pollution in the river.

Figure III.2.
Floating cages in the Batanghari River (Sumatra).

FEEDING

Feed quality and quantity

An appropriate food supply is required for maintaining broodfish in good health. It is well known that food restriction or deficiency in essential nutrients can affect growth and gonad maturation.

P. djambal is susceptible to high fat deposition, which may be unfavorable to gonad development as has been observed for other pangasiid catfishes in Vietnam (Cacot, 1999). It is recommended to rear them on a protein-rich diet with an adequate feeding rate.

P. djambal broodfish can be fed as follows:

- 35% crude protein pelleted feed;
- daily feeding rate depending on fish size (Table III.3).

Table III.3.
Daily feeding rate for *P. djambal* according to its mean body weight.

Body Weight	Daily feeding rate
500 g – 1000 g	2.0%
1000 g – 2000 g	1.5%
> 2000 g	0.8 – 1%

In certain locations or for various reason (cost, supplier, etc.), it is not always possible to obtain and to distribute a high protein diet. Thus, in order to maintain an equivalent level of crude protein, it is recommended to revaluate the ration as detailed below.

Calculations

1) Protein contained in Feed 1 / Protein contained Feed 2 = Protein ratio

35% / 25% = 1.4

2) Protein ratio x Daily feeding with Feed 1 = New daily ration

1.4 x 0.8% = 1.12%

The new daily feeding rate is 1.12% of the total biomass with the Feed 2.

Type of feed	Daily feeding rate	Protein Content
Feed 1	0.8%	35%
Feed 2	1.12% (after calculations above)	25%

Table III.4.
Daily feeding rate for 2 different types of feed given to maintain daily crude protein allowance.

Feeding practice

Although, it is essential to consider the nutritional aspect, various factors such as physical characteristics of the feed, modalities and frequency of distribution, and regular revaluation of the distributed quantity also have an effect on broodfish condition.

- Feed characteristic: since *P. djambal* in captivity feeds on the bottom or in a dark part of water column, it is recommended to use sinking pellets rather than floating ones. The former has to be water-resistant enough to allow the fish to swallow them before desintegration.
- Frequency: 2 times per day and 6 days a week, 1 day of fasting per week is recommended.
- Modalities of distribution: the feed has to be distributed slowly in order to get fish used to artificial feed and allow all fish to eat. At the same time, farmers can observe fish behavior.
- Revaluation of the quantity: as the growth of *P. djambal* is very rapid, it is recommended to sample and weigh the fish every month in order to revaluate the feeding rate and obtain optimal growth and sexual maturation.
- Eliminate parasitic fish: undesirable fish present in the rearing structure could eat a significant part of the distributed feed preventing the broodfish from getting their full ration. To avoid this problem, all parasitic fish should be removed from the pond regularly.

HANDLING AND METHOD TO REDUCE STRESS

Methods used to reduce fish stress in aquaculture have been already well described in the literature, in particular by Woynarovich and Horvath (1980) and Harvey and Carolsfeld (1993).

However, it is important to note that stress resulting from capture and handling may affect gonad maturation and growth. It is known that stress may reduce feed intake, weaken the fish and finally affect the spawning success.

It is obvious that the sensitivity to stress is higher for fish originally caught from the wild, as may be the case for first stock of *P. djambal*, than for fish already domesticated. Domestication is a long-term methods to reduce stress and increase handling tolerance (Harvey and Carolsfeld, 1993). As has been the case for *P. hypophthalmus*, we can expect that sensitivity to stress of captive *P. djambal* will decrease within a few generations.

However, without waiting for domestication, some simple and preventive method can be used to reduce stress and should be rapidly integrated as routine procedures.

Feeding

Risk

Fed fish need more oxygen and are more sensitive to stress than fasted fish. Handling of fed fish can cause unexpected mortalities in the broodstock.

Recommendation

It is imperative that the day of sampling corresponds to a fasting day. It is strongly recommended to stop feed at least 24 hours before capture and handling.

Capture

Risk

During sampling, prolonged period in the net results in rubbing and stripping away the protective mucus of the fish. Overcrowding in the net can also cause injuries made by pectoral spines. As a result, fish could contract infection.

Recommendation

To avoid a long holding period (1 hour or more) in the net, small sized ponds can be used in order to reduce the total fish population, which in most cases needs to be caught at the same time. During sampling operations, the number of fish maintained in the pouch of the net should not exceed 50 individuals. In case of wounds, local disinfectant can be applied and fish released promptly.

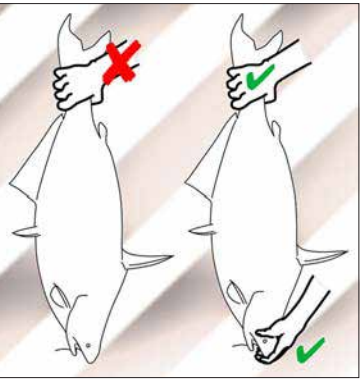
Handling

Risk

Catching fish roughly by the caudal peduncle (tail) is stressful and unsafe. Fish may struggle to escape and may be injured falling on the floor. Moreover this catching method, often observed on fish farms, results in stripping away protective mucus around the caudal peduncle.

Recommendation

In order to better support the weight of the fish and hold it safely, catch its head and caudal peduncle at the same time with care, then place the fish slowly in a moist towel, cover the eyes and carry it “like a baby” (Figure III.3). Plastic or moist material bags are widely used to handle brooders. This operation has to be repeated for each individual.



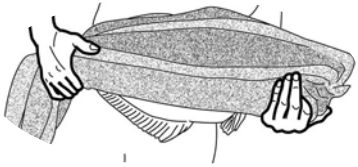


Figure III.3.
Handling recommendation for reducing the risks of stress and knocking.

Selection and sampling

Risk

In routine broodstock management, fish have to be weighed, measured or evaluated for their sexual maturity. During these operations, there are risks of struggle and injury for fish.

Recommendation

From the net, place carefully the fish, free of towel or bag, in an anesthetic bath for few minutes. When fish has calmed down, it becomes easy to manipulate without stress and risk.

Fish may die from staying a long time in the anesthetic bath. It is imperative that fish regain consciousness in water without anesthetic before being returned to their rearing structure. Otherwise, they can stay in the mud at the bottom of the pond and die by suffocation.

Anesthetic use

Two anesthetics were tested on *P. djambal*,

- MS222® (*tricaine methane sulfonate*) at 50 – 100 ppm;
- *2-phenoxyethanol* at 300 – 400 ppm.

The previous doses are given for fish above 2 kg body weight. *2-phenoxyethanol* is also used as an anti-bacterial and anti-fungal bath.

Anesthetic has to be carefully mixed with the water of the tank before placing fish in the bath.

As the effect of anesthetic depends of species, size and temperature, the applied dose may be excessive. The fish behavior in the anesthetic bath should be constantly observed so they can be removed in due time if necessary.

General handling precaution

- Fish should be handled after a short fasting period (24 h).
- Fish should be handled with care.
- Fish should be wrapped in a moist towel before handling.
- Never throw fish into water of rearing structure; place them back gently.
- After anesthesia, give the fish enough time to recover before releasing it.

TAGGING METHODS

Proper management of broodstock requires individual identification of brooders:

- to follow the livestock;
- to record individual events;
- to plan future induced breeding;
- to avoid multiple injections of the same fish;
- to identify and record the best brooders;
- to avoid inbreeding.

It is clear that tagging broodfish presents many advantages and facilitates broodstock management. Fish culturists, from simple to sophisticated ones, have used several different marking or tagging methods. Two of these techniques were tested and developed in *P. djambal*; PIT tags and encoded color spots.

PIT tags

PIT (Passive Inductance Transponder) tags are internal tags relying on high-technology electronic methods. Although more expensive than others, this technique is widely used and presents many advantages:

- easy to implement;
- no tag rejection;
- unique individual code;
- easy to detect and read;
- not perishable.

Figure III.4.
Preparation of PIT tags.

Method of implantation

- after disinfection with alcohol, PIT tag is inserted into an adapted needle;
- PIT tag is implanted in the muscle close to the dorsal fin;
- PIT tag is automatically detected and its number read using a PIT tag reader.

Figure III.5.
Implantation of PIT tags.

Figure III.6.
Reading of PIT Tags.

Encoded spots

Figure III.7.
Dermojet filled with Alcian blue solution.

This marking method was initially developed and used by IRD for other catfish species (Slembrouck and Legendre, 1988; Hem *et al.,* 1994) and consists in individually tattooing blue spots on the skin of the fish belly. These blue spots correspond to 5 $g.L^{-1}$ aqueous Alcian blue solution injected using a Dermojet. This technique is cheaper than the PIT tag and easy to use but the Alcian blue spots are not permanent and tend to disappear with time.

Spots are placed in accordance with a number code (Plate III.1) and allow numbering around 1000 fish. On *P. djambal*, blue spots are visible for a period of 2 – 3 months. However, this is not a major constraint as it is easy and cheap to retag the fish with the dermojet when the blue spots become difficult to see.

Figure III.8.
Tattooing with Dermojet.

ASSESSMENT OF MATURITY

P. djambal males and females do not develop any external charateristic allowing easy distinction of sexes and of sexual maturation stage. Even when females show large and soft belly, it corresponds in many cases to the presence of perivisceral fat.

In this species, males could be identified only when sexually mature by emission of sperm upon hand-pressure onto the abdomen and females when oocytes (follicles) could be sampled by intra-ovarian biopsy.

Although oocytes (follicles) could be able to develop fully mature gonads in captive condition, *P. djambal* does not reproduce spontaneously in the rearing structure. A hormonal treatment is necessary to induce final oocyte maturation and ovulation.

Male

Assessment of sexual maturity of males is much easier than that of females and their maturation stage is determined according to the following scale:

0 Absence of sperm.

1 Presence of a little sperm after squeezing.

2 Noticeable emission of sperm with normal hand-pressure.

3 Abundant emission of sperm with slight hand-pressure.

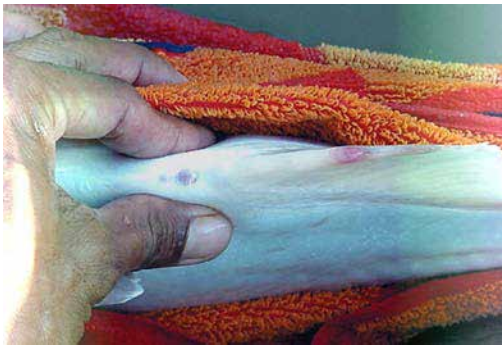


Figure III.9.
Assessment of male maturity.

Female

After intra-ovarian biopsy and clearing in Serra's fluid (30% formalin, 60% ethanol and 10% acetic acid), *P. djambal* oocytes never show migration of the germinal vesicle before the female has received an appropriate hormonal treatment. In contrast to some other fish species, nucleus position in the oocytes is not a criterion of maturity in *P. djambal*.

Modal diameter and homogeneity of oocyte diameter distribution remain the best criteria to determine readiness of *P. djambal* females.

BIOPSY PROCEDURE

Figure III.10.
"Pipelle de Cornier" used for *P. djambal* biopsy.

A polypropylene tube with rounded end originally developed as an endometrial suction curette, called "pipelle de Cornier" (Figure III.10) is inserted into the ovary through the genital opening and gonoduct (Figures III.11, III.12).

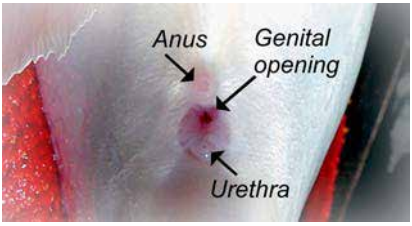


Figure III.11.
Detail of uro-genital papilla.

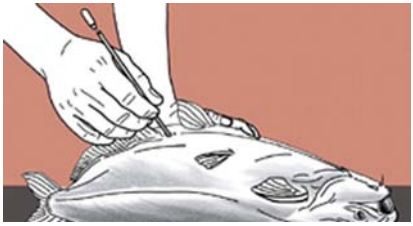


Figure III.12.
Intra-ovarian biopsy of anesthetized *P. djambal.*

A few dozen of oocytes are gently aspirated, scattered on a glass plate (Figure III.13) and measured with a micrometer under a binocular microscope at magnification of 25 X (Figure III.14) to determine the size distribution and modal diameter.

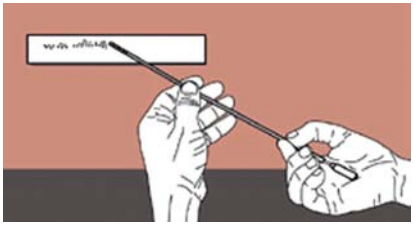


Figure III.13.
Scattering oocytes on a glass plate.

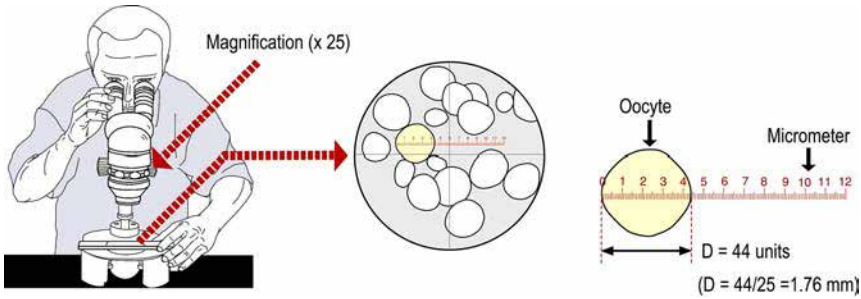


Figure III.14.
Measurement of oocyte diameter after intra-ovarian biopsy.

Biopsies made at regular intervals (e.g. monthly) allow following the evolution of sexual maturation of broodfish and selecting females ready for triggering ovulation.

Oocyte diameter analysis

Correct maturity assessment requires measuring diameter of a sufficient number of collected oocytes for each female. It is recommended to measure the diameter of at least 50 oocytes in order to obtain a representative histogram of size distribution and to determine the modal diameter (Plate III.2.). The latter criterion is considered as the best indicator of ripeness for this species.

Our investigations have shown that *P. djambal* starts to be sexually mature when modal oocyte diameter reaches 1.6 mm and reaches full maturity at modal diameter between 1.7 and 2.1 mm. No ovulation was observed for females with oocytes of modal diameter smaller than 1.6 mm. Oocytes of diameter over 2.12 mm become overripe in most cases and do not ovulate after hormonal treatment (Plate III.2).

The index sheet presented in Plate III.2 shows an example of recording oocyte distribution and of their size evolution after successive biopsies made at regular intervals on one *P. djambal* female. This type of follow-up of broodfish maturity clearly represents useful background information for the farmer and is a key to successful induced spawning.

In order to provide detailed information, each data sheet should specify:

- species, origin, rearing structure and individual identification number;
- date of sampling;
- diameter in mm of each measured oocyte, constructing size distribution histogram as shown in Plate III.2.

Visual aspect of oocyte sample

As the process of gonad maturation is gradual, oocyte size and appearance vary according to their development stage (see Plate III.2). For small-scale farmers, due to lack of equipment, the macroscopic aspect of oocytes is often the only way to evaluate fish maturity. In fact, with some experience, visual examination can also become an acceptable assessment of readiness.

In order to identify the final stage of gonad maturation, we give below some indications for evaluating readiness of *P. djambal* oocytes after sampling by biopsy.

- Color of the sample has to be homogeneous and ivory;
  - if the sample is clear with a few visible oocytes, it means that the gonad is not ripe enough;
  - if the bigger oocytes are clear (translucent), it indicates that over-ripening has started (process of atresia).
- Oocyte sample has to be almost dry or only slightly wet;
  - if the sample contains too much fluid, it suggests that many oocytes are already engaged in the process of atresia.
- An evaluation of the diameter with a centimeter ruler is strongly recommended. Oocyte size has to be homogeneous and the average diameter has to be 1.7 mm or greater;
  - heterogeneous oocyte size indicates that the final phase of gonad maturation has still not been reached.
- Oocytes must be easily separated from each other.

If these 4 points are observed, it means that the gonad has reached a stage of full maturity and that it will be possible to induce final oocyte maturation and ovulation by hormonal treatment.

GROWTH AND AGE AT FIRST MATURITY

Growth performances

The growth of *P. djambal* caught in the wild was followed over the long term in ponds using the rearing conditions (fish stocking density, feeding, etc.) described previously in this chapter.

Starting from a mean body weight of 550 g, *P. djambal* reached 6350 g after 990 days of rearing, corresponding to a mean growth rate of 6.0 g. day^{-1}. The shape of the growth curve was almost linear during the whole rearing period. This makes an important difference with *P. hypophthalmus* for which growth

drastically slowed down above 2 kg mean body weight (Figure III.15.).

Different experiments carried out with fish born in captivity showed that the growth of *P. djambal* was also significantly faster than that of *P. hypophthalmus* during the larval and fingerling stages (Legendre *et al*., 2000).

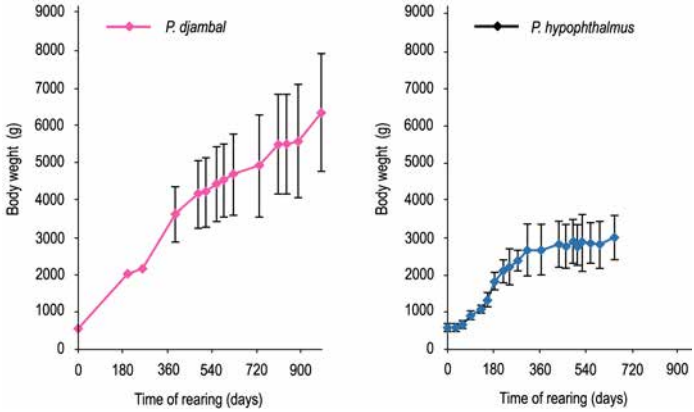


Figure III.15.

Growth of *P. djambal* and *P. hypophthalmus* reared in ponds up to the adult stage (mean ± sd).

Sexual dimorphism for growth

In *P. djambal*, the respective growth of males and females could be compared because all of them were all individually tagged (PIT tags). In this species, the females presented a much faster growth rate than males above 3 kg mean body weight (Figure. III.16.). In males, the lowering of growth corresponded to the period at which most of them reached full sexual maturity.

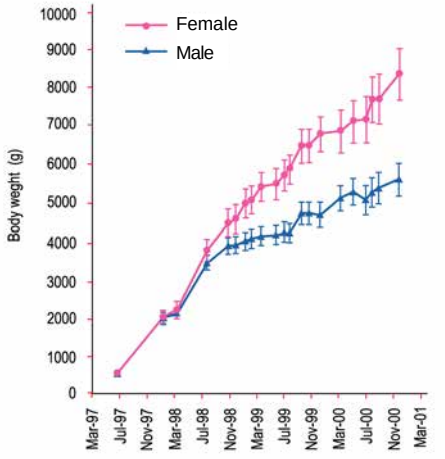


Figure III.16.

Growth of males and females of *P. djambal* in pond (mean ± sd).

Age at first sexual maturity

The age of *P. djambal* at first sexual maturity was estimated from fish groups reared in ponds. Observations carried out either for fish initially caught in the wild or for fish born in captivity led to similar conclusions.

Sexual maturity is reached much earlier in males than in females.

The first mature males were observed at the age of 11 – 12 months and more than 80% of males were fluent (oozing sperm) at two years of age. By this time, males born in captivity were of 2 – 3 kg individual body weight under our rearing conditions.

The first mature females (stage 4) were observed at the age of three years. Nevertheless at 4 years all female broodstock of *P. djambal* could be considered as fully mature. At 3 years, females born in captivity had reached 4 – 5 kg individual body weight in our rearing conditions.

SEASONAL VARIATION OF SEXUAL MATURITY

In *P. djambal*, once the first sexual maturity has been reached, sexually mature individuals (males at stage 2 or 3; females at stage 4) could be found all year long within the broodstock cultivated on Java and Sumatra Islands. However, clear seasonal variations in sexual activity were observed, showing a cycle repeated during four consecutive years of follow up. The proportion of mature females ranged between 50 and 100% during the period from September to March, corresponding to the rainy season, while it dropped to less than 30% between June and August, at the peak of the dry season. A similar tendency was observed in males with a slight lowering in the proportion of fluent fish during the dry season.

Seasonal variations in the quantity and quality of ova collected after induced breeding (see Chapter IV) were also observed. The mean fecundity and hatching rates were about two times lower during the dry season (April to August) than during the rainy season (September to March).

Even if larvae of *P. djambal* can be produced all year long, the most favorable period for production of fry lasts for about 7 months with a rise between November and January.

EQUIPMENT AND TOOLS

Feeding

1. Balance for weighing daily ration of feed (5 kg ± 10 g).
2. Plastic buckets for conveying and stocking feed for each rearing structure.

Fish capture

1. Fishing net fitted with floats on the top part and chain or lead on the lower part; bamboo sticks fastened at both ends; length and depth exceeding one of the dimensions of the rearing structure.
2. Moist towel for transferring broodfish from the net to tank.
3. Plastic bag for short distance transportation.

Handling fish

1. Disinfectant for treatment of the wounded broodfish.
2. Anesthetic; MS222® or 2-phenoxyethanol.
3. Tank for anesthetic bath.

Anesthetizing fish

1. Measuring container to determine the quantity of water in the previous tank.
2. Pocket calculator for calculating anesthetic dose.
3. Graduated syringe for accurate measurement of anesthetic dose.
4. Tank with clean water for fish recovery before return to its rearing structure.

Weighing fish

1. Balance for weighing broodfish (15 kg ± 50 g).
2. Index card for recording fish weight.
3. Plastic container placed on balance for weighing broodfish.
4. Pocket calculator for calculating the new daily ration.

Tagging broodfish

PIT Tag

1. Internal tags.
2. Syringe with adequate needle for inserting PIT tags.
3. PIT tag reader.
4. Alcohol 70% for tag and needle disinfection.

Encoded spot

1. Alcian blue powder.
2. Distillated water for diluting Alcian blue (5 $g.L^{-1}$).
3. Dermojet.

Assessment of maturity

1. Index card to record data for each broodfish (see Plate III.2).
2. “Pipelle de Cornier” or catheter made from adequate polyethylene tubing (3 mm external diameter; minimum 2 mm internal diameter).

3 Glass plate for scattering oocytes from “Pipelle” or catheter.
4 Low power stereo microscope (binocular x 25) with micrometer for accurate measurement of oocyte diameter.
5 Magnifying glass for visual examination of oocytes.

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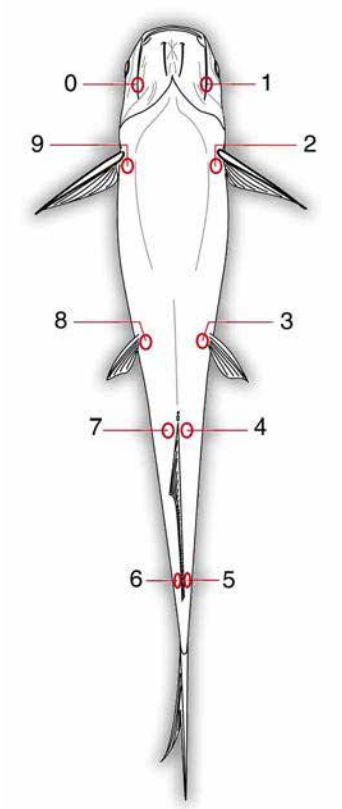
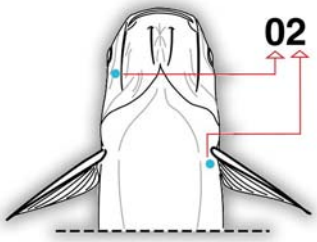
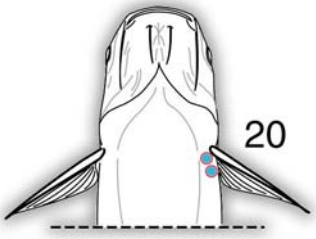


Number correspondence of the tagging spots as a function of their location on the fish body.
Spots are made with a 5 g.L^{-1} Alcian blue solution injected using a Dermojet.

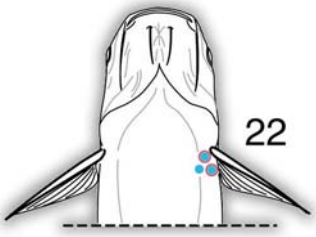
One spot at one place give the units (No. 0+2=02).



Two spots at the same place gives the tens (No. 20).



Three spots at the same place give tens and unit (No. 20+2=22).



Two spots at the same place give the tens, one spot at another place gives the units (No. 20+8=28).

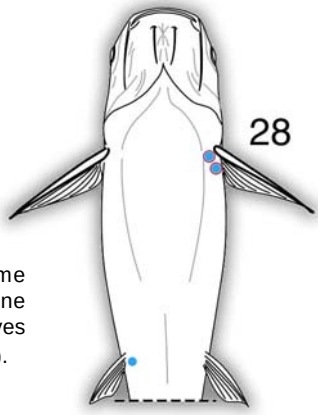
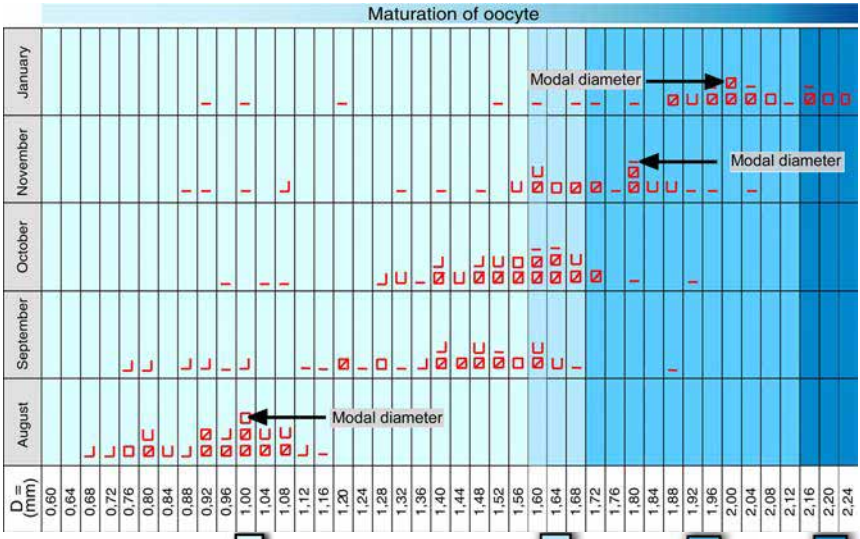


Plate III.1.

Tagging broodfish with encoded spots.
Examples of fish numbering by using different combinations of spots and location.

Species: *P. djambal* Origin: Indragiri River

PIT tag number: 425 916 0D2 8 Rearing structure: Pond 2



Maturing gonad: no ovulation after hormonal treatment.
Visual aspect: oocytes clear and difficult to separate from each over.

Final gonad maturation: 60 – 80% successful spawning after hormonal treatment.
Visual aspect: ivory color, more homogeneous diameter of bigger eggs, most of oocytes can be separated.

Full gonad maturation: 100% successful spawning after hormonal treatment.
Visual aspect: ivory color, homogeneous diameter and all oocytes can be easily separated.

Overripe oocytes: do not ovulate after hormonal treatment.
Visual aspect: mixed clear and ivory color, presence of intra-ovarian fluid and many degenerating oocytes.

Plate III.2.

Record of *P. djambal* oocyte development after successive sampling by intra-ovarian biopsy and measurement with a binocular microscope and micrometer.

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Chapter IV

Induced Spawning

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The method of artificial breeding described in this chapter is presented in chronological order to facilitate the identification of the different steps of the breeding procedure. The involved operations require a minimum of rigour for successful spawning. This is even more important as broodfish have to be handled several times for selection, injections, ovulation checking and stripping.

SELECTION OF MATURE FISH

The first step is to select mature fish in the best condition from the cultivated broodstock in order to obtain the best quality of spawning.

General preparation and recommendation

Practical experience has shown that for selection of brooders, it is preferable to catch a small number of fish at the same time in order to minimize handling and stress. The use of index cards is necessary to record bio-data for every broodfish to increase the knowledge on their biology and to improve the technique.

Material necessary for the selection should be ready (Equipment and tools, Chapter III) prior start of fish capturing. The general handling precautions should be respected (see Chapter III).

After capture, in order to make the selection easier, each fish should be:

- slightly anesthetized;
- weighed;
- evaluated for their sexual maturity (biopsy for females or hand-pressure onto the abdomen for males).

Estimation of male sexual maturity is quickly done (Chapter III) and could be realized shortly after anesthesia and before weighing. Thereafter, ripe males can be isolated directly for reproduction while other fish are returned to their rearing structure.

Oocyte measurement and observation take a few minutes after intra-ovarian biopsy (Chapter III, Plate III.2). Mature females should be placed in a small private cage or pen, then recaptured only for injection. Non selected fish are released in their rearing structure until another checking.

Note on the *P. djambal* readiness (details in Chapter III)

- **Male readiness** is determined by production of milt at stripping using slight hand-pressure on the abdomen (scale 3).
- **Female readiness** is determined after intra-ovarian biopsy, by a homogeneous diameter distribution of oocytes sampled and a modal

diameter >1.7 mm. The bigger oocytes should be ivory and easy to separate from each other. The presence of a noticeable quantity of ovarian fluid in the biopsy is generally indicative of an ongoing process of oocyte resorption (atresia). External appearance (soft abdomen, swollen genital papilla, etc.) is not reliable enough to judge readiness in *P. djambal* females.

How many males per female?

Practically, the quantity of sperm collected from one male is generally sufficient to fertilize all ova collected from one or two females. However, producing a new fish generation from only one couple leads to a reduction in the genetic variability of the strain (consanguinity). This may result in a decrease of zootechnical performance after a few generations as already observed in several fish species including catfishes (Agnese *et al*., 1995).

P. djambal is a newly cultured fish with very good natural performance and excellent potential for aquaculture in Indonesia. If farmers cannot retain natural performance of this strain, the future production of this species is in jeopardy.

In fact, there are two targets for breeding fish, the first one is to constitute a new generation of brooders and the second one is to produce fry to be grown for human consumption. Even if these targets are different, farmers expect zootechnical performance at least as good as that of the parents.

Ideally, for maintenance of maximal genetic variability of new broodstock and prevention of reduced performance, it is recommended to use at least 10 males for 10 females, the sperm of each male being used to fertilize separately ova from each female (Gilles *et al.*, 2001). After hatching, an equivalent number of descendants from each crossbred should be reared together to constitute the future broodstock. However, even if this pattern of breeding is respected, the overall genetic variability of the strain will decrease slowly (around 5%) after each crossbreeding (Chevassus, 1989). This means that it should be necessary to introduce wild blood (wild fish) every 3 generations in order to ensure large genetic variability of the cultivated strain.

For each reproduction cycle of fish for grow-out, we recommend using the sperm pooled from 6 to 10 males for fertilizing ova collected from all the females induced (generally 2 to 4).

HORMONAL TREATMENT AND PROCEDURE

Once the most mature fish are selected, isolated in their pens and the other broodfish released into their rearing structure, the process of induced breeding can be started.

The term "mature" female means that oocyte growth has been achieved and that final oocyte maturation and ovulation can be induced through adequate hormonal stimulation. Various protocols of hormonal treatments were tested for triggering *P. djambal* ovulation (Legendre *et al.*, 2000a). So far the best quality of gametes has been obtained with the use of the following hormonal treatment (Legendre *et al.*, 2002).

Female

The hormonal treatment corresponds to two successive injections:

- one priming injection of hCG (*human chorionic gonadotropin*) at a dose of 500 IU (international unit) per kg female body weight. To prepare oocyte response to the ovulatory treatment (second injection). This priming injection never leads to ovulation by itself;
- one injection of Ovaprim (commercial mix of GnRh and Domperidone)[1] given 24-hours after the hCG injection, at a dose of 0.6 $mL.kg^{-1}$, for triggering ovulation.

Male

In order to increase the quantity of collected semen and reduce its viscosity, males received a single Ovaprim injection of 0.4 $mL.kg^{-1}$ given at the same time as Ovaprim injection of females.

Induced breeding procedure

If the previous recommendations have been applied fish should have already been weighed and isolated in a safe housing structure. That leaves time to calculate the right hormone quantity according to recommended doses, then to proceed to the first hormone injection.

Calculation of the hCG quantity

Product presentation

hCG is available in dehydrated powder form in sterile ampoules of 1500 and 5000 IU. These ampoules are presented in a small box together with 1-mL ampoules of 0.9% saline solution (Plate IV.1). The cost depends on the dollar rate. In March 2003, one ampoule of 1500 IU cost 76,400 IDR (Indonesian Rupiah) and 1 ampoule of 5000 IU, 144,567 IDR.

[1]) 1 mL of Ovaprim® (Syndel Laboratories, Canada) contains 20 µg of GnRha and 10 mg Domperidone.

Example of calculation

In the following example (Table IV.1), we take the case of two females ready to induce, of 4.5 and 6.5 kg body weight respectively. The first step in the induced breeding procedure is to calculate the quantity of hCG (in IU) to inject into each female.

Table IV.1.
Calculation of the quantity of hCG to inject.

	Body weight kg	hCG dosage IU.kg^{-1} of fish	hCG needed IU
Female 1	4.5	500	4.5 x 500 = 2250
Female 2	6.5	500	6.5 x 500 = 3250
Total	11	500	11 x 500 = 5500

Evaluation of the optimal quantity of hormone

To inject these two females in the most economical way, we have to take into account the number of hCG ampoules needed, because an ampoule once opened cannot be preserved for more than a few hours. In order to reduce the quantity of wasted hormone and thus optimize the operational cost, we compare several possibilities of ampoule combinations to reach the optimal ratio of cost to dose (Table IV.2).

Table IV.2.
Optimization between quantity and cost for hCG injection.

	Option 1	Option 2	Option 3	Option 4
Ampoule 1500 IU	3	0	4	1
Ampoule 5000 IU	0	1	0	1
Total hCG obtained (IU)	4500	5000	6000	6500
Correspondence in IU.Kg^{-1} of fish	409.1	454.6	545.5	590.9
	Far from wanted dosage	Near from wanted dosage	Near from wanted dosage	Far from wanted dosage
Total hormone cost* (IDR)	229,200	144,567	305,600	220,967

* In March 2003.

Calculation of the quantity to inject

From the calculation (in Table IV.2), it appears that the best option is to use one ampoule of 5000 IU hCG to inject both fish. This corresponds to the lower cost for a dose of 450 IU.kg^{-1}, still close from the recommended dosage for priming injection. The 5000 IU of hCG can be dissolved in 1 mL of 0.9% saline solution.

The following calculation shows the proper volume of hCG to inject into each female:

Table IV.3. Calculation of the volume of hCG solution to inject for each female.

	Body weight kg	Proportion of solution for each female	Corresponding volume mL	Round figure mL
Female 1	4.5	40.9% (4.5 / 11)	0.409	0.4
Female 2	6.5	59.1% (6.5 / 11)	0.591	0.6
Total	11	100%	1	1

As a general rule, the quantity of hCG to dissolve in 1 mL of saline solution should not exceed 5000 – 6000 IU. If the required quantity of hormone exceeds this amount, the volume of solvent should be increased accordingly.

Calculation of the Ovaprim quantity

Product presentation

Ovaprim is available in liquid form in sterile bottle of 10 mL. Its cost depends on the dollar rate. In March 2003, one bottle of this imported product cost 210,000 IDR.

Example of Calculation

In the following example, we prepare the second injection of the ovulatory treatment to the same females which already received the hCG priming:

Table IV.4. Calculation of the volume of Ovaprim to inject.

	Body weight kg	Ovaprim dosage mL.kg^{-1} of fish	Necessary Ovaprim mL
Female 1	4.5	0.6	4.5 x 0.6 = 2.7
Female 2	6.5	0.6	6.5 x 0.6 = 3.9
Total	11	0.6	11 x 0.6 = 6.6

The Ovaprim volume needed could be directly aspirated from the bottle with a sterilize syringe (Plate IV.2) and the remainder could be preserved in a cool place (refrigerator) for a few weeks.

Preparation of the injection

As hCG is available in powder for dissolving in 0.9% saline solution and Ovaprim is available as a liquid, it is clear that the process of preparation for injection of these two hormones is not the same. Details for specific preparation of each are presented in Plates IV.1 and IV.2.

Nevertheless, common rules have to be respected:

- in order to treat fish with accurate hormone doses, the size of the syringe used should be appropriate to the volume of liquid to inject. For example, 0.9 mL of hormone preparation should be injected with a 1-mL syringe and not with a 10-mL syringe;
- in order to prevent the solution from leaking out from fish body after injection, it is recommended;
  - to use the finest possible needle long enough to allow "deep" intramuscular injection. We recommend using a needle size of 0.70 x 38 mm.
  - To split the injection at different locations of dorsal musculature if the volume exceeds 1 mL for a small female (less than 4 – 5 kg body weight) or 2 mL for a bigger fish. In practice, it is better to prepare in advance the number of syringes needed in accordance to the volume to inject.
- Some days after induced breeding, skin and muscle necrosis is sometimes observed at the injection place. This results generally from infection brought by contaminated syringe or needle, or by products which have expired. In order to prevent such situation, it is strongly recommended to disinfect syringe and needle with alcohol before use or to use sterilized new materials for each series of induced spawning. It is also recommended to use a new bottle or ampoule of hormone each time.

Injection procedure

So far, no scientific comparison has shown a better ovulation or spawning when fish were injected intramuscularly or intraperitoneally. In practice either means of injection could be chosen, each with its own advantages and disadvantages (Harvey and Carolsfeld, 1993). The most important point remains that the total amount of injected hormone reaches the gonads, via blood flow, to trigger the ovulation process.

For *P. djambal*, we chose to deliver the hormone intramuscularly below the dorsal fin (Figure IV.1). At this place, the muscular mass is thick enough

and a deep injection could be done, limiting risk for the hormone preparation to leak out through the injection hole.

Handling for injection without anesthesia can be tolerated by the fish as far as it stays safely in its housing structure. In order to prevent stress (see detail in Chapter III), handle the fish with care, then wrap it gently in moist towel and maintain it in water. Only the dorsal part of the fish should emerge from the water in order to facilitate hormonal injection (Figure IV.1).

Injection should be done gradually. To help the liquid to find its way between muscle fibres, wait a few seconds before withdrawing the needle slowly.

After checking that there is no hormone leaking out through the injection hole, the fish is released in its housing structure, and then observed for a while to make sure that its behaviour is normal.

For females, these operations have to be repeated for the second injection.

Figure IV.1.
Hormonal injection on *P. djambal*.

FINAL MATURATION AND LATENCY TIME

The time lapse between hormone injection and ova collection is a key factor in the success of reproduction techniques involving hormone-induced ovulation and artificial fertilization in fish. In pangasiids, this latency period is defined more precisely as the delay between the second (last) injection and stripping of ova.

The aim of the first injection (hCG) is to prepare the gonad, increasing oocyte sensitivity to the second stage of hormonal treatment (Woynarovich and Horvath, 1980; Cacot *et al.*, 2002). This first injection generally results in a slight increase in oocyte diameter while germinal vesicle (nucleus) of oocytes remains in a central position. The process of final oocyte maturation and then ovulation is triggered entirely by the second injection (Ovaprim).

Following Ovaprim injection, the process of oocyte maturation includes migration of the germinal vesicle to the edge of the oocyte and germinal vesicle breakdown (GVBD). After GVBD, the oocyte is mature and ready for expulsion from the follicle (ovulation); then it becomes an ovum, ready for

fertilization. When the process of maturation is not complete, it is generally impossible to collect ova; eventually a few follicles could be obtained by hand stripping but are unable to be fertilized. For farmers, observation of the nucleus state and of the different phases of its migration allow better understanding of why spawning sometimes does not happen.

In order to observe nucleus position, a few dozen oocytes sampled by intraovarian biopsy can be fixed in Serra's fluid (30% formalin, 60% ethanol and 10% acetic acid in volume) for 5 to 15 minutes. After this period, oocytes become translucent and the nucleus is visible. Even if the nucleus can be seen using a magnifying glass, the use of binocular microscope (x 25) is recommended for accurate observation. The different stages of nucleus migration until ovulation are detailed in Plate IV.3.

Latency Time for *P. djambal*

In fish, delayed collection of gametes after ovulation leads to overripening of ova which can result in low fertilization rates, increase in the number of deformed embryos and lowering of embryo and larvae viability. The delay of ova survival varies according to species.

The process of overripening occurs rapidly in *P. hypophthalmus* (Legendre *et al.,* 2000b*)*. In order to obtain the highest egg quality in this species, the best period to collect ova is of short duration (no more than 2 hours) and occurs just after completion of ovulation.

In *P. djambal*, our observations indicate that a delay of 1 or 2-h after first ova occurrence (see infra) was necessary for collecting eggs of best quality, leading to highest fertilization and hatching rates.

The latency period between the last hormone injection and ovulation was negatively correlated with water temperature (Legendre *et al.,* 2002*)*. The higher the water temperature, the shorter is the latency period. Latency time to collect ova in *P. djambal* varied from 13 to 17 h for water temperature from 27 to 30°C (Table IV.5). It could be estimated by the following relationship: $LT = 20279\ WT^{-2.15}$, with LT, latency time, and WT, water temperature.

Water temperature (°C)	Latency time (h)
27	17
28	15
29	14
30	13

Table IV.5.

Latency time between second injection and ova collection as a function of water temperature in *Pangasius djambal.*

GAMETE COLLECTION AND PRESERVATION

In order to check ovulation and collect ova in good condition, all materials for fertilization and incubation should be ready to use. It has to be cleaned and prepared in advance.

Fish farmers generally use direct fertilization, which consists of stripping males and spreading milt directly over collected ova. This technique entails some risk of activating spermatozoa by urine, lowering their fertilizing ability before they could be properly mixed with ova. In the testis, spermatozoa are immobile. Their movements are initiated once semen is ejaculated and diluted in water. However in *P. djambal* the viability of sperm is of very short duration (about 30 seconds) and once spermatozoa stop moving they lose their fertilizing ability. To prevent this problem, milt should be properly collected and preserved.

Sperm collection and preservation

A delay of about 10 hours from hormonal treatment was sufficient to enhance spermiation in *P. djambal*. In practice, in order to give enough time for collecting sperm from 10 males before start of female checking, sperm collection should be start 9 to 10-h after hormonal injection, i.e. 2-h minimum before checking female ovulation for the first time.

Before starting the collection of milt, most of urine should be expelled from bladder by pressing gently the ventral area just anterior from the genital papilla. Then the fish papilla area and hands of manipulator should be dried (Figure IV.2) in order to prevent eventual mix of sperm with water.

Figure IV.2.
Papilla area is dried with absorbent paper before collection of sperm.

Sperm is collected by gentle squeeze of the abdomen as done for the assessment of maturity (Chapter III). In order to prevent activation of spermatozoa in case of mixing with some urine, the sperm is diluted immediately in an immobilizing solution (Cacot *et al.,* 2003). The most effective way to shorten the delay between sperm stripping and dilution is to

Figure IV.3.
Accurate measurement of the 0.9% saline solution.

Figure IV.4.
Sperm is aspirated directly into a syringe containing saline solution.

aspirate the milt directly into a syringe containing saline solution (0.9% NaCl; Figure IV.3 and IV.4).

The ratio of 1 volume of sperm to 4 volumes of saline solution is used. This dilution rate allows good preservation of sperm quality (fertilization ability) during a period of at least 24-h when dilute sperm is kept in a cool place at 4 – 5°C (refrigerator or cool box). As the sperm preparation is used most of the time for fertilization within a period of 2 to 6 hours after collection that generally ensures the availability of sperm of good quality.

After collecting sperm, each male can be released in its rearing structure until another reproduction cycle. The dilute sperm from all males is preserved in a cool place until fertilization.

How much sperm is it necessary to collect?

The total quantity of sperm needed for fertilization varies according to the total weight of ova collected, which is related to the body weight of induced females. About 1 mL pure sperm (5 mL diluted) is generally used for fertilizing 100 g of ova. So far, the quantity of ova collected from one *P. djambal* female after induced ovulation has not exceeded 10% of its body weight. This observation can serve to estimate the maximal volume of sperm needed in a given reproduction trial, as done in the following example.

If the total weight of induced females is 14 kg, maximal weight of ova that could be expected to collect is 1400 g (10% of female biomass). In order to have enough sperm, it is recommended to fertilize these ova with 70 mL of dilute sperm (5 mL per 100 g ova), i.e. 14 mL of pure sperm collected.

Assessment of stripping time, ova collection and preservation

Depending on water temperature (see Table IV.5), the checking for ovulation has to be started 11 to 12-h after the Ovaprim injection in order to collect ova

at the decisive moment. Checking should be repeated one to three times at 2-h interval when females have not ovulated yet.

As noted previously, females should be handled with care, then wrapped gently in a moist towel, covering the eyes and maintaining the fish in water. The belly has to be maintained out of the water in order to get direct access to the genital papilla.

To check the ovulation gentle hand-pressure on the abdomen towards the genital papilla has to be done carefully and assessment of stripping time is determined according to the following procedure:

- only ovarian fluid or ovarian fluid with about a dozen oocyte are extracted by gentle squeeze of the gonad indicates that the fish is not ready for stripping. The female is released in its housing structure for a supplementary period of 2 hours, until the second checking;
- more than 10 – 20 ova with no or very little ovarian fluid are extracted (Figure IV.6); the female is released for 1-hour and re-captured for direct stripping. It will be the **decisive stripping time**;
- emission of ova in the net without hand-pressure indicates that the female has to be stripped directly. The optimal stripping time is probably past already.

Figure IV.5.

Female requires careful handling for checking ovulation.

Figure IV.6.

Decisive stripping time.

Ova collection

Female should be taken out of the water carefully and the general handling precautions respected (Chapter III).

Once a female is in position for stripping, the papilla area and hands of the manipulator should be dried. If ova come into contact with water for some time, the micropilar canal will close and spermatozoa will not be able to penetrate the ova for fertilization.

After taking precautions, the stripping could be started and ova collected in a dry plastic bowl. Gentle hand-pressure is applied on the abdomen towards the genital papilla. The right time for stripping is characterized by a soft belly

and a continuous jet of ova at each hand-pressure (Figure IV.7). Generally, easy stripping leads to good quality of ova.

When ova are difficult to extract from the gonad and the female presents a rather hard belly, it is best to release the fish in the rearing structure.

Hard stripping generally leads to the collection of a dry mass of ova mixed with some blood (Figure IV.8); the hatching rate obtained from such eggs is generally very low.

Figure IV.7.
Easy stripping

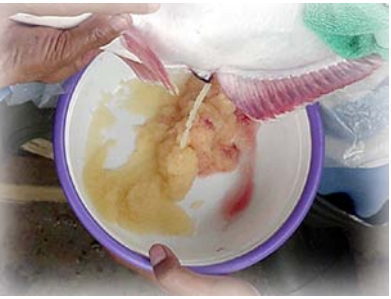

Figure IV.8.
Hard stripping

Our work experience has shown that excessive pressure on the belly could cause internal injuries and the fish could die.

However, when the stripping time is well evaluated and all the procedures described above respected, fatal situations occur rarely.

Before fertilization, collected ova could be preserved for more than 1-h when plastic bowl is covered, placed in shadow and protected against water splash. Indonesian farmers often immerse *P. hypophthalmus* ova in 0.9% saline solution for preservation before fertilization. However such practice should not be used for *P. djambal* ova. In fact, when placed in 0.9% NaCl solution for a few minutes, *P. djambal* ova could not be fertilized anymore, as was the case with freshwater.

To summarize, collected ova have to be preserved in a shady place without adding 0.9% NaCl solution and kept at a distance from any source of water.

EQUIPMENT AND TOOLS

Selection of mature fish

1. All equipment and tools listed in Chapter III should still be available.
2. The number of necessary small private cages or pens for isolating mature fish.

Hormonal treatment

1. Pocket calculator for calculating hormone doses.
2. Watch to note down injection time.
3. Thermometer for water temperature follows-up during latency period.
4. The necessary number of hCG ampoules or Ovaprim bottles.
5. Sterile ampoule or bottle of 0.9% saline solution (0.9% NaCl).
6. Sterile needle (size 0.70 x 38 mm).
7. Sterile syringe from 1 to 5 mL.
8. Alcohol for disinfecting reused needle or syringe.

Gamete collection and preservation

Males

1. Absorbent tissue paper for drying papilla area.
2. Bottle of sterile 0.9% saline solution.
3. Clean and dry syringe of 10 – 30 mL volume partly filled with saline solution for direct dilution of sperm during collection.
4. Plastic tubes with screw top for storing sperm preparation.
5. Small icebox with ice or refrigerator for preservation of sperm preparation.

Females

1. Absorbent tissue paper for drying papilla area.
2. Clean and dry plastic bowl for collecting ova.

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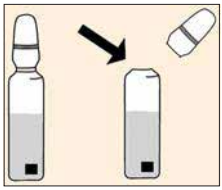
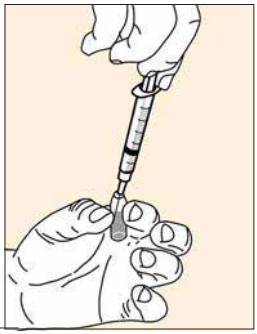
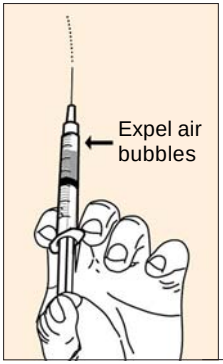


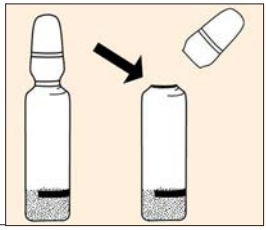


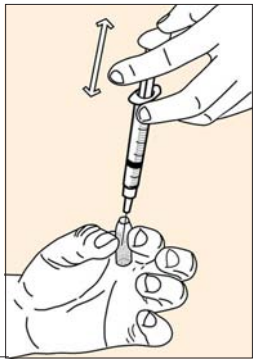
Open 1 ampoule of saline solution and collect the liquid in a 1 mL syringe.



Air bubbles should be expelled from the liquid then saline solution should be adjusted to 1 mL in the syringe using another ampoule of saline solution.

Open ampoule of hCG corresponding to the required quantity (see Table IV.2), then mix the saline solution with hCG powder, which is extremely soluble.

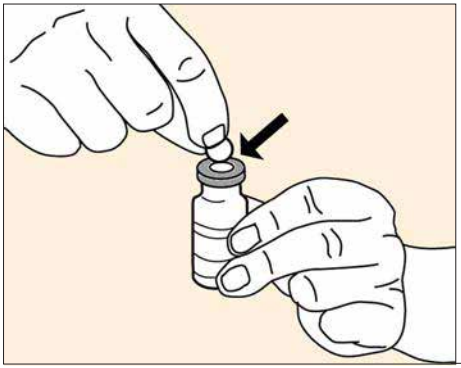




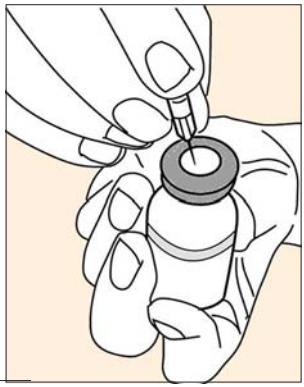
The 1 mL mixture of hCG and saline solution is delicately aspirated in 1 mL syringe. This hormone solution is ready to use, unless some air bubbles remain. In this case they should be expelled accurately with a minimum of hormone solution.

Plate IV. 1.

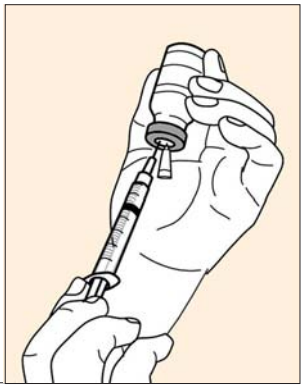
Procedure for hCG preparation.



Open 1 ampoule
of 10 mL Ovaprim.



Ovaprim liquid is thick.
To facilitate
the aspiration of
hormone,
it is recommended to
insert a second
needle through the top.

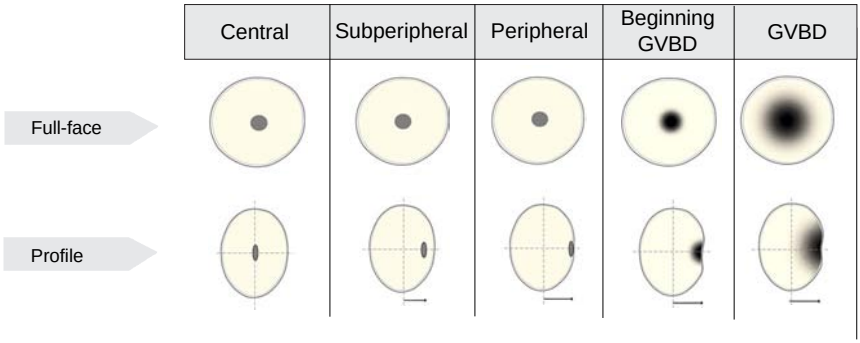


Necessary volume for one female is slowly aspirated in a 5 mL syringe. This hormone preparation is ready to use, unless some air bubbles remain.
In this case, they should be expelled again minimizing lost of hormone preparation.

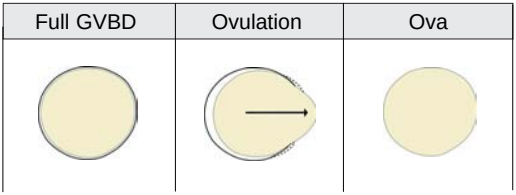
Plate IV. 2.

Procedure for Ovaprim preparation.

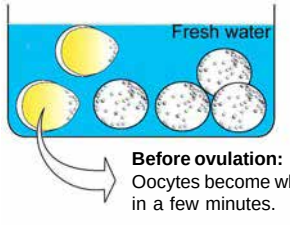
After 5 to 10 minutes in Serra's fluid the ova nucleus is visible. Then, it is possible to observe the difference stages of nucleus migration triggered by hormone treatment. Final oocyte maturation is described below.



Final oocyte maturation is followed by ovulation. After being released from their follicle, ova are ready to be collected and fertilized.

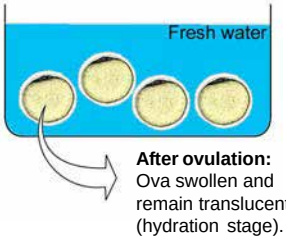


Determining the difference between oocytes in their follicle and ova with the naked eye is not so easy.



Before ovulation: Oocytes become white in a few minutes.

Practical advice: After immersion in fresh water the difference become clear.



After ovulation: Ova swollen and remain translucent (hydration stage).

Plate IV. 3.

Practical advice for observation of final oocyte maturation and for determining the difference between oocytes in their follicle and ova.

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Chapter V

Artificial Fertilization and Egg Incubation Techniques

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The artificial fertilization technique used for *P. djambal* is the dry method, i.e. the sperm is first spread over and mixed manually with collected ova.

To increase the fertilization rate it is recommended to divide collected ova in small batches of 100 – 200 g (100 – 200 mL) in plastic bowls. For fertilization, 5 mL of diluted sperm are poured over one 100-g (100 mL) batch of ova, then mixed delicately with a feather until the sperm is homogenously spread in the ova mass (Plate V.1). Spermatozoa activation is triggered by addition of freshwater. The ratio generally used is 1 volume of freshwater for 1 volume of ova. Freshwater should be added quickly in order to activate all spermatozoa at the same time. One minute of gentle stirring or mixing with a feather is recommended for good fertilization (Legendre *et al.,* 2000; Cacot *et al.,* 2002). Then eggs should be rinsed with clean freshwater to remove excess of milt before transferring them for incubation (Plate V.1).

Eggs of *P. djambal* are sensitive to mechanical shocks. As a consequence, shaking generally results in low hatching rates and increased proportion of deformed larvae. Egg manipulation should be done carefully to optimize hatching rates and numbers of normal larvae.

EGG INCUBATION TECHNIQUES

As is the case in *P. hypophthalmus*, eggs of *P. djambal* are sinking, spherical or slightly oval in shape and become sticky after contact with water. They adhere to each other or to any substrate via a sticky mucous coating covering their entire surface. Due to these characteristics, incubation techniques applied for *P. hypophthalmus* by fish farmers in Indonesia (Kristanto *et al.,* 1999) can also be used for *P. djambal* eggs.

Two of these incubation techniques have been tested and adapted for *P. djambal*; 1) incubation of eggs in monolayer in stagnant or running water and 2) incubation in running water funnels (MacDonald jars) after suppression of egg stickiness (Plate V.4).

As a general rule, it is preferable to incubate eggs in running water to remove continuously the waste materials produced by the eggs (NH_3, CO_2) and to maintain good quality and oxygenation of water. In most cases, running water also helps in limiting fungus development. Nevertheless, in Indonesia, many fish farmers commonly conduct incubation of fish eggs in stagnant water.

Incubation in stagnant water

Incubation in stagnant water is generally carried out in aquaria and does not need expensive equipment. This simple and cheap technology is the most widespread incubation system in Indonesia. However the drawback of this technique is the risk of water pollution by organic matter, particularly that accumulated from dead eggs. To limit this problem a limited quantity of egg should be incubated in each aquarium (maximum recommended of 100 eggs per litre). Therefore, for large scale production of fry, this technique requires a great number of aquaria, as well as a large space in the hatchery.

Structure preparation

In order to avoid a thermal shock, the aquaria must be filled with water well before receiving eggs to equilibrate temperature and increase dissolved oxygen concentration (by airstone).

Water used for egg incubation should be clear and treated with a disinfectant such as formalin to control fungus development (*Saprolegnia* sp.). In order to avoid toxic effects of the drug on the eggs, this treatment should be applied 12 h before placing eggs in incubation. A dosage of 10 to 15 $mL.m^{-3}$ formalin inhibits fungus development and disinfects incubating water without any risk for the eggs and young larvae.

Egg incubation

Homogeneous egg distribution in a monolayer at the bottom of the tank is decisive for the success of incubation. This allows each egg to be in water of good quality. In this way, healthy eggs are not affected by dead eggs starting to decompose.

After fertilization and rinsing off excess of milt, the aeration is stopped in the aquarium and eggs can be delicately spread out with a feather on the water surface. (Plate V.2). It is then recommended to mix eggs and water gently to obtain a homogeneous distribution of eggs in the aquarium. As eggs of *P. djambal* are sinking, the previous homogeneous distribution will allow the eggs to reach the bottom of aquarium in a monolayer (Plate V.3).

After some minutes and eggs have stuck to the glass, the aeration can be progressively opened again without disturbing the incubating eggs.

Incubation in running water funnel (MacDonald jar)

The MacDonald jars used at the RIFA and JFADC stations are funnel type with a spherical bottom made of fiberglass (Plate V.2). Farmers also use other materials such as glass, concrete, plastic and non-corrosive steel.

The MacDonald jar principle consists in keeping eggs in motion via a water inflow through a PVC pipe fixed into the funnel and reaching right down to

the bottom (Woynarovich and Horvath, 1980). It is generally connected to a clean gravity-flow water or a recycling water system, so this technique presents the advantages of occupying limited space in the hatchery and reducing fungus development (*Saprolegnia* sp.) on eggs during incubation.

After hatching, this system also facilitates sorting newly hatched larvae from white (dead) eggs and egg shells.

Removing the stickiness of eggs

After fertilization and before pouring them in the MacDonald jar, eggs have to undergo a treatment with clay to suppress their stickiness (Plate V.4). Actually, after mixing eggs and clay together, small particles of clay adhere to the egg sticky layer, covering their entire surface and preventing them adhering to each other or to any substrate. The suppression of adhesiveness allows keeping the eggs in movement in the water flow during the entire incubation time.

Preparation

The clay suspension is made of 1 kg of red clay (Latosol) in 2 litres of water. The procedure is as follows:

- red clay is cleaned of its impurity (leaf, pebbles, etc.);
- it is then mix progressively with boiling water (ratio of 1 kg for 2 L). Boiling water is necessary to kill microorganisms and parasites. After mixing, the preparation could be boiled again to ensure its sterilization;
- after cooling, the solution is sieved through a 700 µm mesh;
- the sieved mixture is kept in a plastic bucket fitted with strong aeration to obtain homogeneous mixing;
- the preparation is ready to use. If in excess after a given reproductive trial, the remaining clay suspension can be preserved frozen until another induced breeding. The clay suspension should be frozen in small volume to allow thawing according to the quantity needed.

Suppressing the stickiness

After mixing of sperm and ova for artificial fertilization (see above) excess milt should be drained off and replaced by clay suspension following the procedure below (Plate V.4):

- pour about 100 mL of clay suspension on 200 g of eggs;
- delicately mix the eggs and the clay using a feather until they do not adhere to each other, i.e. clay already covers all the sticky mucous;
- then the mixture is transferred into a net to remove the excess clay;
- after rinsing until clean water is obtained, eggs are transferred again to a plastic bowl filled with water;
- eggs are then ready to be placed in the MacDonald incubators.

Structure preparation

When MacDonald jars are connected to a recycling water system:

- a special procedure for first using recycling water system should be followed as described in Chapter VII (see "preparation of rearing structure"). In all cases, incubators must be filled with clean water and run long enough before receiving eggs for equilibrating temperature and reaching maximal level of dissolved oxygen;
- a preventive treatment of formalin at a concentration of 10 to 15 $mL.m^{-3}$ is also recommended in order to disinfect the incubation water.

When incubators are connected to gravity-flow water, water should be:

- free of plankton and waste;
- well oxygenated;
- at stable and adequate temperature (27 – 30°C);
- distributed at a constant flow.

Egg incubation

After suppression of their stickiness, a maximum of 200 g of eggs can be transferred delicately into each jar (capacity of 20 L). Before pouring eggs into their incubator, water flow should be stopped for a while in order to avoid loss of eggs through the water outlet. After eggs have sunk to the bottom, the water inlet can be opened slowly and adjusted for continuously keeping eggs in motion. During incubation, the adjustment of the rate of flow and the centering of PVC inlet pipe are essential for optimizing hatching rates (Plate V.5):

- insufficient outflow or bad centering of the inlet pipe may create a motionless mass of egg which could not be well oxygenated. This could result in mortality of a significant proportion of embryos due to anoxia, with dead eggs turning white;
- too strong outflow shakes up eggs excessively and risks impairing embryo development resulting in increased proportion of deformed embryos and larvae;
- good adjustment of water flow and centering of inlet pipe result in all the eggs getting slow and regular wave motion.

EMBRYO DEVELOPMENT AND HATCHING KINETIC

Embryo development

As the process of embryo development is not the main topic of the present work, we will limit this presentation to some important stages allowing

farmers to recognize them and correctly evaluate egg quality. The duration of incubation (from fertilization to hatching) depends upon water temperature, it decreases as temperature increases (Legendre *et al.*, 1996). The following description gives an example of development time for a mean incubation temperature of 29°C. Corresponding egg development illustrations are given in Plate V.6.

After contact with water, eggs are subjected to a rapid hydration leading to the formation of the perivitelline space. The egg mucus coat also swells in contact with water and becomes adhesive. At this stage, whether fertilized or not, the swollen eggs have a yellowish color and the animal pole is marked as a reddish-brown cap. However, fertilized eggs soon start to develop and the first cleavage (stage two-cells) becomes clearly visible 25 – 30 minutes after fertilization, followed by 4, 8, 16 and 32-cell stages (1.5 to 2 hours after fertilization). From the 32-cell stage, the egg is in morula stage during about 60 minutes and then cells become progressively smaller until the blastula stage (3 to 4 hours after fertilization). Shortly thereafter, the gastrula stage starts, the cell division progresses and cells progressively cover the yolk mass. The last step of the gastrula stage occurs about 12 hours after fertilization and is characterized by the closing of the blastopore.

Thereafter, i.e. 15 to 18 hours after fertilization the embryo appears in the form of a half ring with the head and the tail buds present at the two ends. Afterwards the first segments of the body become rapidly visible while the tail bud starts to grow longitudinally. The cardio-vascular system becomes functional and the embryo starts to twitch its tail occasionally. The first pigmented cells (melanophores) become clearly visible after 23 hours from fertilization and the movements of the embryo become more and more vigorous before hatching.

Differences between fertilized and unfertilized eggs

As noted earlier, once exposed to water, unfertilized eggs start the swelling process and their animal pole is marked by a reddish capsule as is the case for fertilized eggs. Moreover, incubating unfertilized eggs can remain translucent for several hours and can not be easily distinguished from fertilized eggs with the naked eye. It is only after 8 to 14 hours of incubation that most unfertilized eggs become opaque and whitish.

When observed with a low power stereomicroscope (magnification x 25), unfertilized eggs of *P. djambal* do not show any cell divisions. For an accurate and easier evaluation of fertilization rate, it is recommended to observe eggs between the “4-cell” and the morula stages; i.e. from about 30 min to 2 h after fertilization (Plate V.6).Estimation of fertilization rates should be done during these early development stages, because at following stages, i.e.

from late morula to early gastrula (between about 2 to 5 h from fertilization), fertilized eggs again become more difficult to distinguish from unfertilized ones.

Determining fertilization rate allows farmers to rapidly evaluate expected hatching rate from a given spawn. Actually, a low fertilization rate (< 30 – 50%) is generally indicative of poor ova quality when the ova are properly obtained respecting all rules for sperm conservation, ova fertilization and egg incubation. Due to abnormality of embryonic development and increased embryo mortality, low fertilization rates resulting from bad quality of ova generally lead to even lower hatching rates and high proportions of deformed larvae. In such case, it may be more advisable for farmers to clean off eggs and induce new broodfish in order to obtain eggs and larvae of better quality.

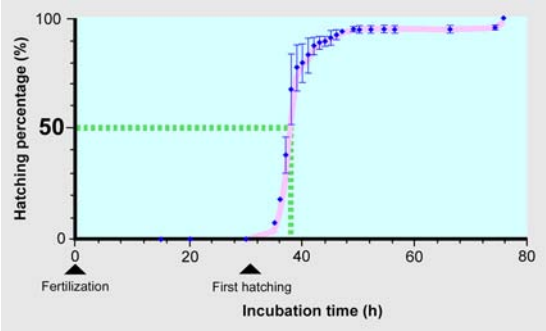
Duration of incubation

In *P. djambal*, as in other fish species, the duration of egg incubation is strongly dependent upon water temperature. Hatching time could be reached faster in warm water and retarded in cold water. At a water temperature of 29 – 30°C, larvae started to hatch by breaking out of the egg shell, about 33 to 35 h after fertilization (Figure V.1). Fifty percent of larvae were already hatched after 37 – 38 h; i.e. 2 – 3 hours after the beginning of hatching.

Larvae do not hatch in synchronization and the difference between the first hatching and the last hatching could be up to 40 h (Figure V.1). However, such long delay generally corresponds to hatching of deformed larvae and 9 to 10 h after the first hatching, more than 90% of larvae had already broken out of the eggshell.

In every case, the incubation temperature should be adapted to the species requirement to obtain the optimal hatching rate. For *P. djambal*, a water temperature of 27 to 30°C was shown to fall within a suitable range.

Figure V.1.
Hatching kinetics of *P. djambal* eggs incubated at a water temperature of 29 – 30°C.



HANDLING AND STOCKING NEWLY HATCHED LARVAE

After hatching, water in incubating structures is usually polluted by decomposing dead eggs and hatching wastes. Water begins to turn unclear, indicating that the environment could become toxic and dangerous for newly-hatched larvae.

To avoid a risky situation, it is necessary to quickly transfer larvae into "temporary" structures containing clean stagnant and aerated water. This could be aquaria (60 to 120 L) or small tanks. At this stage, oxygenated stagnant water is recommended because larvae cannot swim and a small current could be enough to squash them on the outlet net (Plate V.7).

This transfer could start as soon as possible after most larvae have hatched (e.g. 40 h after fertilization or 5 h after first hatching at 29 – 30°C).

"Temporary" structures must be ready long time enough before receiving the larvae to maximize dissolved oxygen concentration and equilibrate water temperature. It is important avoiding thermal shock or stress to larvae.

A few hours after hatching, larval behavior changes and normal larvae become more active and photosensitive (attracted by light). From this moment, it is relatively easy to concentrate normal larvae at one side of the aquarium or tank, and to separate them from those deformed and unable to swim. This operation should be repeated until no more normal larvae gather up. (Plates V.3 and V.7). As the first hatching occurred about 40 h before the last hatching, larvae obtained from a same group of eggs are not exactly at the same stage of development.

This step is important for beginning larval rearing in good condition because most abnormal larvae are doomed to die before 2 – 3 days of age and decomposing bodies can pollute the rearing environment, favoring bacteria and fungus development.

Technical guidelines

Before use, all the material should be cleaned, disinfected and dried.

- After fertilization and stocking eggs in incubation system, i.e. around 40 h (at 29 – 30°C) before collecting larvae, fill up "temporary" containers with clean freshwater and implement a strong aeration. This period is long enough to equilibrate oxygen and temperature;
- to avoid stress, before moving newly hatched larvae into the "temporary" containers, reduce aeration and control temperature. Water temperature

should be in the recommended range (see Chapter VI, Table VI.2) and close to that of incubation water (± 1°C);

- fill up a plastic bowl with clean water from the "temporary" tank and implement a gentle aeration, then catch larvae with care from the incubation structure with an adapted plankton net (80 µm) and transfer them slowly into the bowl (Plate V.7);
- when enough larvae have been caught, slowly empty out the bowl into a "temporary" container (Plate V.7);
- in "temporary" tanks, clean a part of the bottom by siphoning to remove white eggs and abnormal larvae. One to two hours after moving larvae, give some light on this clean bottom. Wait for about half an hour to concentrate normal larvae in the beam of light (Plate V.3 to V.7);
- while waiting for larvae aggregation, fill up a plastic bucket with clean water from the future rearing tank and implement a gentle aeration. Water temperature has to be in the recommended range of 27 – 31°C with no more than 1°C difference from water of the "temporary" containers. Siphon larvae slowly with a plastic pipe into the bucket. This operation has to be repeated until all normal larvae have been siphoned (Plate V.7);
- before transferring larvae in each rearing tank, their number has to be determined by counting or at least accurately estimated in order to adjust feeding and water renewal during larval rearing (Plate V.7).

EQUIPMENT AND TOOLS

Artificial fertilization

1. Clean and dry plastic bowl for dividing collected ova.
2. Clean and dry syringe of 10 – 30 mL capacity for evaluating volume of sperm collected.
3. Measuring cup or bowl for freshwater.
4. Chicken feather for mixing eggs, sperm and freshwater.
5. Clean freshwater for rinsing eggs and eliminating excess sperm after fertilization.

Incubation in stagnant water

1. Aquarium 60 x 50 x 40 cm.
2. Air blower with airstone in each aquarium.
3. Plastic bucket for filling aquaria.
4. Tank for storing clean water.
5. Chicken feather for dispersing eggs into aquaria.

Incubation in running water funnel

1. Recycling water system with bio-filter and mechanical filter or clean gravity water.
2. MacDonald jars installed as explained in Plate V.5.

Removing the stickiness of eggs

3. Sterilized clay suspension (1 kg for 2 L of water).
4. Plastic bowl and feather for mixing.
5. Small net and clean freshwater for removing excess clay.
6. Measuring cup or bowl for pouring the same quantity of eggs in each jar.

Handling and stocking newly hatched larvae

1. Aquaria with airstone and clean water.
2. Plankton net (80 µm mesh size) for catching larvae.
3. Plastic bowl for transferring larvae to the aquaria.
4. Flashlight for concentrating normal larvae in the beam of light.
5. Plastic pipe for cleaning the bottom of white eggs and deformed larvae and then siphoning normal larvae.
6. Plastic bucket for transferring normal larvae to their rearing structures.

Estimation of fertilization rate

1. A low power stereomicroscope (magnification x 25).

Water quality control (recommended)

1. Oxygen kit or oxygen measuring device.
2. Thermometer.

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Kristanto, A. H., J. Slembrouck, J. Subagja and M. Legendre, 1999. Effects of egg incubation techniques on hatching rates, hatching kinetics and survival of larvae in the Asian catfish *Pangasius hypophthalmus* (Siluroidei, Pangasiidae). In: *The biological diversity and aquaculture of clariid and pangasiid catfishes in Southeast Asia*. Proc. mid-term workshop of the «Catfish Asia Project» (Editors: Legendre M. and A. Pariselle), IRD/GAMET, Montpellier. 71-89.

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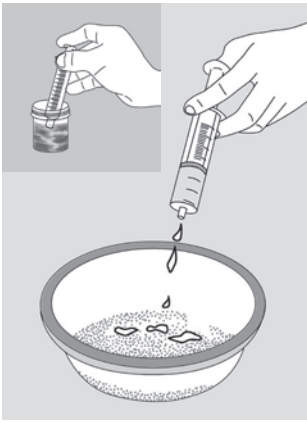
Legendre, M., L. Pouyaud, J. Slembrouck, R. Gustiano, A. H. Kristanto, J. Subagja, O. Komarudin and Maskur, 2000. *Pangasius djambal*: A new candidate species for fish culture in Indonesia. *IARD journal*, 22: 1-14.

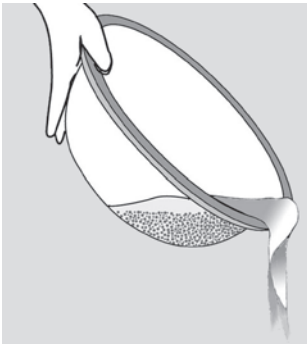
Woynarovich, E. and L. Horvath, 1980. The artificial propagation of warm-water finfishes – a manual for extension*. FAO Fish. Tech. Pap*., 201: 183 p.

To optimize fertilization rate, divide ova into small batches of 100 – 200 g (100 – 200 mL).



- Pour 5 mL of diluted sperm for 100 g (100 mL) of ova.
- Mix delicately until sperm is well spread.

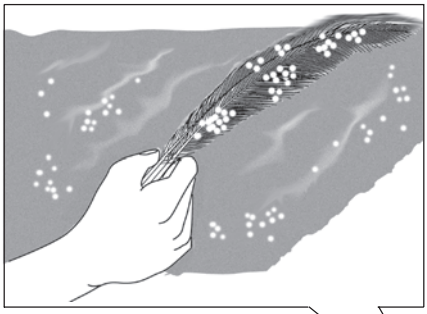
- Activate spermatozoa by addition of 1 volume of freshwater for 1 volume of ova.
- Freshwater has to be poured quickly to activate all spermatozoa at the same time.



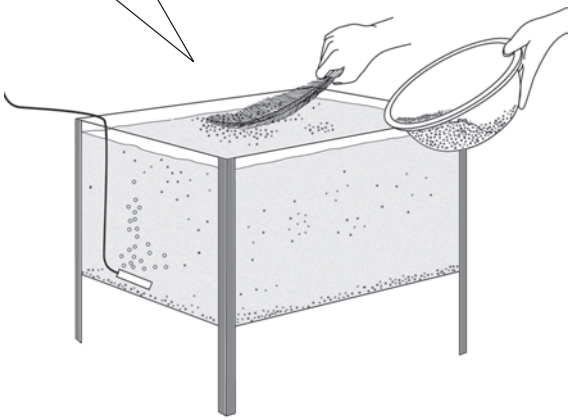
- Mix slowly for 1 minute.
- Rinse with freshwater to remove excess milt before transferring eggs to incubators.

Plate V.1.

Procedure of ova fertilization.



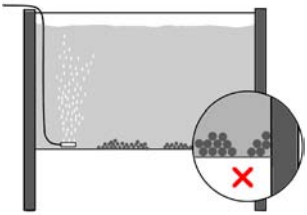
- After fertilization, handle eggs delicately using a feather.
- Disperse up eggs into aquarium.
- Mix eggs and water to obtain a good distribution of eggs over the entire water column. Then they will sink with an homogeneous distribution to the bottom and adhere to the glass surface.

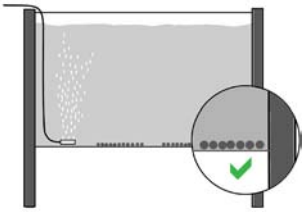


- After suppressing their stickiness (Plate V.4), transfer rinsed eggs into a plastic bowl filled with clean water and slowly pour them into the MacDonald incubator.

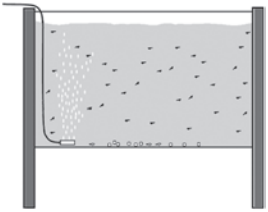
Plate V.2.

Transfer of the fertilized ova to the incubation structures.

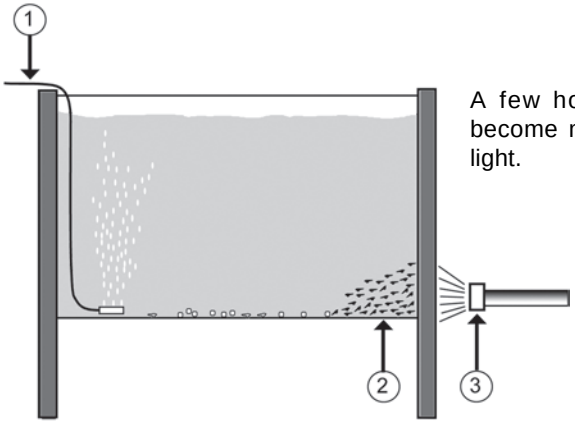




Distribution in a monolayer on the bottom allows each egg to get good water quality and improves hatching rate.



Larvae start to hatch after 33 to 35 h of incubation at 29 – 30°C and after a while swim throughout the entire column of water.



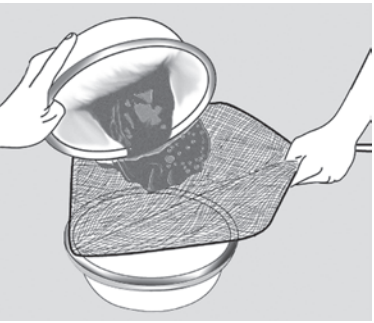
A few hours after hatching, larvae become more active and attracted by light.

1. Airstone.
2. Normal larvae gather in the beam of light.
3. Flashlight.

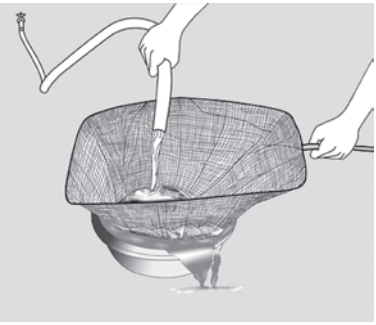
Plate V.3.

Incubation in stagnant water.

- Pour 100 mL clay solution on 200 g of eggs.
- Mix delicately with a feather until eggs do not adhere to each other.



- Delicately transfer the eggs in a small net to remove the excess clay.

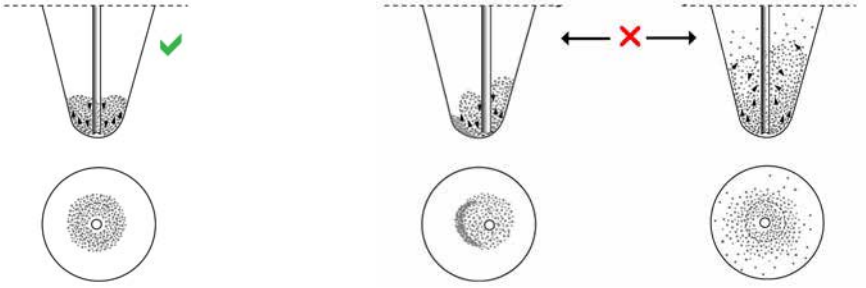


- Rinse with a slow rate of flow until clean water is obtained.

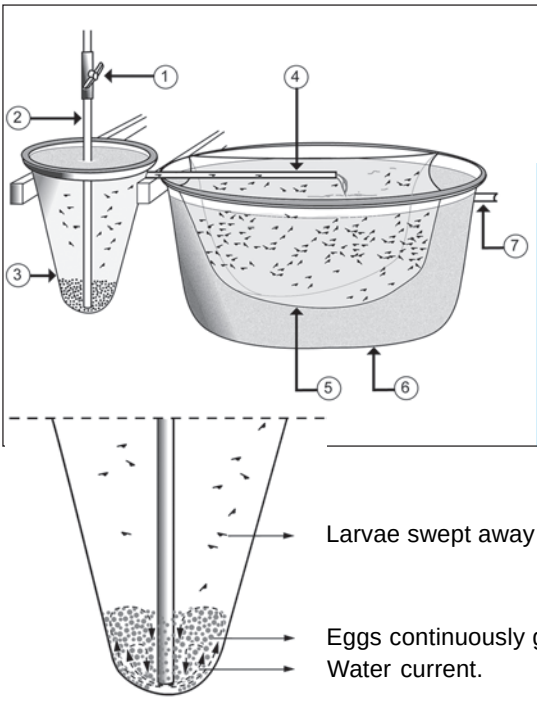
- Transfer eggs into a plastic bowl filled with clean water.
- Eggs are ready to be incubated in MacDonald jars.

Plate V.4.

Procedure for suppressing stickiness of eggs.



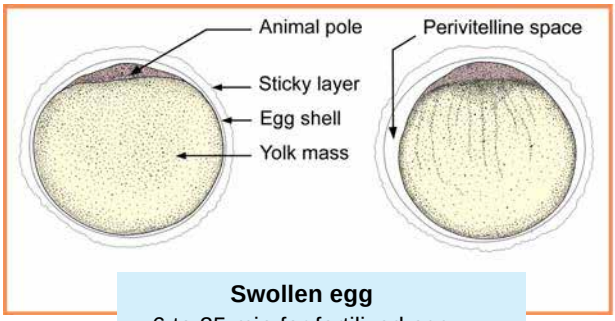
1. Adjusting water flow allows the surface of the egg mass to get slow wave motion.
2. Bad centering of the inlet pipe does not allow setting all the eggs in motion.
3. Strong water flow shakes up eggs and risks increasing embryos abnormality.



1. Outflow adjustment.
2. PVC pipe.
3. Eggs in motion.
4. Over flow.
5. Small size net receiving newly hatched larvae.
6. Fiber tank.
7. Over flow.

Plate V.5.

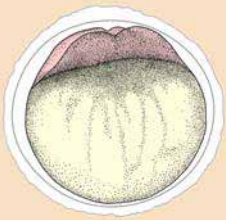
Incubation in MacDonald jar.



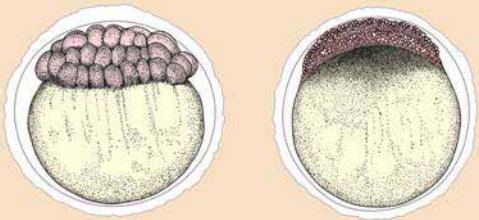
Swollen egg
- 6 to 25 min for fertilized egg.
- Such aspect could last for more than 8 h for unfertilized egg.

2-cell stage
25 to 40 min

Best stages for evaluating the fertilization rate



4-cell
30 to 55 min



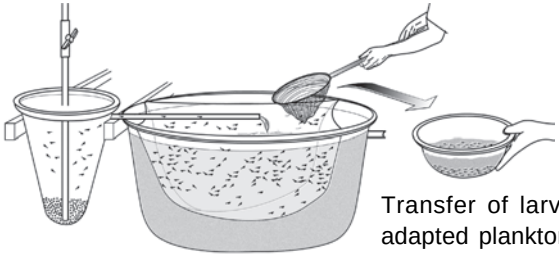
Morula
1 to 2 h

Gastrula
(middle time)
about 7 h

Closing of the Blastopore
12 to 18 h

Plate V.6.

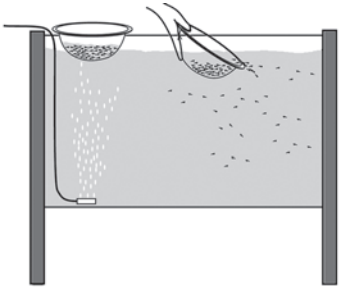
Some early stages of *P. djambal* embryo development.

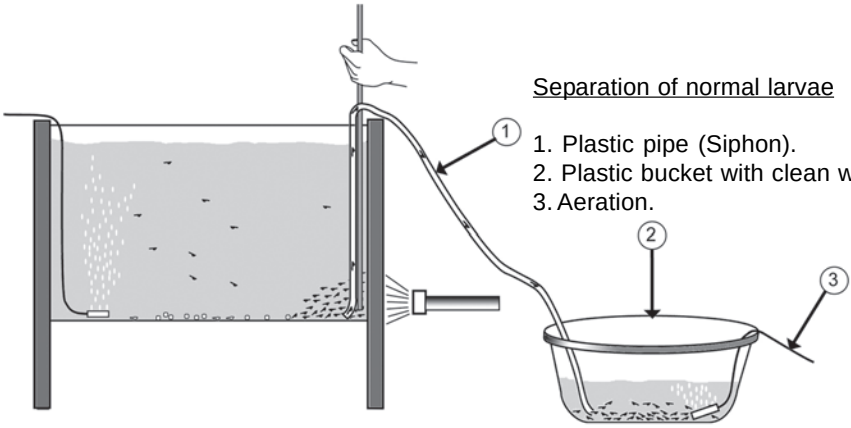


Transfer of larvae with adapted plankton net.

To avoid risks, it is necessary to transfer larvae soon after hatching into “temporary” structures containing clean aerated water.

Equilibrating temperature is strongly recommended, before slowly emptying out the bowl.





Separation of normal larvae

1. Plastic pipe (Siphon).
2. Plastic bucket with clean water.
3. Aeration.

Before being transferred to their rearing structures, larvae should be counted for better management of feed distribution and water quality.

Plate V.7.

Harvest of newly hatched larvae.

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Chapter VI

Larval Biology

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Techniques for larval rearing of farmed fish are specific and must be adapted to the particular behaviour and biological requirement of fish during their development. Based on our observations, the present chapter provides information on various aspects of development, requirements and specific characteristics of *P. djambal* larvae. Although more detailed investigations are required, these initial data allow a better understanding of the management of *P. djambal* larvae and fry during their two first weeks of life. The methods of larval rearing are detailed in Chapter VII.

LARVAL CHARACTERISTICS

Biological characteristics of *P. djambal* larvae and fry, from hatching to 15-day-old, are presented in Table VI.1.

Born from larger eggs, newly hatched larvae of *P. djambal* are bigger than those of *P. hypophthalmus*. They possess a large yolk reserve and usually present no cannibalistic behavior. However, in case of food shortage during the first days of life, some rare occurrence of cannibalism could be observed. Due to their initial difference in size, *P. djambal* larvae were also bigger (about 3 times) than those of *P. hypophthalmus* at the onset of exogenous feeding. As a consequence, larval rearing of the former species was easier and high growth and survival rates were regularly obtained (Legendre *et al.,* 2000).

Table VI.1.
Some characteristics of *P. djambal* larvae.

Characteristic	
Total length of larvae at hatching (mm)	4.7
Duration of yolk sac absorption at 28 – 29°C (days)	2.5
Total length of larvae at first feeding (mm)	8.6
Body weight at first feeding (mg)	4.5
Behavior of larvae	No cannibalism
Survival rate at 15 days of age (%)	70 – 94
Body weight at 15 days of age (mg)	190* – 380**

* in stagnant water
** in recycling water system

LARVAL DEVELOPMENT

As hatching of larvae is not synchronous but spread over a period of several hours, the age of a group of larvae refers to the moment at which 50% of larvae have hatched. In the example of hatching kinetics shown previously (Figure V.1), this "T_0" or hatching reference was reached after

about 37 – 38 h of egg incubation at a temperature of 29 – 30°C. The period between hatching and 24-h after hatching is considered as “day 0” and “day 1” starts 24-h after hatching.

Compared to *P. hypophthalmus*, *P. djambal* larvae developed an earlier dark pigmentation which even appeared on the embryos before hatching. During the first week, this pigmentation was restricted to the anterior half of the body. Eleven days after hatching, the fins were almost totally formed and the juveniles presented the general morphology of the adult (Plate VI.1.)

Supplementary observations were also made on the gut development of young fish from post hatching up to the age of 21-day. In *P. djambal*, the digestive tract was not subjected to a major anatomic evolution as is the case in *P. hypophthalmus*. In the relatively large-size post-hatched larvae of the former species, the stomach was visible since the first day. Only some minor morphological changes occurred during development and after 5-day of age the gut already presented the general morphology of that of a 21-day-old juvenile.

As *Artemia* nauplii represent a rather expensive feed, other types of feed were tested as possible substitutes during larval rearing of *P. djambal*. The time of first ingestion of each feed type by the larvae is presented in Table VI.2. It was shown that feed intake could be delayed by 4 to 6-h depending on the available type of feed.

The first feed intake of *P. djambal* larvae fed *Artemia* sp., or *Moina* sp. (49 h at 27 – 30°C) occurred before the yolk sac was totally absorbed (see Table VI.1).

Table VI.2.

First feed intake in *P. djambal* larvae fed with different types of feed.

Feed type	Time after hatching (hours)
Artemia sp.	49 h
Moina sp.	49 h
Daphnia sp.	53 h
40% protein dried diet	55 h

CHOICE OF FEED FOR LARVAL REARING

A comparison of growth and survival rates of larvae fed either *Artemia* sp., *Moina* sp*., Daphnia* sp., tubifex (blood worms) or a 40% protein dried diet was carried out until 11 days after hatching. Although, no differences in survival rates were found between larvae receiving the different types of feed, larvae fed *Artemia* nauplii showed 3 – 4 times faster growth.

Difference in growth rate between larvae receiving the different feeds could be explained at least partly by a faster and greater ingestion of *Artemia* nauplii from the first feeding (3 times) to the 8th day after hatching (20 times) in comparison with other feed types.

Although all feed type tested could be used for larval rearing of *P. djambal* in terms of survival, these results demonstrated that *Artemia* nauplii were clearly more efficient than other feeds. Similar observations were reported for *P. bocourti* (Hung *et al.,* 2002).

STOMACH EMPTYING

It has been shown for different fish species that the optimal frequency of feed distribution during larval rearing depends on age. Indeed, the speed of stomach emptying is related to stomach development and varies as a function of age. Some investigations previously done on *P. djambal* larvae showed also that the period of time between a meal and the total stomach emptying was about 4 – 5 hours until 5 days and increased up to 7 hours after 8 days. It is obvious that these biological changes have an influence on the feeding behavior and on routine work during larval rearing.

WEANING TIME

Previous studies have shown that larvae of *P. djambal* reared in recycling water system and fed with *Artemia* nauplii displayed optimal growth and survival rates. This optimal zootechnical performance was maintained when the larvae were weaned after 4 days only instead of a longer period of one week or more. As a consequence, *Artemia* substitution by an artificial diet from the 5th day of feeding allowed a significant decrease in operating cost of larval rearing.

WATER QUALITY

The parameters of water quality given in Table VI.3 correspond to those observed during various larval rearing of *P. djambal* carried out at JFADC or at RIFA stations. The list of parameters is not exhaustive and among them, only the tolerance to salinity was tested experimentally. Although the lethal limits of other parameters were unknown, respecting the ranges given in Table VI.3 could give good results in terms of growth, survival rates and health of *P. djambal* larvae.

During larval rearing particular attention should be paid to the dissolved oxygen concentration because the requirement of larvae is quite high.

Rearing them below an oxygen concentration of 3 mg.L^{-1} is risky. Besides, from the second day after hatching, *P. djambal* larvae like to swim against the current which keeps them in the most oxygenated part of the rearing tanks.

Table VI.3.
Environmental parameters for rearing *P. djambal* larvae.

Environmental parameter	Observed Range
Temperature (°C)	26 – 31
pH	6 – 8.9
Oxygen (mg.L^{-1})	> 3
Ammonia [NH3] (mg.L^{-1})	< 0.2
Nitrite [NO2] (mg.L^{-1})	< 0.1
Salinity (g.L^{-1})	0 – 4

This particular behavior could be used as a criterion to evaluating the level of oxygen available in the rearing structure. When the oxygen concentration was high enough, the larvae were well spread out in the tank. By contrast, when the concentration of oxygen was too low, the larvae were concentrated in the current resulting from aeration or water inlet.

In terms of temperature, good performance of larvae was obtained in the range tested (Table VI.3). However larvae reared below 28°C were subjected to higher risk of infection by parasites, such as *Ichthyophtirius multifiliis* (white spot disease). In this case, it is recommended to increase artificially temperature up to 28 – 30°C or to administer some preventive treatments (see Chapter VIII) for avoiding infection.

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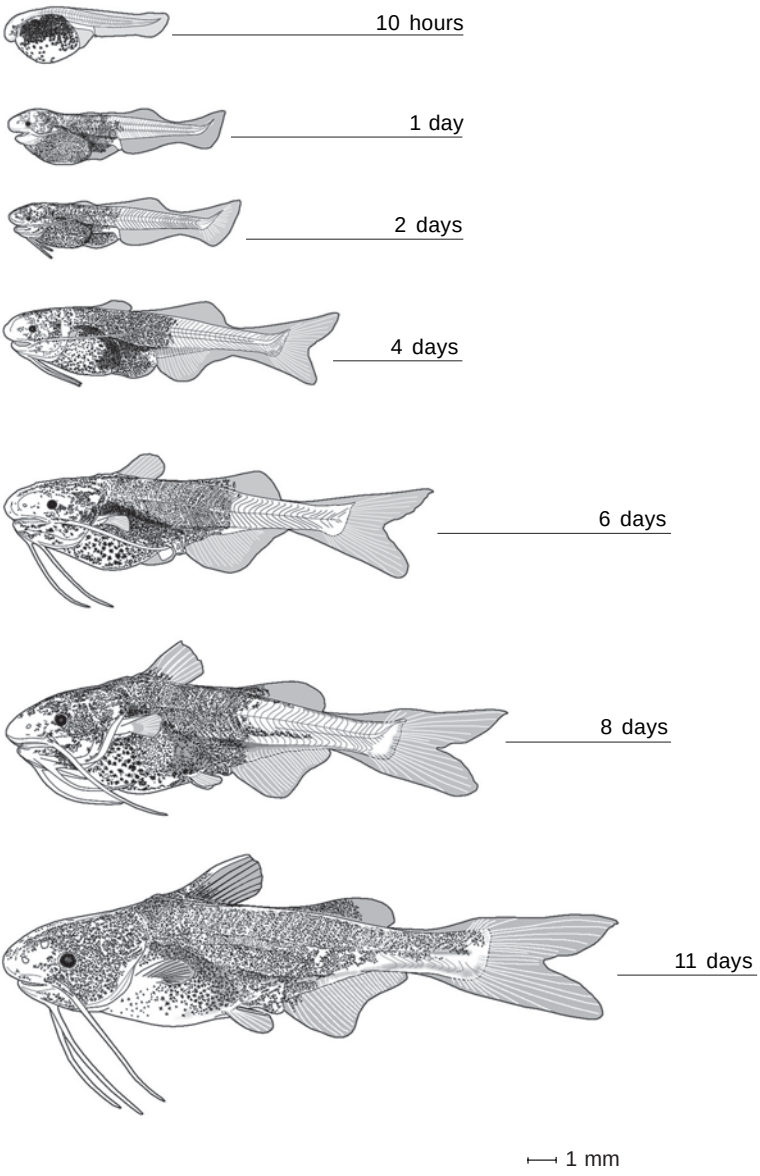

Plate VI.1.

Different stages in the early development of *P. djambal*.

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Chapter VII

Larval Rearing

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The large number of fish farms and the variety of their size, working context and cultivated species have resulted in a great diversity of fry production systems in Indonesia. Although larval rearing could be done successfully in ponds for different fish species, as recently developed for *Pangasianodon hypophthalmus* in Vietnam, this technique has not been used to a large extent for *P. djambal* so far. Some investigations on rearing larvae of this species in ponds led to high variability in terms of survival rates but they remained preliminary. More work is needed to improve and adapt this way of producing *P. djambal* fry.

In Indonesia, fish larvae are generally reared in tanks or aquaria using either stagnant or recycling water.

In this country, mainly for economical reasons, small and middle scale fish producers use the stagnant water aquarium option for rearing larvae. Recycling water systems, synonymous with high technology, generally require a higher investment than stagnant water rearing systems. However, in some situations, using a recycling water system may become a necessity for farmers. This may result either from long term or recurrent shortage of water or from the poor quality (turbidity, oxygen, temperature, ammonia, etc.) of water available in the area, requiring previous treatments.

As the choice of the level of technology used for seed production depends on farmers' needs and possibilities, larval rearing for *P. djambal* larvae has been developed successfully using either stagnant water (Day *et al.*, 2000) or recycling water systems (Legendre *et al.,* 2000). Both technologies will be described in the present chapter together with their associated routine activities.

DESCRIPTION OF REARING STRUCTURE

Although, the sizing of rearing structure is not the main purpose of this guide, some practical indications in this field are given in Appendix I for the recycling water system technology. Indeed it is important to note that the rearing volume needed for seed production either in stagnant or recycling water systems is directly related to the stocking density of fish and the way they are fed. Whatever the technology used, rearing structures are designed for a given maximal stocking density of larvae. Exceeding this number or biomass of fish may lead to a lowering of water quality and the failure of rearing.

In order to make the larval rearing management easier to understand, the following sections describe the working principles of each system.

Recycling water system

Recycling water systems allow rearing larvae in running water, as an open water system. Thanks to mechanical and biological filters, the recycling water is continuously rid of its impurities and toxic dissolved compounds (ammonia particularly) resulting from food waste, fish urine and feces. As the amount of food waste and toxic substances depend directly on the number of reared larvae, the volume of filters must be increased with increasing larval stocking density. This technology also permits decreasing quantity of water supply, controling temperature variations more easily, increasing the stocking density and treating eventual infestation by parasites or bacteria without changing water. The monitoring of the rearing environment has an obvious impact on the performance of the larvae particularly for growth.

A recycling water system is a chain of water treatments and each link corresponds to a specific function. Numerous pieces of equipment are available for each function, but in this practical guide we describe the main steps via the presentation of an original system already used in Indonesia (Appendix I).

Stagnant water system

Rearing in stagnant water means that there is no permanent water flow and water is regularly changed by siphoning. The maintenance of the level of dissolved oxygen in water is ensured by airstones placed in the rearing structure.

Generally carried out in aquaria in order to keep an eye on larvae, the rearing in stagnant water does not require expensive equipment and is well adapted for small and medium scale production. This simple and cheap technology is the most widespread for fry production in Indonesia.

The routine work must be meticulous. Uneaten feed and feces of larvae should be remove manually every day. Moreover, in order to reduce the amount of dissolved toxics, between 50 and 75% rearing water must be changed daily by siphoning. This technique requires a good management of water throughout the larval rearing period.

PREPARATION OF REARING STRUCTURES

Before stocking larvae, all structures have to be ready in order to avoid pathology, stress and escape of newly hatched larvae from rearing structure. Whatever the technology used, all rearing structures should be cleaned and disinfected before filling for a new rearing cycle.

Initial use of recycling water system

Recycling water systems need a special procedure for their very first use in order to prepare the biological filter in good condition. The biological filter can purify ammonia and nitrogen wastes only after the development of nitrobacteria (*Nitrosomas* and *Nitrobacter*). This process needs around 10 to 15 days after the system has been filled with water and started.

It is known that excretion of ammonia and nitrogen wastes at a level exceeding the filter treatment ability can lead to lethal concentrations for the larvae. In order to avoid a risky situation, the biological filter needs progressive conditioning.

In practice, the recycling water system must be filled with water about 2 weeks before starting the first larval rearing. During this period, in order to create favorable conditions for good development of "nitrobacteria", the following procedure should be followed.

- First, the concentration of ammonia is increased by adding a food bag of about 100 g of ground pellets per m^3 of water. This bag has to be placed before or directly on the mechanical filter in order to prevent pollution of the biological filter with suspended particles. From desegregating pellets, ammonia and other nitrogen-waste compounds are freed, dissolved in water and stimulate a natural development of the "nitrobacteria";
- after one week, remove the waste pellets if any and place another bag with ground pellets (100 $g.m^{-3}$);
- two weeks after having filled the rearing structure, rinse with clean water in order to change the total volume. The biological filter has already been colonized by nitrobacteria and the rearing structure is now ready to receive larvae.

Once the biological filter works and the first cycle of rearing has been achieved, the second cycle of larval rearing can be started without any special procedure, except for cleaning and disinfecting the structures before introducing new larvae (see Appendix I).

Stagnant water system

Rearing structures in stagnant water do not need special procedures for the initial use. Except for very new containers which need a period of washing to avoid toxic elution. However this technique needs a sufficient stock of clean water and requires very good water management during the entire rearing time. Aquaria or tanks must be filled with water well before receiving larvae in order to allow equilibration of oxygen and temperature, and to avoid thermal shock and stress for the larvae.

STOCKING DENSITY IN REARING STRUCTURES

The stocking density is the number of larvae reared per litre of water. Unknown and too high density may engender an imbalanced water quality, slow growth, low survival rate and great size heterogeneity of remaining fish. As noted previously, each rearing structure is designed for a maximum fish stocking density. Whatever the technology used, this maximal value should not be exceeded.

Using a low stocking density does not allow operation of the farming system at its full capacity and results in reduced production.

The initial number of larvae transferred to the rearing structures has to be known by counting, or accurately estimated, for a proper subsequent management of feeding and good water quality maintenance in each tank.

To optimize larval production, the stocking densities recommended for *P. djambal* during larval rearing are the following:

- in stagnant water: 15 fish per litre until 8-day of age, then 5 larvae per litre from the age of 8 to 18-day;
- in recycling water: 30 fish per litre until the 15th day of age.

Age (days)	Average weight in recycling water system (mg)	Average weight in stagnant water (mg)
2	5.4	5.7
3		
4	16	11
5		
6	47	20
7		
8	97	30
9		
10	130	65
11		
12	210	100
13		
14	380	190

Table VII.1.

Growth of *P. djambal* larvae in recycling and in stagnant water systems.

GROWTH OF LARVAE

Mean growth performance of *P. djambal* larvae reared in recycling water (RIFA) and in stagnant water (JFADC) are compared in Table VII.1. These data were obtained by individual weighing of larvae every two days using an accurate balance. Although every farmer could not invest in such an expensive measuring device, accurate follow up of growth rate allows one to calculate and readjust the daily feeding rate.

Table VII.1 shows a slower growth in stagnant water illustrating the limits of this rearing technique in comparison to recycling water systems. Nevertheless, the survival rates obtained in both situation (stagnant or recirculated water) are similar and greater than 75%. These results demonstrate that, even if the growth is not optimal in stagnant water, this technique developed by the JFADC remains well adapted for this species and could be used by small scale farmers in Indonesia.

FEEDING PROCEDURES

Artemia nauplii

For each cultured species, optimal feeding levels have generally been established and are specified as a number of nauplii per larva and per distribution or per day. It has been demonstrated that larval rearing success is considerably impaired when optimal feeding levels are not respected. As larvae need a given number of *Artemia* nauplii per distribution, it is recommended to estimate every day the harvested quantity of *Artemia* nauplii produced in the hatchery, in order to manage properly the ration of the day. Incubating, harvesting and counting methods for *Artemia* are described in Appendix II.

P. djambal larvae have to be fed with *Artemia* nauplii from 48-h post-hatching (see Table VII.2) until:

- 5-day of age, i.e. 4-day of feeding, in recycling water;
- 8-day of age, i.e. 7-day of feeding, in stagnant water.

In recycling water

From the very first meal, the quantity of *Artemia* nauplii distributed to the larvae should be respected and increased daily as shown in Table VII.2. This table gives the recommended number of nauplii for one meal and for one larva according to age. The evaluation of the volume of water containing the *Artemia* nauplii to use for feeding the larvae is also explained in detail in Appendix II.

In a recycling water system, it is recommended to stop the water flow for 30 minutes at each feeding time in order to maintain the living prey in the tanks.

The *Artemia* nauplii number recommended per larva has to be multiplied by the total number of larvae grown in each tank. An example is given in Table VII.2 for one tank of a recycling water system stocked with 7500 larvae.

The quantity of *Artemia* nauplii per distribution given in Table VII.2 was evaluated for a feeding schedule of 7 meals per day. The recommended feeding frequency is to feed larvae every 3 hours from 6:00 to 00:00.

Table VII.2.
Calculation of the *Artemia* nauplii quantity for one meal.

Age (hours)	Age (days)	Number of *Artemia* nauplii per distribution and per larva	Quantity of dried diet	Number of *Artemia* nauplii per distribution for 7500 larvae	
				Calculation	Number
48 - 73	2	20	0	20 x 7500	150 000
72 - 96	3	50	0	50 x 7500	375 000
96 - 120	4	70	0	70 x 7500	725 000
120 - 144	5	100	trace	100 x 7500	750 000

In stagnant water

In order to preserve water quality, the number of *Artemia* nauplii is distributed as required (*ad libitum*). As stated above, the number of *Artemia* nauplii per larva should be increased daily until the weaning time. Therefore, for evaluating the right number of prey to distribute farmers should observe accurately the behavior of larvae to see if they are filled up or not.

To obtain optimal performance, the following feeding frequency is recommended:

- between the 2nd and 8th days of age, fish are fed 5 times per day, every 4 hours between 7:00 and 23:00 with a fasting period between 23:00 and 7:00.

Weaning time

In many fish species weaning time is a very delicate period because the anatomical and functional development of the gut is not totally achieved yet. In *P. djambal*, substituting *Artemia* nauplii with a new artificial diet does not cause any problem. However, it is recommended to get the larvae used to their new diet before stopping distribution of *Artemia* nauplii.

In recycling water

From the 4th day of feeding, when using the recycling water technique, it is advisable to distribute a small quantity of artificial diet half an hour before feeding the larvae with *Artemia* nauplii. As stated previously, total substitution of the *Artemia* nauplii with artificial diet from the 5th day of feeding significantly decreases operating cost of larval rearing (Chapter VI).

In stagnant water

Nowadays, artificial diet is not commonly used to feed larvae in stagnant water to avoid water pollution. Therefore, *Artemia* nauplii are substituted by *Tubifex* sp. (blood worm) after the 8th day after hatching.

Artificial diet

The following comments concern only larvae reared in recycling water because artificial diet is generally used in this system.

An appropriate protein content and size of particles are required for successful weaning. Particle diameter of the initial dried diet should be well adapted to the mouth of larvae to permit or facilitate ingestion and also to aid digestion. The size of the initial dried diet recommended for *P. djambal* weaning should be between 270 and 410 µm.

Since a deficiency in essential nutrients can affect growth, it is recommended to rear larvae on a balanced protein-rich diet (40 – 45% of crude protein). The best way to feed larvae is to distribute artificial diet as required by the fish (*ad libitum*). However sometimes the limit between *ad libitum* distribution and over-distribution is very thin. An excess of food in a rearing container can reduce water quality, resulting in disease or fish mortality.

Therefore, we recommend to give a daily ration of 20% of the biomass from the 6th to the 9th day; 15% from the 10th to 13th day and then 10% from the 14th day (Table VII.3). After this period, the feeding rate will be decreased step by step according to age.

Calculation of the feed quantity (Table VII.3)

- Mean body weight of larvae must be known and multiplied by the total number of larvae to obtain the total biomass.
- Total biomass is multiplied by the daily ration in % to obtain the daily quantity of artificial diet to distribute (ration).
- Daily quantity of artificial diet calculated above must be divided by the number of distribution done during the day to obtain the quantity of feed per distribution.

However, as stomach emptying is totally different after 8 days (Chapter VI), it is recommended that larvae be fed 6 or 7 times a day (as for *Artemia* nauplii) until the age of 7-day and 5 times a day from the 8th day.

Table VII.3.

Calculation of the feed quantity for *P. djambal* larvae.

Age	Mean body weight (mg)	Total biomass for 7500 larvae		Daily quantity for 7500 larvae		Quantity of dried diet per distribution for 7500 larvae	
		Calculation	Biomass (g)	Calculation	Ration (g)	Calculation	Quantity (g)
6	47	7500 x 0.047	353	20% x 353	71	71 / 7	10 – 11
7					71		10 – 11
8	97	7500 x 0.097	728	20% x 728	146	146 / 5	29 – 30
9					146		29 – 30
10	130	7500 x 0.130	975	15% x 975	146	146 / 5	29 – 30
11					146		29 – 30
12	210	7500 x 0.210	1575	15% x 1575	236	236 / 5	47 – 48
13					236		47 – 48
14	380	7500 x 0.380	2850	10% x 2850	327	327 / 5	57 – 58

Blood worms *(Tubifex* sp.*)*

Due to their low price and their abundance in some Indonesian areas, *Tubifex* are the most widespread wild food used for feeding fish larvae or fry in stagnant water. However, *Tubifex* are not available everywhere in Indonesia and their abundance depend on the season. Even if their price remains lower than that of dried diet, it is very variable according to season and locality.

It is known that *Tubifex* can carry parasites for fish such as myxozoa or cestodes (De Kinkelin, 1985) and can trigger disease. Moreover, these worms are often collected in sludge or in pluvial evacuation, and then put in plastic bag for transportation at ambiant temperature sometimes exceeding 30°C. Although, farmers wash *Tubifex* sp. with clean running water and add airstone before feeding to larvae, the risk of parasitic and bacterial contamination exists.

For these reasons, and since in recycling water artificial diet is very efficient, we advise not using *Tubifex* in this structure.

If *Tubifex* is to be used, we strongly recommend disinfecting *Tubifex* with formalin in the following way before distribution:

- put *Tubifex* in a formalin solution at 50 ppm.; i.e. 50 $mL.m^{-3}$;
- mix for 2 minutes;
- rinse at least 3 times with clean water;
- distribute to larvae.

Generally, in stagnant water, farmers prefer *Tubifex* to dried diet. In fact, distributed alive, the worms group together and form a ball at the bottom of the rearing tank and stay alive a long time, which is a great advantage:

- easy to control the ration (as long as some *Tubifex* remain it is not necessary to re-distribute);
- no pollution of the rearing water (live-food);
- larvae eat when they want (self-feeding).

Chopped or ground *Tubifex* could also be used for very young larvae, but in this case rearing water is quickly polluted when stagnant.

In JFADC, for *P. djambal* larvae live *Tubifex* were successfully used to substitute *Artemia* nauplii from the 7th to the 16th day of age.

CLEANING AND WATER MANAGEMENT

In order to preserve water quality and keep larvae in good health, it is strongly recommended, from the 2nd day of rearing, to remove the food wastes and feces daily from the bottom of the tank by siphoning. This cleaning operation should be done before the first feeding in the morning.

As indicated previously (Chapter VI), during larval rearing particular attention must be paid to oxygen concentration because requirement of this species is quite high.

In recycling water

Specific routine activities and water management related to mechanical and biological filters are explained in Appendix I.

To keep good quality of water in the rearing tank, the flow rate must be increased progressively according to the age of larvae. In fact, very young larvae of *P. djambal* habitually swim against the current created by the water flow. This behavior probably makes exchange easier with oxygen. Now, if the current is too strong, larvae wear themselves out to swimming or are squashed on the outlet net.

In fact, without a measuring device to control water quality, the watchfulness of the farmer is important determining when the water flow is:

- too low:
  - larvae are concentrated on aeration or water flow;
  - water becomes whitish.
- too strong:
  - larvae tire themselves swimming;
  - larvae concentrated or squashed on the outlet net;
  - food wastes and feces are totally evacuated by the current.
- well adjusted:
  - larvae are well spread out around the tank;
  - larvae swim slowly against current;
  - water is clear;
  - food wastes and feces are partly concentrated near the outlet.

To avoid stress to larvae, problems of water quality must be anticipated and water flow increased every 2-day according to the following rates:

- 25% of water volume of the tanks changed **per hour** from the 1st to the 2nd day of rearing;
- 50% from the 3rd to 5th day of rearing;
- 100% from the 6th to 15th day of rearing.

Water flow is considered here by the percentage of rearing water changed in the tank during one hour while it is generally stated in litre per hour or per minute.

Although, the previous expression is currently used by farmer, we have to be careful using this expression, because for the same rate of water exchange, water flow is different depending upon the volume of the tank. The following explanation gives two examples of calculations for converting the percentage of water exchange into flow rate.

Calculations

An experimental tank with a rearing volume of 30 L and water change of 25% per hour, has a water flow of 7.5 $L.h^{-1}$, i.e. 25% x 30 = 7.5 $L.h^{-1}$.

A production tank with a rearing volume of 1000 L and water change of 25% per hour, should have a water flow of 250 $L.h^{-1}$; i.e. 25% x 1000 = 250 $L.h^{-1}$.

In stagnant water

Since the rearing is done in stagnant conditions, the water is removed by siphoning during cleaning wastes. Siphoning should be done slowly with a plastic pipe into a bucket. To avoid sucking up larvae during the cleaning, the end of the siphon should be fitted with an outlet net large enough to avoid larvae squashing against it.

To limit stress, the quantity of water renewed should be adapted to the age of the larvae and can be programmed as follows:

- 50% of water change per day from the 2nd to the 6th days of rearing;
- 75% from the 7th to 16th day of rearing.

EQUIPMENT AND TOOLS

Recycling water system

1. Recycling water system with bio-filter and mechanical filter (equipments are listed in Appendix I).
2. Rearing tanks with adjustable inlet and outlet nets of suitable diameter.
3. Air blower with airstones in each rearing tank.
4. Watch to measure the water flow in each tank.
5. Calculator for calculating water flow.

Stagnant water

1. Aquarium 60 x 50 x 40 cm.
2. Air blower with airstones in each aquarium.
3. Plastic bucket for filling aquarium.
4. Tank for storing clean water.

Water quality control (recommended)

1. Oxygen kit or oxymeter.
2. Ammonia kit.
3. Nitrite kit.
4. pH kit or pH meter.

Handling and stocking newly hatched larvae

1. Aquarium with airstone and clean water.
2. Plankton net (80 µm mesh size) for catching larvae.
3. Plastic bowl for transferring larvae to the aquarium.
4. Flashlight for concentrating normal larvae in the beam of light.
5. Plastic pipe for cleaning the bottom of white eggs and abnormal larvae and then siphoning normal larvae.
6. Plastic bucket for transferring normal larvae in rearing structures.

Feeding

Artemia

1. Equipment described in Appendix II.

Artificial diet

1 Balance (500 g ± 1 g) for weighing daily feeding ration.
2 Plastic bucket for stocking feed for each rearing structure.

Blood worm

1 Small tanks with water flow and airstone for stocking *Tubifex*.
2 Formalin for disinfecting.
3 Syringe for measuring formalin.

Cleaning

1 Plastic pipe with a large outlet net for siphoning the bottom.
2 Plastic bucket for catching the siphoned water.

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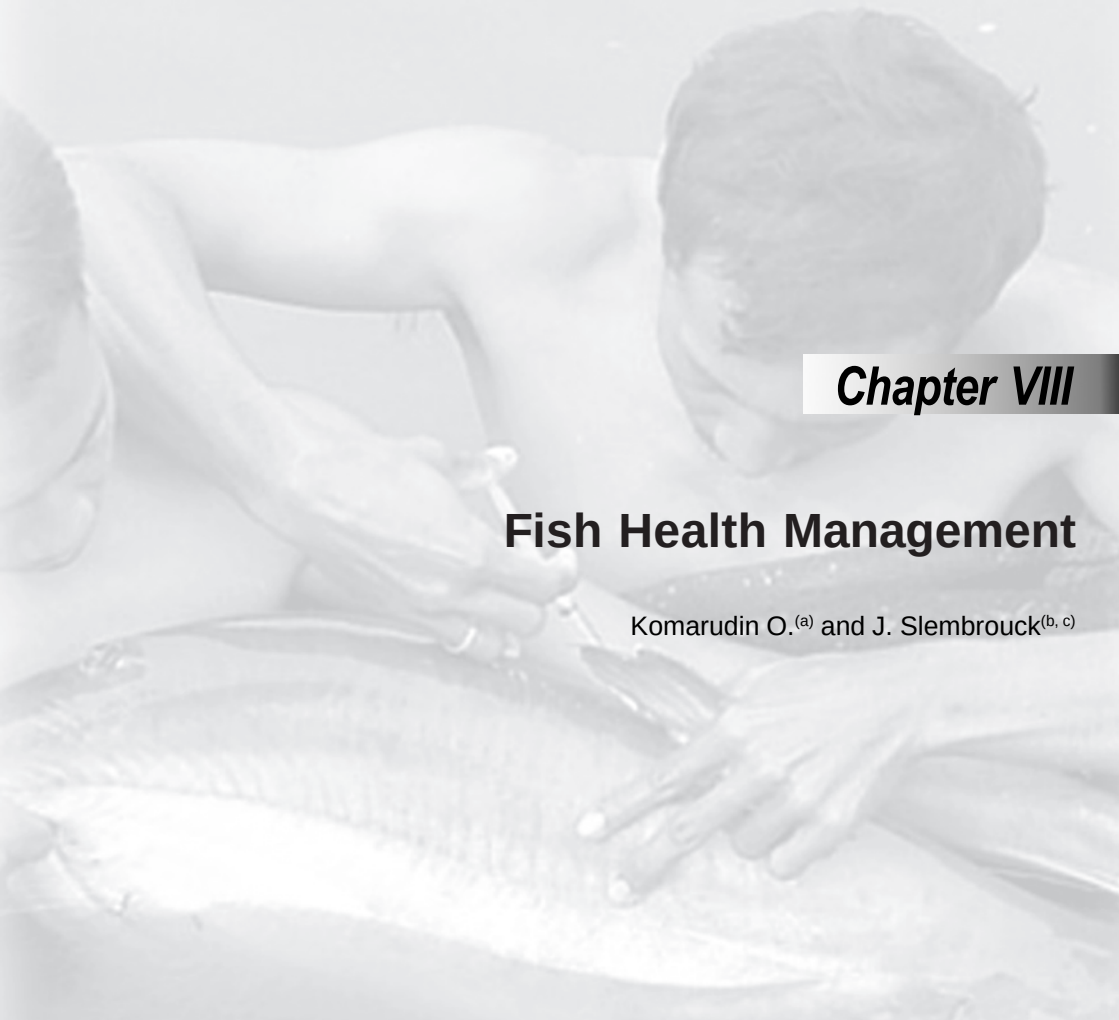

Chapter VIII

Fish Health Management

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Due to the difficulties in diagnosis, implementation of appropriate treatment and identifying causes, fish disease is a major problem faced by producers. The economic consequences for fish farmers are significant either through the loss of production (mortality and low growth) or the cost of treatment.

Generally, stress may decrease the threshold of fish resistance and is considered to be one of the main causes of fish disease in intensive culture systems. However, fish stress in culture could be avoided in many cases. Much work has demonstrated that healthy fish cannot be seriously infected by pathogen, while weak fish will be easily infected (Woynarovich and Horvath, 1980).

It is important to remember that the stress may also reduce feeding and can seriously affect the spawning success for brooders (Chapter III).

THE ORIGIN OF STRESS

Water quality

Inadequate rearing water quality (high organic mater content, presence of ammonia or nitrite, low dissolved oxygen concentration, inappropriate pH, quick and high variation of temperature, etc.) force fish to maintain their metabolic balance, weakening them and resulting in disease susceptibility.

Water pollution from chemical sources could also be the cause of sudden and apparently unexplained mortalities, and susceptibility to disease, particularly when fish are reared in open water or with water from a river or reservoir.

Rearing conditions

In intensive culture, stocking densities are often high with respect to carrying capacity of the rearing structures. Therefore, fish are readily exposed to stress caused by the confinement, thus increasing the susceptibility of the fish to pathogen infection. High stocking density is also favorable in spreading of diseases, as close contact between individuals facilitate the transmission of pathogens.

Bad management of routine activities and feeding could also cause weakness through undernourishment or unbalanced water quality.

Handling

Capture, handling and transport could affect gonad maturation and growth and may cause diseases (Chapter III).

CONTROL AND PREVENTION

The behavior of the fish has to be observed carefully. If visual observation reveals some abnormal fish behavior, further evaluation should precisely determine whether it is due to the presence of pathogen or to poor water quality conditions. This examination has to be carried out as soon as possible to avoid further negative effects to the cultured fish. If pathogens are involved, treatment has to be administered immediately while looking for the origin of the infection.

Several methods should be used to prevent and control infection by pathogens:

- before using them, rearing structures have to be cleaned, disinfected and dried; drying of earthen ponds after harvesting fish has to be carried out regularly;
- routine disinfection of equipment will greatly help to prevent pathogen contamination;
- keep the cultured fish in optimal rearing conditions (right stocking density, good water quality, appropriate feeding procedure);
- preventive anti-parasitic or anti-fungus treatments should be administered regularly;
- fasting day before handling and transportation of fish;
- vaccination of fingerlings will be also effective to stimulate immune response and to prevent infection and spread of disease.

Methods for disinfection

- Earthen pond

The pond should be completely emptied and then kept drained for 5 – 7 days. If the bottom could not be completely drained, spread quicklime at a rate of 200 – 250 $g.m^{-2}$.

- Smaller structure

Concrete tank, fiberglass tank or aquarium can be disinfected with formalin or chlorine bleach.

- Equipment

All other materials (landing net, bucket, plastic pipe, etc.) could be disinfected by dip for 20 – 30 seconds in a solution of 6 ml of chlorine bleach in 1 litre of freshwater.

CHOICE AND GENERAL ADVICE FOR FISH TREATMENT

Observation of fish behavior will lead farmer to make a rapid diagnosis of the disease required to prescribe a course of treatment. This therapy should be quickly administrated, well targeted and given at the right dose in order to stop disease development.

The choice of chemotherapeutic agents should be based on the following criteria:

- not prohibited;
- right drugs for the right disease (requires knowledge of the drug and its dosage);
- easily available and cheap;
- does not affect humans consuming the fish.

Three methods have been used administering therapeutic drugs to *P. djambal*: by injection, blended with feed or mixed with water (De Kinkelin *et al.,* 1985). These methods are used to control the spread of pathogens and reduce the intensity of the infection. However, each drug has specific use recommendations made by the manufacturer.

Injection

Generally used for broodfish, this technique is practiced when there are no other available methods. Actually, handling fish for the injection increases stress. However, it is the only way to treat valuable fish reared in cage or in pond, and which refuse to eat. Injection of drugs makes sure that the medicine penetrates the body.

The drugs used for injection are antibiotics, vaccines or vitamins and are injected intra-muscularly or intra-peritoneally.

Blended with feed

This method does not stress fish. It is generally used to administer antibiotics and vitamins powder. During the treatment, the daily feeding rate of fish has to be decreased to 1% per day to make sure that all feed distributed is entirely ingested. The blending in feed is done as follows:

- daily quantity of feed is calculated (1% of the biomass) and weighed;
- daily quantity of drug is calculated from the total biomass and weighed;
- calculation: Total fish biomass (kg) x dosage of the product (mg per kg of fish per day);
- place together in a small container;

- mix food and product together;
- add 0.1 L of vegetable oil (palm or soybean oil) for 5 kg of feed;
- mix feed and oil together until all antibiotic powder is sticking on pellets;
- distribute the blend slowly to make sure that the feed is entirely ingested.

Mixed with water

This method is generally used to treat ecto-parasites or external bacterial infection. Although simple to administer, bathing with chemotherapeutic agents needs care to avoid risk during the treatment.

Advice for bath treatment

Before anti-parasitic treatments, fish must not be fed.

In order to prevent fish from swimming in dangerous concentration during the treatment, it is not advisable to put drugs directly in the rearing structures. We strongly recommend to take a sample of rearing water (10 L), making a stock solution that will be distributed among the treated structures. The stock solution will help the homogenization of the treatment into the water.

Not all drugs listed below are dangerous for fish at the recommended dosage. However, a calculation error is always possible. For this reason, fish behavior should be checked during treatment. If fish show stressful reactions, the chemical should be rapidly removed by water exchange with clean freshwater. It is also recommended to increase air flow during the treatment because some drugs can decrease the dissolved oxygen in the water.

Before using a new treatment for small fish, it is recommended to test the drug in order to determine lethality of the prescribed dose and tolerance time. For this purpose, some buckets are filled with 10 litres of clean water; the drug is added into each bucket at the dose recommended by the manufacturer. Into each bucket 10 fish are placed. During the test period fish behavior is observed to determine whether they get stressed or die. If the time recommended for treatment does not fit with the condition resulting from these observations the effectiveness of the drug against the pathogen has to be determined.

Difference between disinfectant and antibiotic treatments

Disinfectant

Used for treatment against ecto-parasites, fungi and external bacteria. Administered by bath or dip, they give quick results. One treatment is generally not enough to kill the pathogen. Therefore, it is recommended to

repeat it one or two times on alternating days in order to avoid new developments.

Although numerous chemotherapeutics are available worldwide for aquaculture, formalin is still considered the most common drug to treat external and gill parasitic infestation. Formalin is chosen not only for its efficiency, but also for its availability and low price. Mixed with "Malachite Green Oxalate" (MGO), its wide effectiveness seems to be reinforced.

Regular preventive treatments with formalin are recommended. No resistance was developed by the pathogen for this drug.

Water management

Our experience and various reports have shown that formalin or MGO utilisation in a recycling water system does not affect the biological filter at the dose recommended. As this drug is completely degraded after 26-h at the dose of 25 $mg.L^{-1}$ in bathing treatment (Tonguthai, 1997), it is not necessary to change water after preventive treatment in a recycling water system. On the other hand, for curative treatment, it is recommended to change water after 24-h of bath treatment in order to remove a maximum amount of pathogen, particularly with *Ichthyophtirius multifiliis*.

In a stagnant water system, as 50 to 75% of the water is changed every 24-h, the routine activities remain unchanged whatever the treatment.

Antibiotic

Never to be used for preventive treatment, antibiotic is administered to fish for controlling bacterial diseases through injection, bath or blend in feed. It is important to point out that:

- bacteria are sensitive to specific antibiotics;
- bacteria become resistant to drugs when antibiotic therapy is not well targeted or not administered for the proper time.

In order to avoid such problems, it is the best to first identify the species of bacteria and then do a sensitivity test to antibiotics before selecting and using the drug. However, obtaining such results takes 6 – 8 days and these analyses are not necessarily available close to fish farms in Indonesia. Therefore, in case of bacterial disease, it is preferable to use a wide spectrum antibiotic such as Oxytetracycline (Terramycin) rather than losing the production.

Antibiotics should be used at the right dose and for sufficient duration in order to ensure the total elimination of bacteria. Antibiotic therapy should be administered for 6 – 8 days and could be repeated after one-week break in order to avoid a new development of pathogen. Aborting treatment before the

6th day risks creating bacterial immunity, even though mortality may have decreased and fish health improved rapidly.

The final very important point is that antibiotic residues in the flesh could affect human consumers. In fact, depending on temperature and the type of antibiotic, fish are free of residues about one month after the end of treatment. During this period, fish should not be sold for human consumption.

Water management

The use of Oxytetracycline in a recycling water system does not affect the biological filter at the recommended dosage. Nevertheless a slight slowing down in nitrification efficiency is observed after 5 days (Blancheton and Melard, 1990). As the fish daily feeding rate is considerably reduced during the treatment, this temporary reduction in efficiency of the biological filter has no consequence on the fish culture.

It was demonstrated that the half-life of this antibiotic is 128 h at 15°C (Blancheton and Melard, 1990); we can assume degradation is faster in a tropical climate (27 to 31°C). As antibiotic purchase is very expensive for farmer and dry season sometimes imposes water rationing, a procedure for long term bath in recycling water system could conserve both antibiotic and water. The following procedure and doses could be applied without changing water over 7-days:

- 20 mg.L^{-1} for the first bath treatment;
- 48 h after, give a dose of 15 mg.L^{-1};
- 48 h after the second dose, give a dose of 10 mg.L^{-1};
- 48 or 72 h after the third dose, water should be changed completely.

The same procedure and dosage could be used in stagnant water with water changed every 48 h when water is not restricted. However, during treatment the daily feeding rate should be decreased to 1% of fish biomass per day.

PATHOGEN FOUND ON *P. DJAMBAL*

So far, three main pathogens has been found to infect *P. djambal* in culture conditions. The following give elements to identify them, clinical symptoms, prevention and efficient treatments and their dosages.

Bacteria: *Aeromonas hydrophyla*

Short rods, 0.7-0.8 x 1.0-1.5 µm, motile, with single polar flagellum, gram negative.

Not visible with the naked eye, this bacteria is the most common cause of hemorrhagic septicemia, the infection generally follows stress.

Prevention: maintain good conditions of rearing and avoid unnecessary stress of fish.

Clinical sign: abnormal behavior; swim slowly; refuse to feed; hemorrhage; discolored and eroded fins; skin lesion sometimes reaching deep into the muscle.

Treatment: antibiotic such as Oxytetracycline administrated by injection, bath or orally.

Dosage: **Never stop** the treatment before 6 – 8 days, if necessary it could be repeated after a one-week break.

- 10 – 20 $mg.m^{-3}$ (ppm) of active substance, in bath for 24 hours,
- 50 – 75 $mg.kg^{-1}$ of fish per day, blended in feed, taken orally,
- 50 $mg.kg^{-1}$ of fish per day, by injection for brooders.

Protozoa: *Ichthyophtirius multifiliis*

Body of mature stage is round with a diameter of 500 – 1000 µm, covered with cilia, macronucleus large and horseshoe-shaped. External parasite, visible to the naked eye. Called "Ich" in Indonesia, young parasites colonize gills or insert themselves under the mucus layer. This parasite is the most dangerous for fingerlings and can lead to mortality up to 100% in the case of heavy infection.

After 7 days at 25°C, each *Ichthyophtirius multifiliis* becomes mature and releases small cysts in the rearing water, which sink to the bottom. Then after few hours, they give birth to a thousands of descendants. The latter, called Tomite, infest the fish again. The infectious stage, called "white spot disease" is reached rapidly. Chemical treatments are effective on the free-swimming stage only (Tomite). Multiplication is very rapid below 28°C, but at higher temperatures, the risk of infection is considerably reduced.

Prevention: Good rearing conditions; temperature of rearing water should be higher than 28°C; running water to remove Tomite; preventive bath treatment.

Clinical sign: Appearance of white spots on skin or fins, irritant effect, infested fish become lethargic and do not respond to stimuli correctly.

Treatment: Bath with a stock solution of Malachite Green Oxalate (4 g) diluted in formalin (1 L).

Dosage: This treatment is the most efficient against "white spot disease"; however exceeding the recommended dosage is very dangerous for fish. In case of a new stock solution, it is strongly recommended to test it on a sample of fish first.

- During the first 10 days of larval rearing: 5 $mL.m^{-3}$ for 24 h, once a week, as preventive treatment.

- Over 10-day-old: 10 mL.m^{-3} during 24 h, once a week, as preventive treatment.
- In case of infection: the dosage of 20 mL.m^{-3} should be applied for 24 h, and repeated 3 times after 24-h break (total duration 5 days). During the 24-h break, the water should be changed entirely whatever the rearing method used.

Note: Lethargic or heavily infested fish should be eliminated from the rearing tank in order to reduce infestation.

Monogenean: *Thaparocleidus*

Worm without segment, small size (< 3 mm). External parasite, generally fixed on the gills (Komarudin and Pariselle, 2002).

These species of Monogenean grow in number very quickly in intensive culture (Thoney and Hargis, 1991). The beginning of the infection is invisible with the naked eye. Afterwards, farmers should open the fish operculum to inspect for the presence of Monogenean on the gills. For an accurate diagnosis, farmers could sacrifice some fish and observe gills with a low power stereo-microscope.

This infection causes respiration deficiency for the fish and tends to reduce growth, with evident negative impacts on fish production. Bacterial infection could be a side effect.

Prevention: disinfection of rearing structures; draining of earthen pond or treatment with quicklime before starting a new production cycle; avoid mixing fish of very different size in culture; regular observation of fish behavior; regular preventive treatment.

Clinical sign: at the beginning of the infection, no outward signs; operculum spread in advanced stage; fish gathered near the water inlet or oxygen sources; appetite loss.

Treatment: bath with formalin.

Dosage:

- 25 mL.m^{-3} for 24 h, once a week, for preventive treatment;
- 40 mL.m^{-3} for 24 h in case of infection, need to repeat the treatment once after 24-h break.

EQUIPMENT AND TOOLS

Control and prevention

1 Low power stereo microscope (binocular) for accurate observation of parasites (recommended).

2 Stock solution of disinfectant and antiparasitic agents (formalin, MGO) for preventive treatments.

Disinfection

1 Quicklime, formalin, chlorine bleach.
2 Measuring bucket or cup for water volume measurement.
3 Syringe for measuring drugs.
4 Plastic bucket for mixing drugs with water.

Injection

1 Sterile syringe with suitable needle.
2 Ampoule of Oxytetracycline in liquid form (injectable antibiotic).

Blending drugs with food

1 Balance for weighing food and drugs generally in powder form.
2 Syringe for measuring oil.
3 Plastic container for blending food, drugs and oil.

Mixing drugs with water

1 Balance for weighing drugs in powder form (Oxytetracycline, Malachite Green Oxalate).
2 Syringe for measuring drugs in liquid form (formalin).
3 Plastic bucket for preparing stock solution.
4 Measuring cup for distributing among the structures to treat.

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RECYCLING WATER SYSTEM: RUDIMENTS

A recycling water system is a chain of water treatment, each part corresponding to a specific function. Numerous equipments types are available for each function but in this work we will describe the rudiments of this technology through a simple system already used in Indonesia for the larval rearing of Pangasiidae and other fish species.

The six main elements of a recycling water system are described hereafter.

- Pump: to maintain a good water quality, the pump chosen for the system must have enough power to recirculate the water volume of the rearing tanks about 3 times per hour.
- Mechanical Filter: Mechanical filters are used to remove organic particles such as uneaten feed and feces in order to clean rearing water. This type of filter is generally located as close as possible from the outlet of the rearing tank.
- Biological filter or nitrification unit: Ammonia and nitrogen compounds excreted by the fish in the culture medium result also from the decomposition of feces and uneaten feed. Their concentration in water should not exceed lethal levels. These compounds can be removed or transformed to non-toxic materials with the help of a biological purification system.
- Oxygen supply: Oxygen is consumed by the fish, by bacteria in the biological filter and by decomposition of organic waste products. As low oxygen levels will reduce growth and feed conversion rates, it is important to maintain the dissolved oxygen concentration at a sufficient level, generally above 5 $mg.L^{-1}$ at 28 – 30°C. Oxygen can be easily added in the system using blowers, making a water fall or increasing the water flow.
- Pathogen regulation: Disinfection of water is necessary for larval rearing. For recycling water, technologies such as sterilization by ultraviolet, chlorine or ozone could be used. However they are expensive and difficult to implement for many farmers. In order to avoid bacteria, fungus or parasite development in the rearing water, we recommend to give a preventive disinfectant bath each week (Chapter VIII).
- Thermal regulation: Energy conservation is one of the advantages of recycling system. Once the tank water has reached the optimal temperature, a small amount of energy is sufficient to maintain temperature. Temperature could be maintained by thermoelectric resistance or by thermal insulation.

EXAMPLE OF A SIMPLE RECYCLING WATER SYSTEM

The structure presented here was designed to develop an intermediary step in the production of fingerlings, helping to promote a familiy and urban fish production. This “home industry” system is workable with cheap local equipment available everywhere in Indonesia and could be installed easily and quickly in a room of the house. This unit was designed to rear a maximal number of 5000 larvae per tank during a period of 3 weeks, after which fish reach a body length of about 1 inch.

Description and management of the system

The description of the “home industry unit” functioning is summarized here with reference to the schematic representation of the system presented in Figure A1-1.

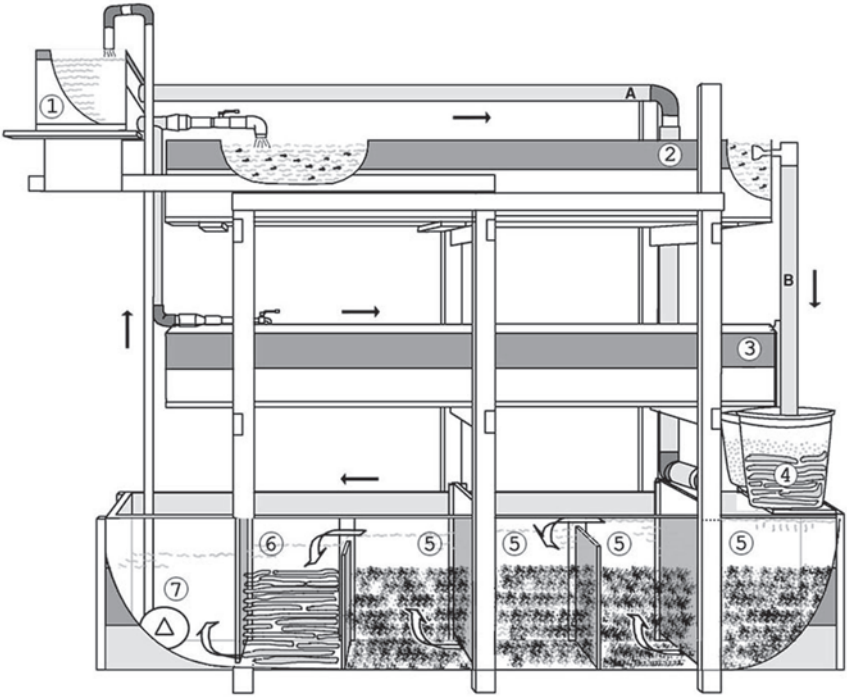

Figure A1-1.
Opened view and water circulation (arrows) of the recycling water system (home industry type) proposed by the “Catfish Asia Project”.

The numbers on the graph refer to the following elements and functional steps:

1. From the water tower (30 L), clear water is distributed by gravity to the rearing tanks or returned to the biological filter through the piping **A**. This "return" pipe is used for over-flow level control and for water oxygenation.

2 & 3. The two rearing tanks are built with wood, plastic foil and linoleum. Each tank has a water capacity of 250 L and is designed for a maximum of 5000 larvae, i.e. a maximal stocking density of 20 larvae per litre.

With the help of a stopcock, water flow can be regulated up to 750 L per hour allowing 3 complete volume exchanges per hour. The level of water is maintained by overflow (piping **B**) with an outlet covered with mosquito net to prevent escape of larvae from the rearing tank.

4. With the help of piping **B**, waste water (including excess of feed and feces) is remove by gravity to the mechanical filter made with foam-plastic mattress in plastic bucket.

5 & 6. Total overflow from piping **A** and **B** reach the biological filter with a stable outflow. Keeping a constant water speed in the filter increases the capacity of biological treatment. Compartments were made in the filter in order to increase the time during which the water is in contact with the biological filtering support. The initial four compartments (5) are filled with fibres from palm-tree *(Kakaban)* as support for the biological filtration and the fifth one (6) with foam-plastic mattress in order to filter impurities from palm-tree fibres.

7. After biological filtration, the water quality has been improved and water can be pumped up to the tower and distributed again in the rearing tanks. The water level in the pump compartment must be high enough to avoid pump cavitation. The pumping compartment is used also for refilling the system with new water and as control of the water level.

CLEANING AND WATER MANAGEMENT OF THE SYSTEM

Mechanical filter

Cleaning of the mechanical filter is required daily. For this purpose, two sets of plastic bucket fitted with foam-plastic mattress should be used;

one in service and one clean. This allows quick exchange of the filter for a clean one every morning. Afterwards, the dirty filter must be cleaned, dried and stocked for the next morning.

Biological filter

During the filtration process, the biological filter trap some waste from water and also nitrobacteria excrete mud. This detritic material should be cleaned by siphoning the bottom of the biological filter compartments after each cycle of larval rearing, i.e. every 3 or 4 weeks.

Level of water

To obtain the best dynamics of water, the level has to be 1 – 2 cm under the separation that is between compartment 5 and 6. In fact, the level has to be high enough in order to avoid pump cavitation. The pump compartment is used for filling the water supply and as a control of the water level.

Pathogen regulation

Regular preventive treatment with formalin is recommended as no resistance by the pathogen was observed with this drug. The dosage for recycling water system is explained in detail in Chapter VIII.

ARTEMIA NAUPLII: HATCHING, HARVESTING AND DISTRIBUTION

For a long time, freshly hatched *Artemia* nauplii have constituted not only the best, but also in most cases, the only available source of live food for larval stages of most cultured species of fish and crustaceans.

Ovoviviparous in certain environmental conditions, *Artemia* generally spawn inactive dry cysts, which remain "dormant" as long as they are kept dry. The "dormant" cysts are at the origin of the worldwide distribution of *Artemia* (Sorgeloos *et al.*, 1986) thanks to a vacuum packing. On top of that, the very simple procedure for hatching *Artemia* cysts gives an additional advantage to this brine shrimp.

Although Indonesian production of *Artemia* is starting, most *Artemia* used in this country is imported from Great Salt Lakes (U.S.A.). It makes them at very high cost for farmers.

Although more expensive than the other food types, the results of previous investigations on *P. djambal* larvae demonstrated that *Artemia* nauplii were clearly the most appreciated first prey.

So far from the experience gained on this fish, we recommend starting the farming of *P. djambal* larvae using *Artemia* nauplii as the first feed. In order to decrease the operating cost, limiting the period of feeding with *Artemia* nauplii to the four first days of feeding allows improved initial larval rearing (see Chapter VI and VII).

GUIDELINES FOR *ARTEMIA* CYSTS INCUBATION

Optimal hatching conditions

The standard incubation technique described below is widely used. It was developed by Sorgeloos *et al.* (1986), and adapted to local conditions by the team of "Catfish Asia project".

Although the technique of *Artemia* cysts incubation is very simple, not respecting the following guidelines may lead to lowering of nauplii yield.

- The standard procedure using plastic or fiber container from 20 up to 75-litre capacity is recommended. In fact, it is better to use smaller rather than larger containers because of potential problems of aeration, water pollution or harvest. Good repartition of air bubbles is required to maintain cysts in movement. For this purpose, funnel shaped tanks are currently used as incubation and hatching containers. For cheaper

investment, small-scale farmers use plastic buckets or recycled 4-gallon drinking water bottles.

- *Artemia* cysts could be incubated in natural seawater (salinity: 35 – 40 $g.L^{-1}$), however hatching rate is generally increased at 5 $g.L^{-1}$. The latter salinity is particularly recommended for producing *Artemia* nauplii used for feeding larvae of freshwater fish.
- To maintain a good level of oxygen during incubation, it is not advisable to exceed a density of 5 g of *Artemia* cysts per litre.
- Hatching containers have to be exposed to light continuously as the illumination of the cysts is essential for a maximum hatching rate.
- Continuous aeration from the bottom of the container ensures that all cysts are kept in suspension and guaranties good oxygen supply. For a better homogenization of cysts and oxygenation of water, airflow should be increased when the volume of water is increased. Optimal airflow is 7 $L.min^{-1}$ for 20 litre incubation tank and 20 $L.min^{-1}$ in 75 litre incubation containers.
- The optimal range of water temperature for cyst incubation is 25 – 30°C and temperature should be maintained as constant as possible to obtain the best hatching rates. Temperature of incubation water could be kept constant by a submersible water heater or by keeping hatching containers in an air-conditioned room.

Although following these standard conditions for incubation of cysts should optimize hatching rate, each provider gives some practical and specific indications. Indeed, the number of cysts per gram, the optimal temperature and the duration of incubation may vary depending on the origin of the cysts. As the duration of incubation is related to water temperature and origin of *Artemia*, we recommend that the farmer, for a good timing of the nauplii production, check the time of hatching when using a new cyst brand for the first time.

HARVEST TECHNIQUE

After complete hatching, it is necessary to stop aeration for 5 to 10 minutes in order to allow the nauplii to concentrate in the lower part of the funnel tank. The empty cysts are buoyant and come to the surface. Unhatched cysts and heavy particles sink to the bottom just under the nauplii. As *Artemia* nauplii are phototropic (attracted by light), their concentration can be strongly increased by lighting a part of the tank.

Undesirable particles should be removed in order to avoid mixing cysts and nauplii. Two ways are used and can be summarized as follows:

- for the 20-litre transparent plastic bucket floating cysts are removed;
- for the tanks of bigger volume, the sinking particles are drained off from the very bottom by siphoning.

The collection of nauplii is done by siphoning using a plastic pipe placed in the middle of the gathered nauplii. They are concentrated on an 80 – 125 µm screen placed at the siphon outlet in a plastic bucket in order to maintain nauplii in water. Siphoning should be stopped when the concentration of nauplii in the collected water is very low and before the number of cysts begins to increase.

The water color indicates the concentration of nauplii and the proportion of cysts. Since, nauplii give a bright orange color to the water and the cysts a light brown color. A blending of nauplii and cysts give an intermediate color.

A second harvest of newly hatched nauplii could be eventually done about 10 – 15 minutes after the first one.

In order to avoid pollution by hatching wastes and bacterial development, collected *Artemia* nauplii should be rinsed in the 80 – 125 µm screen with clear water and stocked in a plastic bucket filled with water containing 5 $g.L^{-1}$ of salt with aeration.

STORAGE, COUNTING AND DISTRIBUTION

Storage

Development of *Artemia* at temperature 28 – 30°C is very rapid and their energy reserves decrease rapidly. In fact, it was demonstrated that the result of larval rearing is significantly lower when using nauplii older than 10-h.

Storage with insufficient aeration during a few hours can cause nauplii mortality, particularly at a temperatures up to 25°C .

To avoid such situations, we recommend distributing *Artemia* nauplii as soon as possible after hatching or storing them with smooth aeration at low temperature (0 – 4°C) for the shortest time possible and never more than 48-h.

Counting

There are two ways for evaluating number of nauplii; 1) estimating the theoretical number from the number of cysts placed in incubation, 2) counting the number of nauplii harvested after dilution to reduce their abundance.

As the first way depends on the hatching rate, the actual number of collected nauplii could be very far from that estimated from the initial number of cysts. The second way which is more accurate is recommended and should respect the following steps:

1 increase aeration in the plastic bucket to obtain an homogenous dispersion of nauplii into the water;

2 take a sample of 10 mL of the nauplii suspension with a measuring pipette;

3 dilute the pipette contents in 1 litre of clean water;

4 carefully mix the suspension to obtain an homogeneous dispersion of nauplii;

5 from the 1 L nauplii suspension take a new sample of 10 mL with a clean measuring pipette;

6 in a beam of the light count the number of nauplii caught in the last sampling;

7 put the pipette contents back in the 1 litre stock;

8 operations 4 to 7 should be repeated 9 times in order to obtain a reliable estimation;

9 the average of the 10 counting multiplied by 10 (to correct for dilution), give the number of nauplii per mL stored in the plastic bucket;

10 this concentration (nauplii per mL) multiplied by the volume of the plastic bucket gives the total number of harvested *Artemia* nauplii.

Accurate evaluation of the *Artemia* nauplii quantity to distribute

For reasons of economy, it is very important to evaluate mortality of larvae during rearing in order to distribute not more than the necessary *Artemia* nauplii quantity. Indeed, if during the first 2 days of rearing the mortality rate reaches 30%, the quantity of nauplii distributed should be counted for the remaining larvae (70% from initial number), i.e. 5250 larvae from 7500.

The following example gives the procedure used for an accurate estimation of the quantity of *Artemia* nauplii to distribute to a batch of 7500 larvae starting from a stock at a concentration of 500 nauplii per mL.

1. To calculate the *Artemia* nauplii quantity to distribute at the first feeding, the number of *Artemia* nauplii necessary for one larvae per distribution (given in table VII.2) is multiplied by the total number of larvae reared in the tank,

i.e. 20 x 7500 = 150 000 *Artemia* nauplii for one distribution (Table A2.1).

2. To calculate the volume of the *Artemia* nauplii solution to distribute, just divide the previous total number of *Artemia* nauplii (150 000) by the concentration of harvested *Artemia* nauplii previously estimated from the stock suspension (500 nauplii per mL),

   i.e. 150 000 / 500 = 300 mL of the stock suspension of harvested *Artemia* nauplii.

Table A2.1.

Method for calculating the *Artemia* nauplii number to give per feeding according to three parameters: concentration of harvested *Artemia* nauplii, age and number of larvae.

Age of larvae	Time of feeding	Number of *Artemia* per mL	Number of *Artemia* per larvae and per feeding	Number of *Artemia* for 7500 larvae	Volume of *Artemia* solution to use for one meal	
First *Artemia* harvest with a concentration of 500 nauplii per mL						
First feeding [1]	06:00	500	20	150 000	150 000 / 500	300 mL
2 days	09:00	500	20	150 000	150 000 / 500	300 mL
	12:00	500	20	150 000	150 000 / 500	300 mL
	15:00	500	20	150 000	150 000 / 500	300 mL
	18:00	500	20	150 000	150 000 / 500	300 mL
New *Artemia* harvest with a new concentration of 800 *Artemia* nauplii per mL						
2 days	21:00	800	20	150 000	150 000 / 800	188 mL
	00:00	800	20	150 000	150 000 / 800	188 mL
3 days	06:00	800	50	375 000	375 000 / 800	469 mL
	09:00	800	50	375 000	375 000 / 800	469 mL
	12:00	800	50	375 000	375 000 / 800	469 mL
	15:00	800	50	375 000	375 000 / 800	469 mL
	18:00	800	50	375 000	375 000 / 800	469 mL
New harvest of *Artemia* nauplii						

[1] 48 h after hatching.

Distribution

The assessment of *Artemia* nauplii harvested allows estimating the daily number of prey distributed. From the estimation of nauplii concentration, the total number of prey collected is known according to the volume of stock suspension.

In order to avoid pollution and bacterial development from hatching waste, collected *Artemia* nauplii should be rinsed before distribution as follows:

1. take the necessary volume of nauplii for feeding;
2. rinse nauplii in the 80 – 125 µm screen with clear water;
3. in order to keep the same concentration of nauplii, fill a plastic bowl with the same volume of clear freshwater as taken previously;
4. drain off the washed nauplii and place them in the plastic bowl using the screen;
5. the initial volume and nauplii concentration are preserved;
6. feeding can be carried out.

EQUIPMENT AND TOOLS

Artemia cyst incubation

1. *Artemia* cysts.
2. Fiber or plastic container of 20 litre or 75 litre.
3. Clean freshwater.
4. Salt.
5. Light source.
6. Air blower with airstone.
7. Small scales to weigh *Artemia* cysts or measuring cup.

Harvest

1. Plastic pipe for siphoning.
2. Light for gathering *Artemia* nauplii.
3. 80 – 125 µm screen for concentrating nauplii.
4. Plastic bucket with over flow for maintaining the level of water in previous screen.
5. Salt water at minimal concentration of 5 $g.L^{-1}$.

Storage

1. Air blower with airstone for aerating nauplii.
2. Block of ice or refrigerator for slowing down development of nauplii.
3. Plastic bucket.
4. Salt water at minimal NaCl concentration of 5 $g.L^{-1}$.

Counting

1. Air blower with airstone for homogenizing nauplii.
2. Measuring cup with 1 litre of clean salt water at a salt concentration of 5 $g.L^{-1}$.
3. Measuring pipette of 10 mL.
4. Light source for counting nauplii.
5. Pocket calculator.

Distribution

1. 80 – 125 µm screen.
2. Clear freshwater for washing out salt from nauplii.
3. Measuring plastic bowl.

REFERENCE

Sorgeloos, P., P. Lavens, P. Leger, W. Tackaert and D. Versichele, 1986. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. *Prepared for Belgian Administration for Development Cooperation and the Food and Agriculture Organization of the United Nations.* State University of Ghent, Belgium. 319 p.

Technical Manual For Artificial Propagation Of The Indonesian Catfish, *Pangasius djambal*

Although 14 pangasiid species have been recognised in the Indonesian ichtyofauna, Pangasianodon hypophthalmus *initially introduced from Thailand was until recently the only pangasiid cultivated in Indonesia. Aiming to utilize local biological diversity, the potentialities of Indonesian* Pangasius *species for fish culture have been investigated since 1996 throughout the «Catfish Asia» research program supported by European Union. Among these species,* Pangasius djambal *Bleeker, 1846, was pre-selected as a new aquaculture candidate for its large size (more than 20 kg individual body weight), for its broad geographic distribution and for its popularity among consumers from Sumatra or other Indonesian islands. Zootechnical evaluation of this species has demonstrated its valuable characteristics for aquaculture. Artificial breeding was firstly achieved in 1997.*

Based on six years of working experience, this technical manual attempts to provide fish farmers and scientists with elements on the biology of P. djambal *and all technical guidelines allowing to succeed in its artificial propagation. After providing an identification key to* P. djambal*, practical aspects related to fish transportation, broodstock management, induced spawning, artificial fertilization, egg incubation, larval rearing and fish health management are developed.*

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