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PROVINCIAL AQUACULTURE DEVELOPMENT PROJECT

LAO PDR

SUPPORT FOR TECHNICAL SERVICES

Small-Scale Fish Hatcheries for Lao PDR

Based on the work of

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The Provincial Aquaculture Development Project (LAO/97/007) is a nationally executed, UNDP funded project working in five provinces in Lao PDR. Through its activities LAO/97/007 aims to :

1. ***Improve fish fry production from government hatcheries through structural improvements and training***

2. ***Support fish fry production by farmers and entrepreneurs through the extension of simple appropriate technology.***

3. ***Develop the capacity of Department of Livestock and Fisheries staff to plan and conduct extension of fish culture techniques to farmers.***

4. ***Form farmers groups and extend improved fish culture techniques as part of the Department of Livestock and Fisheries extension process.***

5. ***Assist farmers and small-scale hatchery entrepreneurs to undertake aquaculture activities through provision of fish fry, broodstock and facilitate access to credit.***

LAO/97/007 is working with the Provincial Livestock and Fisheries Section and farmers groups in: Oudomxay, Sayaboury, Xieng Khouang, Savannakhet and Sekong Provinces. Additional technical assistance and training is also provided to Livestock and Fisheries staff and farmers in other provinces.

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"*Small-scale Fish Hatcheries for Lao PDR*" is also available in Lao.

SMALL-SCALE FISH HATCHERIES for Lao PDR

By

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INTRODUCTION

Lao PDR is a land locked country located within Southeast Asia and has a wide range of climatic environments. The country is composed of mountainous upland regions and lowland plains with a total area of approximately 236,800 km^2. Upland areas constitute approximately 75% of the total area of the country, with the lowlands occupying approximately 60,000 km^2. The country has 57 major rivers mainly running from the north to the south of the country. The lowland areas of Lao PDR are suitable for aquaculture, although aquaculture is practised in some of the upland areas.

Lao PDR has a monsoon season that lasts between May to October allowing rice cultivation in rainfed or irrigated paddies. Upland hill rice cultivation is also widely practised. During the monsoon season the Lao people obtain a significant amount of their nutrition from aquatic animals and fish. These are hunted in paddy fields and water bodies created by the monsoon rains. After the monsoon, the topography of the country is such that waters recede rapidly into the main river bodies and there is an extended dry season where many water bodies dry up completely. During this period there is a shortage of fish and aquatic products, although the Lao tradition of preserving fish in times of abundance compensates for this to some extent. Fish preservation techniques include: fermenting, drying, smoking and salting. Since fish is popular to the Lao people, it often commands a high market price, which makes the capture and culture of fish economically attractive.

Fish fry demand in the Lao PDR is high for stocking into both ponds and rice-fish systems, however fry/fingerling production is low. Most fry produced in Lao PDR originate from the Provincial government hatcheries, which have an overall production level of less than 15 million.

The species currently produced by Lao hatcheries include Common carp, Tilapia, Common silver Barb, Rohu, Mrigal, Bighead carp, Silver carp and Grass carp. There are at least six *Puntius* species indigenous to Lao PDR that show promise as aquaculture species. Besides the barbs, there are also several other possible species that may be suitable for hatchery production; these are: *Pristolepis fasciatus* (Lao name 'Pa gah'), Giant gourami (*Osphoronemus gouramy*) and Snakeskin gourami (*Trichogaster pectoralis*).

The Lao Department of Livestock and Fisheries estimates that nationwide, demand for fish fingerlings is approximately 52 million. There are significant imports of fry and fingerlings from Thailand, Vietnam and China, but this still does not satisfy demand. A reflection of this demand is the high price that fry in Lao PDR can fetch relative to the surrounding countries (typically 50 – 300 % higher). The problem with fry and fingerling shortages is partly due to the lack of hatcheries in the country, but also due to the difficulties in communications between provinces. In this respect large centralised hatcheries are not particularly suitable for fry production due to the logistical difficulties with delivery to the farm locations. Small-scale hatcheries can be effective in producing low cost fry in the locations where fry/fingerlings are required.

An additional attraction of mini-hatcheries is that they are appropriate for low investment situations and thus present income generation opportunities for lower income farmers.

As a result of this situation, the government of Lao PDR places a high priority on improving fry production from small-scale hatcheries in Lao PDR.

Importance of Small-scale Hatcheries

Small-scale hatcheries are an attractive development activity since they present relatively low risk to farmers and can return their investment quickly for the following reasons:

1. Small size results in low construction cost
2. Small size is easy to manage at family scale
3. They do not always require sophisticated equipment (pumps, aerators, electricity etc.)
4. The range of species that can be produced are not restricted by hatchery size
5. They can be located almost anywhere that has a suitable supply of water
6. They can be located close to the farmers that will purchase the fry

CHAPTER 1 - SMALL-SCALE HATCHERY REQUIREMENTS

1.1 Water and water quality

Water is essential for all aspects of fish production – from production of suitable broodstock to egg production, fry and nursery production. Small-scale hatcheries must have a supply of good quality water for successful production and ideally must have a supply of water all year round. Suitable inlet water quality parameters for hatchery production are:

- pH between 6.5 – 8.5 (pH below 7.0 will require storage in a reservoir and the addition of some lime)
- Pond water transparency of 30 – 60 cm (inlet water to hatchery can be clearer)
- Temperature between 23 – 32^{o}C (higher temperatures are acceptable but not desirable)
- Oxygen concentration not less than 3 ppm (this can be difficult to achieve if water temperatures are high)
- Alkalinity between 100 – 200 mg.l^{-1}.

In the consideration of appropriate water sources it is desirable to use water from lakes or rivers that have some degree of water exchange. The presence of healthy populations of wild fish is a good indicator that the water is of reasonable quality.

Water supplies that may be contaminated by urban or industrial effluents are not suitable for fish hatcheries since this water is often polluted with organic matter or toxic chemicals. Water that originates as run-off from agricultural land may be unsuitable if the agricultural method involves the use of chemicals and pesticides. Apart from potential contamination of wastewater from housing and towns, these situations are not common in Lao PDR at the moment.

Figure 1. Water supplies from paddy fields can be muddy due to paddy ploughing during monsoon season

River water in Lao PDR undergoes wide changes in quality between the dry and the monsoon seasons. In the monsoon season many water sources become heavily loaded with suspended solids and this water can be unsuitable for egg hatching and fish fry production (Figure 1).

The water source should ideally be higher than the nursery tanks so that water can be supplied under some pressure to assist aeration and ensure adequate water flow. Typically the height of the water source or reservoir pond should be more than 2 metres above the top of the nursery tanks (Figure 2).

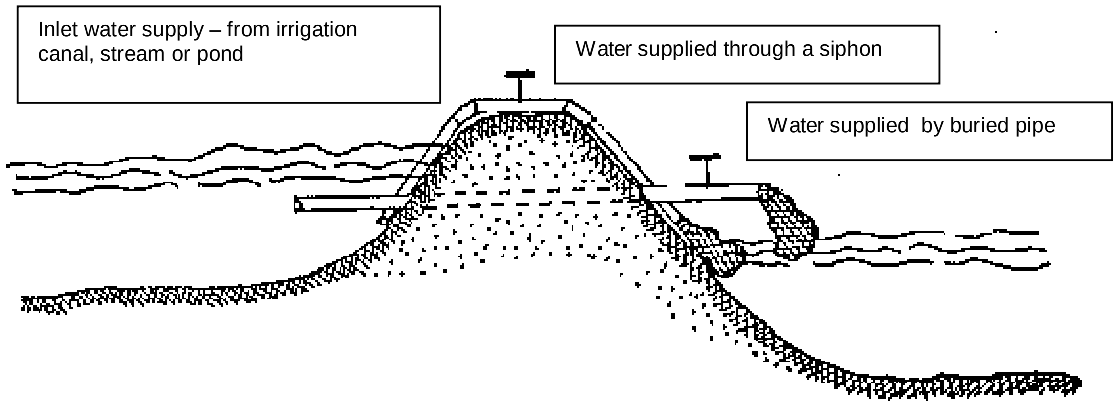
Figure 2. Excellent water source from clear-water stream (dry season flow)

Water inlets for hatcheries can be divided into two basic types:

- Water inlet higher than the hatchery reservoir and tanks (recommended for Lao PDR). This type of inlet is typical of irrigation canals, hillside streams and terraced paddy fields (Diagram 1, Figure 2).
- Water inlet lower than reservoir requiring pumping to obtain water. This type of inlet is common in lowland areas where water levels in lakes, rivers and streams are often much lower than the surrounding land. In this case water must be pumped upwards and stored in a reservoir (Diagram 2).

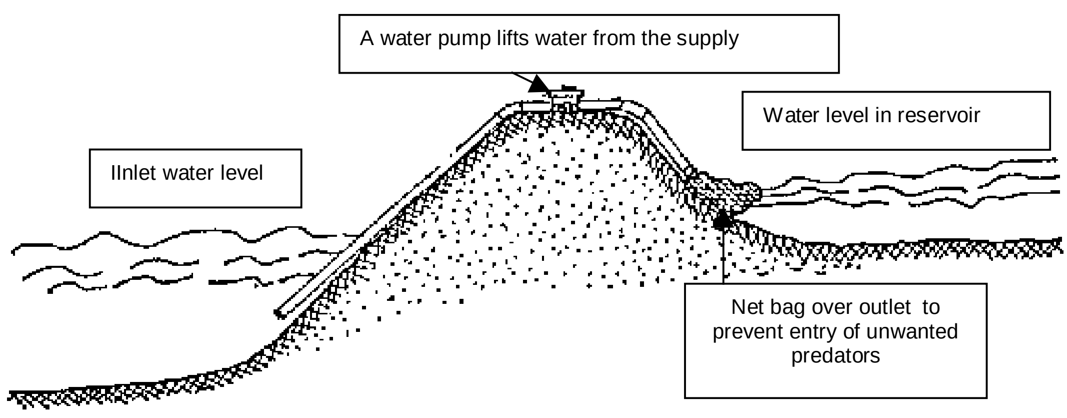
The type of water supply presented in Diagram 1. can use a buried pipe to transmit water to the reservoir and the water will be pressurised. If a siphon system is used water can be moved uphill if necessary provided the outlet of the pipe is lower than the inlet. For siphon pipe type water transfer strong-walled flexible pipe should be used (soft walled pipe will collapse under the suction).

Diagram 1. Water inlet higher than the hatchery



Where water must be pumped upwards to a water storage pond/tank as represented in Diagram 2., the cost of water supply must be considered. This includes the price of the pump and the fuel/electricity required for operation. For some types of pump a foot valve on the pipe inlet is desirable to prevent the water draining out of the pipe when the pump is stopped.

Diagram 2. Water supply is lower or at the same level as the hatchery.



1.2 Earthen ponds

Culture in earthen ponds is the best method for maintenance of good quality, healthy broodstock. Earthen ponds are also very suitable for nursing of fry until they reach a size of 2 –3 cm (or approximately 1 month old). This is because earthen ponds contain a great deal of natural feed that can supplement applied feeds to the pond.

Reservoir

This is used to store inlet water before use in the hatchery and nursery. Inlet water sometimes has a high level of suspended solids, which it is desirable to settle out. In situations where water supply is not continuous, a reservoir will allow hatchery operation to continue without interruption. A small-scale hatchery should ideally have a reservoir of 40 x 40 m (1600 m^2) with a depth of about 1.5 m. the reservoir can be used as a broodstock pond in small operations.

Broodstock culture ponds

These ponds can be the same size of the reservoir (1600 m^2) and should be approximately 1.5 – 2.0 m deep. If they are too shallow, the broodstock growth will be poor and the ponds will tend to dry up completely in the dry season. Ideally the hatchery should have two ponds, but if broodstock can be obtained from outside the farm, maintenance of broodstock may not always be necessary.

Nursery ponds

These ponds should be no larger than 40 x 40 m and should be 1 – 1.5 m deep. Smaller ponds can be used if only small quantities of fry are produced (i.e. from natural or semi-natural spawning techniques). Two or more nursery ponds are desirable.

1.3 Hatchery building and equipment

Hatchery building

This should have an area sufficient to cover all of the hatchery tanks and can be constructed with available materials. Wood, bamboo, thatch or corrugated iron can be used for the construction. Thatch is both cheaper and cooler than corrugated iron and the roof should be at least 2 metres above the tanks. The use of the building is to shade the spawning and hatching tanks. High investment in this structure is not desirable until the hatchery has started to yield reliable profits.

Water supply and storage tank

Water needs to be supplied to the spawning and hatching tanks under pressure. This can be achieved in several ways.

- Water in a reservoir pond, or water supply to hatchery, is higher than the hatchery spawning and hatching tanks and supplied directly by gravity. A coarse screen should be used to prevent entry of undesirable particles and potential predatory organisms.
- The water for the tanks is stored in a tank higher than the spawning and hatching tanks (ideally more than 2m difference in height). This may require a pump to lift the water if the reservoir pond is not higher than the spawning and hatching tanks. The volume of this tank should be approximately 1,000 – 2,000 litres (Diagram 3).
- Water is supplied under pressure from a reservoir tank by using a water pump (this requires electricity). This tank can be large (2,000 – 6,000 litres) and should be located under the roof to prevent the water from being heated by direct sunlight (Diagram 4).

Diagram 3. Water supply to hatchery tanks from a reservoir tank higher than the hatchery tanks

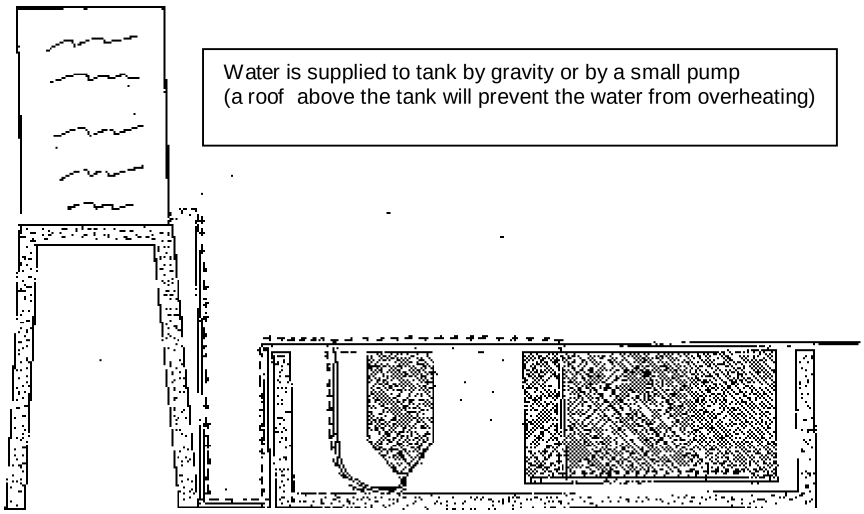
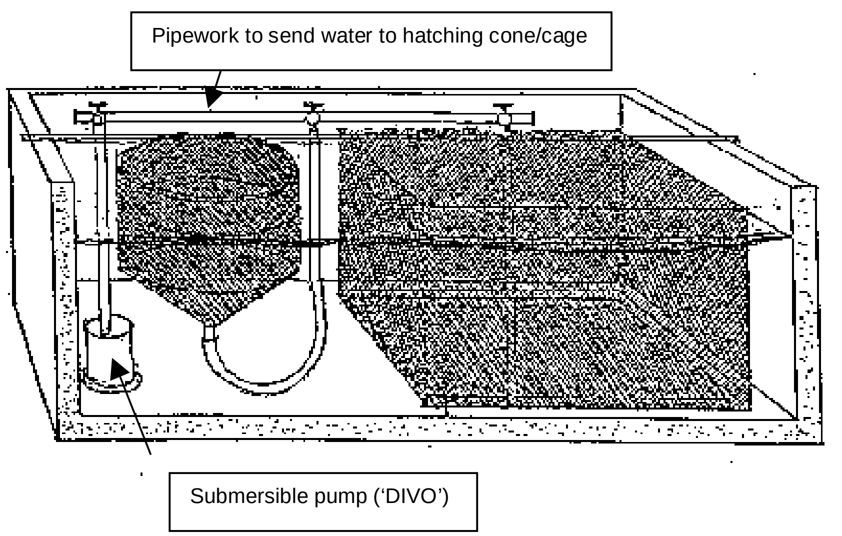


Diagram 4. Water is supplied to hatching containers by a submersible pump inside the cement tank (this requires electricity)



Egg hatching and nursery tanks

Tanks are typically constructed from bricks faced with cement. It is important not to let the tanks dry out completely for long periods since this will cause the cement to crack, affecting water retention and strength of the tank walls. Other construction materials include wooden walls with a plastic liner to retain the water. Fibreglass water storage tanks are available, but are often too expensive for these purposes.

These tanks can be constructed from the same materials as above. They are used for hatching eggs and also for the first nursing period of the hatched fry. The size and shape of the tanks depends upon the hatching method. Typical sizes are 2 x 3m with a depth of approximately 1 m. slightly deeper tanks are desirable if the hatching cone method is to be used (see later text). Two or more tanks of this type are desirable.

PVC pipes and fittings

PVC pipework is cheap and widely available in Lao PDR. It is used for supply of water between ponds and reservoirs and also supply to hatchery and nursery tanks. If PVC pipe is not available, flexible plastic hose can be used or even bamboo piping.

Water supplied to a hatchery via channels is not recommended since there is no pressure to spray water and enhance oxygenation. Open channels often cannot supply water for upwelling hatching cones also.

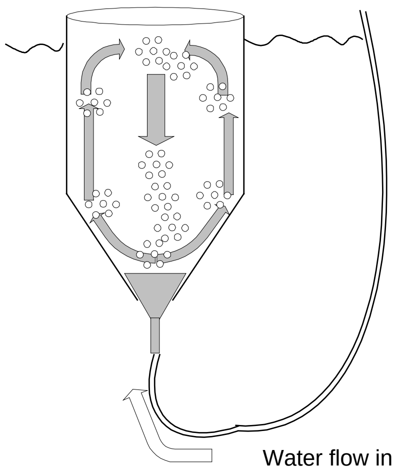
'Orlon' hatching cones

These hatching cones allow water to upwell and circulate eggs during incubation. This supplies oxygenated water continuously and also cleans the eggs and prevents clumping and suffocation. The hatching cone is approximately 50 – 70 cm high and approximately 40 cm wide. 'Orlon' cloth is available from Vientiane and widely available in Thailand. Other types of cloth are suitable but may become clogged if the water supply has high suspended solids. Between 2 – 6 hatching cones would be required by a small-scale hatchery (Diagram 5).

'Orlon' Hatching cages

These cages can be used for nursing newly hatched fish fry. They can also be used for incubation and hatching of fish eggs. For species which have sticky eggs these cages can also be effective for hatching. Typically a cage is 2 x 1 m and approximately 50 – 60cm high. These cages can be placed inside the concrete nursery tanks and supplied water by spraying to increase oxygenation. A PVC pipe frame placed in the bottom of the cage can be used to supply water to ensure that eggs and fry are upwelled gently. As with the hatching cones, 2 – 6 cages are required by a small-scale hatchery (Diagram 6).

Diagram 5. 'Orlon' upwelling hatching cone



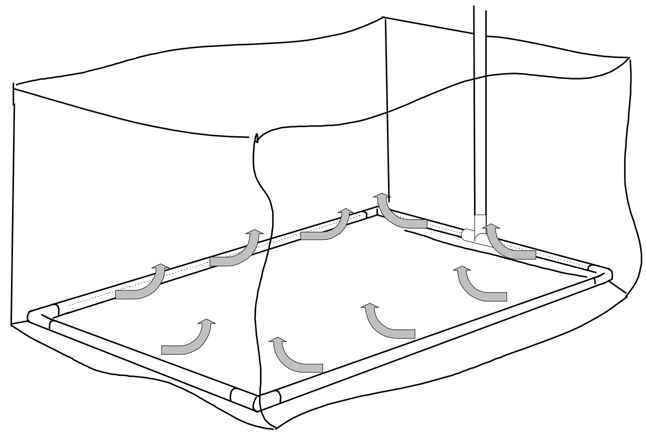


Diagram 6. 'Orlon' cloth hatching cage
(Note: water flow through holes in PVC pipe frame)

Electricity supply

Many potential hatchery locations in LAO PDR do not have an electricity supply. In these circumstances water must be supplied by gravity from a water source above the level of the hatchery. This is usually a stream or irrigation channel. Upland areas with terraced rice fields have a good potential since water flows downwards so the hatchery can be positioned below the rice paddy. Pipework can be used to supply water from a stream to the hatchery.

Where electricity is available, hatcheries can benefit form the use of low cost electrical water pumps and also aeration equipment. Aeration and water pumping can significantly improve the production and survival of fish fry from a hatchery and are very desirable if available.

The installation of electrical equipment can increase the hatchery production cost significantly. Where electricity is not available, small 220V AC generators powered by flowing water can be used (e.g. Xieng Khouang and Oudomxay). Since the water supply that runs these generators is also suitable for small-scale hatcheries, this has good potential for upland areas.

The use of 12V car batteries for small aerators has also been successful for short periods if a mains battery charger is available locally.

Pumps

Water pumps are required for some small-scale hatcheries for moving water as follows:

- Pumping water from water supply to storage tank/reservoir
- Pumping water from reservoir to hatchery/nursery tanks
- Pumping water into and out of nursery ponds

If the hatchery is constructed on sloping land and the water supply is higher than the hatchery, pumps may not be necessary (see Diagram 1).

Aeration

Aeration of water for fish culture is essential for good production. Water splashing and water movement can increase aeration sufficient for fish production. If the water supply to the hatchery is correctly constructed then aeration is often not a problem, provided that water supply is continuous. In hatcheries with limited water supply or water flow, aeration can be enhanced by using electrical aerators. There are several types of aerator that can be used in small-scale hatcheries according to the amount of investment available and electricity supply.

- Compressor type – this is often used in Lao PDR for motorcycle and tyre repairs. The compressor type of aerator supplies a low volume of air at high pressure and is not generally recommended. It is however, widely available in Lao PDR and therefore can be used if mains (220V) electricity is available.
- Aquarium type aerator – these are used for small aquariums and can be used for small applications. These types of aerators are usually 220V mains electricity, although 12V versions are also produced. This type of aerator can only be used for one tank so if several tanks contain eggs or fry more than one is required. This can increase costs of hatchery construction.
- Air blower type – this is the best form of aerator since a high volume of air is delivered allowing aeration of the entire hatchery. The cost of purchase of this type of aerator, and the requirement for 220V electricity deters their use.

Diagram 7. If no aeration or water flow is available – manual stirring of the water for aeration can be performed

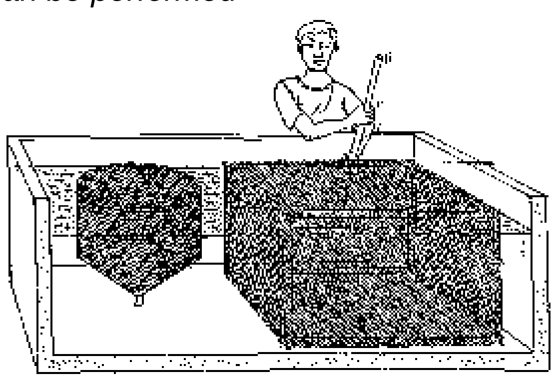
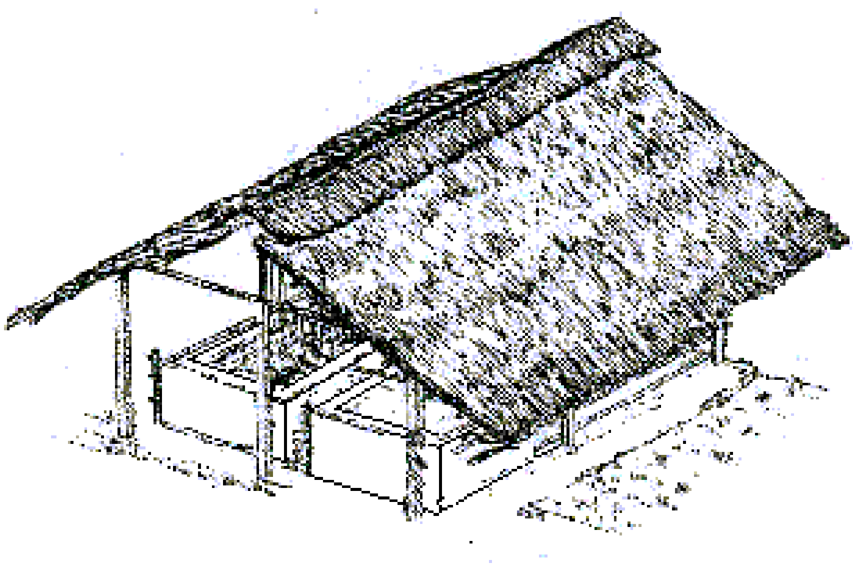


Diagram 8. Small scale hatchery building with cement tanks



CHAPTER 2 - BROODSTOCK

2.1 Source of Broodstock

Good quality mature broodstock are essential for successful production of fish fry and they can be obtained from the following locations:

From broodstock culture ponds

Ideally, hatcheries should have their own broodstock culture ponds so that they have a ready supply of broodfish for spawning. Broodstock ponds also allow the hatchery to ensure that the nutrition of the fish is adequate and the fish are of good quality. Maintenance of broodstock ponds involves expenditure throughout the year, which may be undesirable for small operations (in these cases the broodstock ponds are usually fish culture ponds). In Lao PDR the extended dry season can be a constraint to the all-year culture of broodstock if water is not available. In cases such as this, the broodstock must be obtained from outside of the farm.

From fish culture ponds

During the spawning season fish can be taken from culture ponds if they are large enough and have reached maturity. The size at which fish reach maturity varies between species and ideal broodstock sizes are presented in Table 2. Care must be taken to ensure that broodstock females are mature (usually indicated by a swollen abdomen) and not just too fat (this is not a common feature of Lao fish since they are unlikely to be overfed).

From the wild

Mature fish can be caught in the natural spawning grounds during the spawning season. This requires some local knowledge of the species and its habitats. Wild fish can be caught in rivers, lakes and streams. Care must be taken not to damage the fish during catching and transport to the hatchery since this will reduce their quality for spawning. Fish should be transported in bags filled with oxygen, or in water filled tanks together with some form of oxygenation. The fish should be transported during the coolest part of the day to avoid overheating.

From live fish markets

Some fish species (particularly catfish, snakeskin gouramy, common carp, Tilapia) and transported live to markets for sale. The fish should be in good condition and not bruised or damaged. If possible the fish should be checked for maturity although this is difficult in the case of catfish due to their shape and hard abdomen. The fully mature fish should be purchased during the spawning season for that species.

Table 1. Stocking rates and feeding rates for broodstock

SPECIES	STOCKING RATE	FEEDING	
		TYPE OF FEED	FEEDING RATE (% OF BODY WEIGHT)
Common silver barb	Separate sexes 1,000 pieces/1600 m^2	Catfish starter feed, Sprouted corn, Rice bran, Broken rice	1-2 %
Common carp	Separate sexes 800 pieces/1600 m^2	Herbivorous fish pellet Sprouted corn Rice bran, Broken rice	1-2 %
Rohu Mrigal	200 pieces/1600 m^2 (female to male=1:1)	Herbivorous fish pellet, Sprouted corn, Rice bran, Broken rice	2 - 3 %
Silver carp Big head carp	Sexes can be mixed 160-200 pieces/1600 m^2	Herbivorous fish pellet, Sprouted corn, rice bran, broken rice	1 - 2 %
Grass carp	Sexes can be mixed 160-200 pieces/1600 m^2	Grass Sprouted corn	1 - 2 %
Striped catfish	Sexes can be mixed 400 pieces/1600 m^2	Catfish growout feed	3 %
Walking catfish	Sexes can be mixed 16,000 pieces/1600 m^2	Catfish growout feed	2-3%

2.2 Broodstock culture

Culture of fish for broodstock has a different objective from culture for consumption. Fish cultured for consumption are often fat due to the nature of the feeding. This is not common in Lao PDR where feeds are applied sparingly to ponds (if at all). Culture of fish for use as broodstock requires the following features:

Culture pond

The broodstock culture pond should be of an appropriate size and depth for the species cultured. This means that fish that mature at a large size (*e.g.* Chinese carp and Indian carp) require a relatively large pond area for growth and maturation. This is often difficult to achieve in Lao PDR and results in slow growth rates for these species and the quality of broodstock is poor as a result.

Stocking rate

Broodstock are cultured at lower densities than those used for fish for consumption. Stocking rates vary and are presented in Table 2.

Feed

Feed for broodstock should be of good nutritional value and should be provided in sufficient quantity for good growth. Poor quality feed will affect the maturation of broodstock and will result in poor spawning success. Herbivorous fish require a protein level in their diet of approximately 20% and should be fed at a level of 1 - 2 % of body weight per day (see Table 1). Typical feeds include rice bran, fresh plants, sprouted cereals (corn, wheat). Grass carp can be fed grasses and other vegetation. Application of manure to maintain a good water colour is also recommended since this enhances the natural production of the pond. Since broodstock ponds are stocked at low density natural feeds play an important role in the condition and quality of the broodstock.

Culture environment

The broodstock pond must not be a stressful environment for the fish. The water in the pond must not become deteriorated with wastes (*e.g.* from overfeeding). The ponds should be reasonably deep so that sufficient water is maintained all year round and does not become excessively hot (1 - 2 metres). Some water supply to the pond (either pumped from a water supply, or inflow from a stream, river or irrigation canal) is desirable in this respect. If the pond soil tends to be acidic, this can be neutralised by addition of lime.

Management of fish

Since some species are able to reproduce in a culture pond under the right conditions (*e.g.* silver barb, common carp), separation of the sexes is necessary at the start of the spawning season. If the sexes are not separated the females may spawn in the pond and thus become unfit for use in the hatchery.

2.3 Broodstock selection for spawning

Good quality broodstock should be used in fish hatcheries to ensure good spawning and fry survival. Damaged or diseased fish should not be used. The scales and fins of the fish should be intact and the fish should not be deformed (evidence of genetic or nutritional disorder). It is important that the fish are old enough to be mature to ensure good egg production. Some hatcheries use fish that are too young and are not fully mature or barely mature, the result is poor spawning and fertilisation success. The criteria for mature broodstock suitable for spawning are presented in Table 2.

Table 2. Age, weight and fecundity of mature broodstock fish

Species	Maturation age	Maturation weight	Fecundity (eggs/kilo weight)
Common silver barb	6 months - 1 year	0.4 - 0.8 Kg	320,000 eggs / kilo 1,500 eggs / gram
Common carp	6 months -1 year	0.3 - 1.0 Kg	80,000 eggs / kilo
Mrigal	2 - 5 years	1 - 2 Kg	110,000 eggs / kilo 900 eggs / gram
Rohu	Over 2 years	1 - 2 Kg	200,000 eggs / kilo
Silver carp Big head carp Grass carp	2 years	1.5 - 2 Kg	200,000 eggs / kilo 700 - 750 eggs / gram
Striped catfish	Over 2 years	Over 2 Kg	200,000 eggs / kilo
Walking catfish	Over 1 year	Over 0.2 Kg	40,000 eggs / kilo 465 - 656 eggs / gram

All broodstock fish do not mature and ripen simultaneously and therefore it is important to know how to select mature fish for breeding. Using immature fish, or fish with unripe ovaries will result in poor spawning success and low egg and fry production. This is wasteful of broodstock and will affect the profitability of the hatchery. Broodstock suitable for spawning can be selected as follows:

Female characteristics

Ripe mature female fish will appear to have a swollen belly. If the belly of the fish is gently squeezed it will feel soft and yielding (this is because the muscles of the belly are thin and distended). Gentle squeezing will also cause the genital/anal vent to protrude.

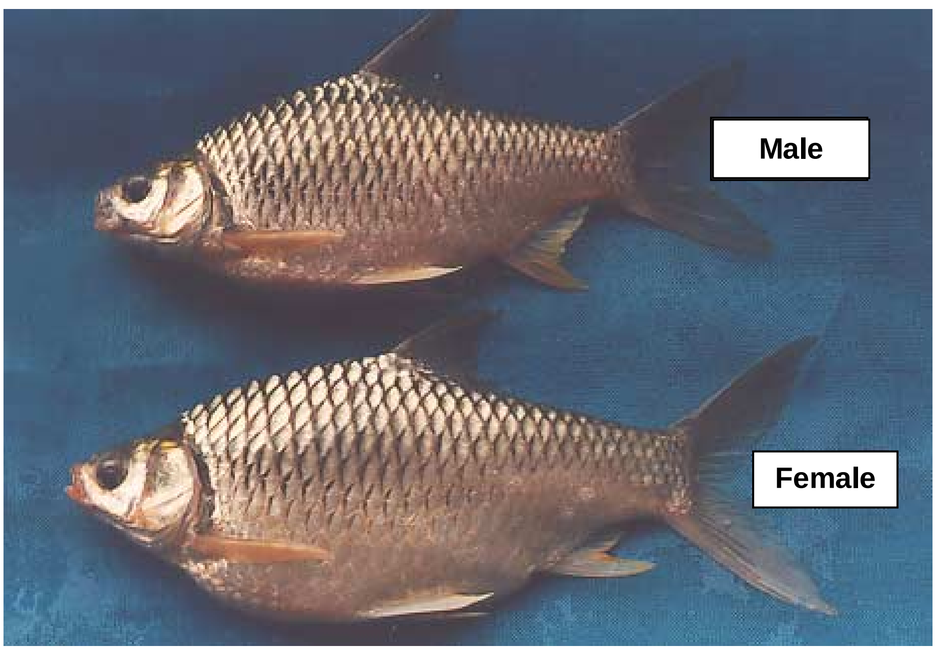
In some species the genital/anal vent will appear darker than usual (often a red or pink colour).

Male characteristics

Suitable male broodstock should be mature and not overly fat or thin. If they have scales near the pectoral fin, these will have a rough feeling when rubbed with the hand (*e.g.* silver barb, common carp). In many species, mature males can be quickly checked by squeezing the belly to cause secretion of milky white milt. If the milt flow readily then the male fish is suitable for use (*e.g.* silver barb, Chinese carp, Indian carp).

Fish species that have hard testis (*e.g.* catfish and striped catfish) will not release milt when squeezed. In this case the fish are selected by age and weight and the testis must be removed by cutting open the fish and removing the testis. Sperm activity can be checked by observation under a microscope if available.

Figure 3. Comparison of external appearance of mature male and female Puntius gonionotus broodstock.



2.4 Spawning season for wild fish

Most freshwater tropical fish species lay their eggs during the monsoon season. This is because rainfall increases the available habitats and nutrients for the hatching fish fry. The water quality of many water bodies usually improves, with increased oxygen, cooler temperatures. Hardness and pH of the water also change with the inputs of rainwater runoff. These changes in environmental factors trigger the maturation of the females so that they can lay their eggs during this favourable season. When attempting to manage reproduction of fish for aquaculture, this seasonality of reproduction must be taken into consideration. It is difficult to obtain successful spawning of fish before their natural breeding season since the condition of the broodstock fish is not sufficiently developed to produce viable eggs or sperm. Similarly, if the season has passed fish that have not spawned will tend to reabsorb their eggs to preserve nutrients. They cannot then be used until the following year.

Table3. Spawning seasons and optimal egg maturity for common aquacultured fish species.

Fish species		Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Common silver barb	Breeding possible		◄—	—	—	—	—	—	—	—	—►		
	Eggs optimal			◄···	···	···	···	···►					
Common carp	Breeding possible		◄—	—	—	—	—	—	—	—	—	—►	
	Eggs optimal			◄···	···	···	···	···	···►				
Rohu Mrigal	Breeding possible				◄—	—	—	—	—►				
	Eggs optimal					◄···	···	···►					
Grass carp	Breeding possible				◄—	—	—	—	—►				
	Eggs optimal					◄···	···►						
Silver carp Bighead carp	Breeding possible				◄—	—	—	—	—	—►			
	Eggs optimal					◄···	···	···	···►				
Striped catfish	Breeding possible				◄—	—	—	—	—	—	—	—►	
	Eggs optimal						◄···	···	···►				
Walking catfish	Breeding possible		◄—	—	—	—	—	—	—	—	—	—►	
	Eggs optimal				◄···	···	···	···	···	···	···►		

Table 3 above presents the approximate spawning periods for some common aquacultured fish species. The broken line indicates the season and the unbroken line indicates the period for optimal egg maturity.

CHAPTER 3 - FISH REPRODUCTION

In most tropical countries, spawning of fish takes place during the monsoon season, due to the abundance of water and natural food during this period. This provides the best available environment for the survival of fish fry. Some fish species are able to spawn almost all year round (*e.g*. Common carp). Many fish species are unable to spawn naturally in culture ponds since the pond environment is different to the conditions in rivers or water bodies where these fish spawn naturally (*e.g*. Chinese carp, Indian carp). Where pond conditions reflect natural conditions closely, fish reproduction can occur naturally (*e.g*. Common carp and Silver barb and other related *Puntius* species).

In situations where fish species grow well in ponds, but cannot reproduce naturally, some intervention is required. The simplest for of this intervention may be the provision of flowing water, splashing of water to mimic rain or provision of spawning substrates. More complex interventions include additional actions such as the injection of hormones to induce the maturation of the eggs in the fish ovary and stimulation of spawning activity. It is therefore essential to understand the particular requirements of each fish species so that efficient spawning and fry production is achieved.

There are three principal methods for seed production.

3.1 Natural reproduction

This is the easiest method and involves placing the male and female fish in a culture pond where they can spawn naturally. After spawning the male and female fish are removed and the pond is then used as a nursery for the fry produced. Alternatively the fry produced are removed and placed in a nursery pond for on-growing. The species that can be produced in this way are Tilapia (*Tilapia spp.*), snakeskin gouramy (*Trichogaster pectoralis*) and guppies (ornamental fish)

3.2 Semi-natural reproduction

This method involves placing the male and female fish in a pond or tank, where the environment can be controlled. Control of the environment includes spraying water onto the pond/tank surface to increase oxygenation and to mimic rainfall. Improved water quality conditions and temperature change can induce the fish to start breeding behaviour.

The selection of ripe male and female broodfish is important for the success of reproduction since unripe or immature fish will not be induced to spawn in this way.

Some fish have sticky eggs that must be attached to spawning substrates for successful fertilisation and hatching, therefore physical materials must be placed in the pond. Such substrates include bunches of string, tree roots, grasses used to mimic growing grass. Other species required a surface for attachment such as cement tiles or plastic pipes.

The species that can be successfully spawned using this method are common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*), walking catfish (*Clarias batrachus*) and the sand goby (*Oxyelotris marmoratus*)

3.3 Artificial fertilisation

This has become a common practice worldwide with the development of hormonal induction techniques for species that can mature, but cannot be bred naturally in fish ponds. Typically species that require hormonal induction of spawning are those fish species whose natural spawning habitat is in rivers or moving water. Parental attention of these species is usually non-existent and eggs tend to be small and planktonic. A common feature of these species is high fecundity and hatching fry require careful attention for the first week after hatching to ensure good survival.

The principle of hormonal induction is to induce the eggs in the ovary of mature female fish to ripen. When the eggs are ripe the fish will spawn and fertilise if held communally under appropriate conditions, or the eggs and milt (sperm) can be stripped from the fish for external fertilisation. The fertilised eggs produced in this manner are incubated and hatched in controlled conditions and there is careful attention to the fish fry after hatching. Typically, the fry are fed supplementary feed and held in tanks for a week before release into prepared nursery ponds.

Some of the principle cultured species that require hormonal induction are: Common silver barb (*Puntius gonionotus*) and other indigenous *Puntius* species common to Lao PDR, Silver carp (*Hypophthalmichthys molitrix*), Bighead carp (*Aristichthys nobilis*), Grass carp (*Ctenopharyngodon idellus*), Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), Striped catfish (*Pangassius sutchi*) other catfish species (*Clarias spp.*).

Of these three methods of fish fry production, artificial fertilisation requires the most management attention and range of equipment. The cost of equipment and requirement for hormones may limit the potential of this method in Lao PDR in the short term, although these materials are inexpensive and all readily available in Vietnam and Thailand.

3.4 Advantages of Artificial Fertilisation

- Allows the mass production of fish eggs and fry under controlled conditions, because the selection of broodstock and spawning process can be controlled.
- Allows the production of species that will not spawn naturally in ponds or rice fields
- This control is important in the effective management and economics of hatchery operations.
- Allows efficient use of a small number of broodstock
- Allows the production of even sized fish
- Diseases and parasites can be better controlled
- Management of broodstock can avoid inbreeding and allows selection of desirable traits
- Hybrids can be produced (*e.g.* catfish hybrids)

CHAPTER 4 - SEMI-NATURAL REPRODUCTION USING THE COMMON CARP.

The culture of common carp (*Cyprinus carpio*) is widespread in Lao PDR because of its tolerance to adverse environmental conditions and the ease with which it can spawn in a range of habitats. The traditional rice-fish culture systems of Xieng Khouang province and other northern areas show that this species has been present for an very long time. The farmers have developed indigenous methods for production of fry in their rice fields so that they can have fish all year round. The production of fish fry in this system is low and could be significantly improved by the use of some basic techniques for enhancing fry production and survival

Figure 4. The common carp (Cyprinus carpio)

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4.1 Breeding characteristics

In Lao PDR common carp usually start to breed when temperatures start to rise at the end of the cold season during February - March. They will continue to breed throughout the monsoon season until October or later. The optimum season for carp breeding is between March and September.

Common carp mature at a relatively small size - this is another of their advantages for use in Lao PDR. Typically a female carp will start to spawn at an age of approximately 6 months and a weight of 300 grams. As a result of this, it is important that the male and female fish are separated early in the year (February) so that they do not start to spawn naturally in the ponds before they can be used in the hatchery.

Since the spawning season for common carp is longer than that of other species it is possible for a well-fed female to spawn up to three times in one year. Common carp are relatively fecund with egg production approximately 80,000 eggs / kilogram of female.

4.2 Difference between the sexes

The immature male and female common carp appear extremely similar and thus it may be difficult to distinguish between the sexes. As the fish mature at the start of the breeding season, the differences between the sexes becomes more apparent, particularly the genital-anal vent of the fish (Figure 5).

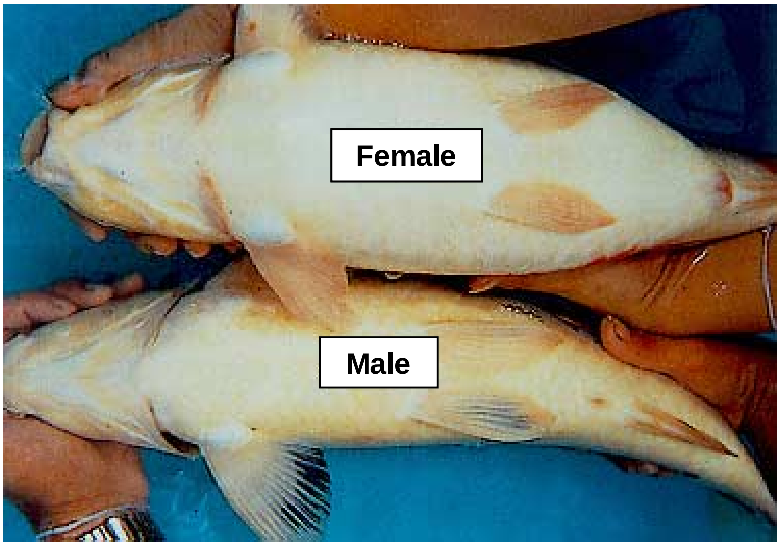
Females

- The vent will appear swollen and pink in colour
- The body will appear rounded and swollen
- The abdomen wall will feel soft and yielding when gently squeezed
- In some cases eggs will be released from the vent when the abdomen is squeezed

Males

- The body will appear longer and thinner
- The abdomen feels firm and hard when squeezed
- When the abdomen is squeezed towards the vent milky white milt will be released
- The scales near the operculum and pectoral fin appear rough when rubbed with the hand

Figure 5. Difference between male and female common carp.



4.3 Preparation of spawning tank/spawning pond and spawning substrate

Common carp can be spawned in either earthen ponds, concrete tanks or net cages held in ponds (hapas). Concrete tanks are the most suitable, but may be limited to well established hatcheries. The concrete tank should be 6 - 12 m^2 and approximately 1 metre deep.

For small farmers, net hapas suspended in ponds are the cheapest and most suitable alternative. The size of the hapas should be approximately the same as for concrete tanks (6- 12 m^2, 1 metre deep). Some form of water flow to the pond is desirable since this will assist the oxygenation of the water and also stimulates the fish to spawn

Common carp require a substrate to stimulate spawning and also for egg attachment. In the wild this is usually grass in flooded areas or waterweeds. In tanks or cages artificial substrate is used and this is usually made of clumps of shredded plastic string, fine tree roots, waterweeds or any other material that resembles grass. The clumps of substrate should be approximately 20 cm long and when spread out occupy a diameter of about 10 - 15 cm.

The clumps of artificial substrate are attached to ropes set in a square frame (Figure 6 - spawning frame) that is suspended in the tank/cage.

If the artificial substrate is not used the carp may not be stimulated sufficiently to spawn.

Figure 6. Spawning substrates attached to a frame for carp spawning in concrete tanks

Figure 7. Frames placed in a cement tank for common carp spawning (Note: water sprayed into tank to simulate rainfall)

4.4 Selection of broodstock for spawning

Ripe females that are ready to spawn are selected according to the criteria listed above (*i.e.* swollen abdomen, soft when squeezed and yellow eggs may be discharged. The vent is pink and swollen). Matures males should discharge milt when the abdomen is squeezed towards the vent.

4.5 Stocking of male and female common carp for spawning

One female fish should be stocked with 2 - 3 male fish to ensure fertilisation of the eggs. Stocking of the fish into the spawning tank/cage should be performed in the late afternoon early evening since spawning occurs during the nighttime, when temperatures cool down slightly.

The water depth in the spawning tank should be 40 - 50 cm to ensure that the spawning substrate fills half of the available space. This ensures that the fish contact the substrate, which is necessary to stimulate spawning.

For efficient spawning, water should be splashed into the tank/cage to simulate rainfall (Figure 7). This splashing of the water will also increase the oxygen level in the water, which will also encourage successful spawning. Water flow to the tanks can be provided by a small pump or piped water from a water source higher than the spawning tank/cage. This method is quite suitable for ponds in terraced rice fields where water flows downhill and can be piped into ponds for spawning.

The spawning takes places during the night and starts with the male fish rubbing the abdomen of the female with its head (which has the roughened scales). This activity stimulates the female to lay her eggs by swimming through the artificial substrate. The eggs are sticky and attach to the substrate. The male follows the female and release milt to fertilise the attached eggs.

Early the following morning the broodstock fish should be removed from the spawning tank, or alternatively the artificial substrate and the attached eggs should be moved to another tank for incubation and hatching. This is because common carp will eat all of the eggs within 1 - 2 days, if left in the same tank.

Eggs left to incubate will hatch after about 48 hours at a temperature of 28 - 31 ^{o}C (although this may be faster or slower according to temperature). During the incubation time it is important to have some form of water flow to maintain oxygen levels so that the eggs do not suffocate. This is often not done in hatcheries in Lao PDR and will lower the survival of the eggs and reduce the number of fry produced significantly.

4.6 Nursing common carp

Fry start to hatch after approximately 2 days and will not eat for the first 2 - 3 days after hatching. This is because they still have an internal yolk sac that provides food. When this is exhausted the fry will start to eat and it is essential that they have feed available during this period. In hatcheries where the water for the hatchery is taken from a pond containing greenwater, the first feed of the fry will probably be natural zooplankton such as *Moina*. Additional artificial feed can be added such as boiled mashed egg yolks (see section 7.6 for details).

Fish fry should be fed mashed boiled egg yolks 3 - 4 times a day (or every 6 hours) for the first 6 - 7 days after they start feeding. After seven days the fry can be transferred to a nursery pond that has been cleared of predators (insects and other fish) and that has a water depth of 50 cm or more. The fry can then be fed on feeds such as finely milled rice bran, finely milled soy waste, finely milled pig or chicken starter feed. The ratio of rice bran to starter feed should be approximately 2:1. The feeding rates for fry of different ages are presented in the section 7.6.

Fish fry should be fed twice a day (morning and evening) until the fry are 2 -3 weeks old and have reached a size of 2 - 3 cm. Fish of this size can be sold for stocking to ponds, or can be nursed for a further month in net cages until the fish are large enough to stock into rice fields.

It is important to note that water levels in the nursery ponds should be maintained during the nursery culture period and if possible some water should be exchanged.

CHAPTER 5 - ARTIFICIAL FERTILISATION OF SILVER BARB

Species suitable for small-scale hatchery production in Lao PDR include the indigenous Barb species (*Puntius spp.*) , Common carp and possibly some of the Chinese and Indian carp species. The attraction of the common carp and the barb species is that they mature at a small size (200 - 300 grams) which means that broodstock sized animals can be produced within one year of culture. In Lao PDR this means that broodstock fish can be produced every year from the growout culture of that year. This is in contrast to the Chinese and Indian carp, which mature at sizes over one kilogram and require at least 1.5 years or more before they can be used as broodstock. This is important in those cases where water shortages in growout ponds are a constraint to all year culture of fish.

Figure 8. Puntius gonionotus - a good candidate for mini-hatchery production

The barbs are attractive as aquaculture species since they are indigenous and there are at least six reported species for Lao PDR. These fish grow well in both pond and rice-fish systems. They have not been widely used as an aquaculture species so far because of the limitations of technical knowledge in spawning and nursery production.

Currently more attention is being paid to the Barb family as potential aquaculture species suitable for extension to small farmer operations and as an alternative to the Common carp.

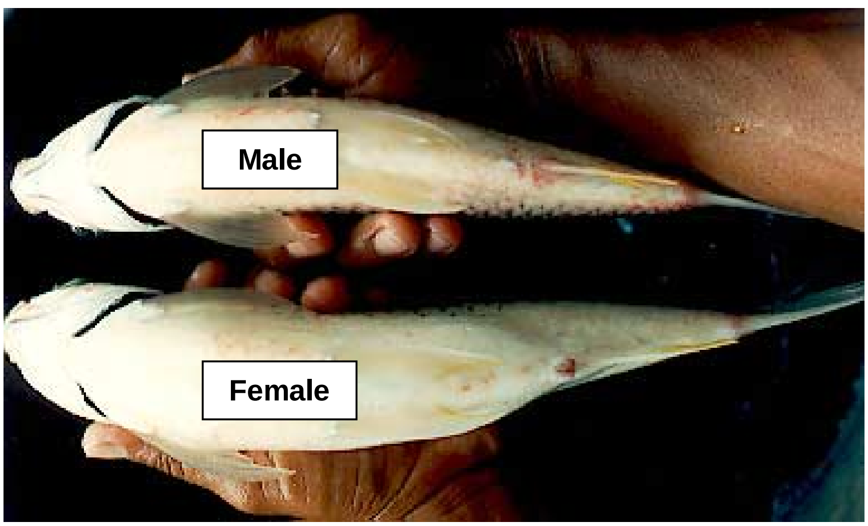
The principles for culture of *Puntius gonionotus* are the same as those for both Chinese and Indian carp. There are differences between species relating to spawning season, hormone requirements for spawning and the times to spawning and hatching. Where there are differences between species, these have been presented in the form of look up tables.

5.1 Broodstock selection

Selection of mature broodstock of *Puntius gonionotus* is similar to the method described for common carp. Male and female broodstock fish should be separated early in the spawning season to prevent premature spawning in the ponds. This will waste eggs and fry since survival will be extremely low.

The female *Puntius* will have a swollen abdomen and this will feel soft when squeezed gently, the abdomen wall will feel thin and non-muscled. The vent on the female will also be swollen and appear reddened. Eggs are not released when squeezed since although the female is mature the eggs are not fully ripened. This is the difference between species of fish that spawn naturally in ponds (*e.g.* common carp) and those which do not (*e.g. Puntius spp.*, Chinese carp and Indian carp).

Figure 9. Broodstock Puntius gonionotus (Note small size).



Male *Puntius* will have rough scales in the in the region of the pectoral fins and the indication of maturity is the release of milt when the abdomen is squeezed towards the vent.

Once the broodstock fish have been selected they can be held in a net cage in a pond before injection with hormone, or they can be placed in a tank in the hatchery. The tank or cage should not be too small since this will stress the fish, especially if water flow is low. Some splashing of water, aeration or water flow is desirable to ensure that the fish do not suffocate.

5.2 Hormones used for induction of spawning

The first hormone to be widely and successfully used for induction of fish spawning was carp pituitary gland extract. This method involved the collection of large numbers of pituitaries from mature carp, homogenisation and then injection to female fish to induce spawning. Typically two injections are required to induce spawning and an interval of time is required between the injections. The timings of the injections are important to ensure successful spawning as is the use of the correct dosage.

The advantage for the carp pituitary method was that farms were able to produce their own pituitaries from fish held on the farm. In Lao PDR this method is not suitable due to the shortage of large broodstock sized fish from which to take the pituitary. This is because most farms do not have the large numbers of mature fish that are required to provide the pituitaries. The drying up of many fishponds during the dry season further limits the availability of large broodstock sized fish. Those fish that are maintained throughout the year are required for use as broodstock and cannot be sacrificed for their pituitaries.

Another potential source of pituitaries are local fish markets. In countries where carp pituitary extract is widely used, fish heads are often discarded and the pituitaries can be collected (*e.g.* from fresh fish markets, fish farms). However, in many Asian countries, fish are purchased with the head on and this limits the availability of pituitaries. In Lao PDR the lack of large sized fish due to the short growing season and drying up of many culture ponds is a further limitation to this method.

Human Chorionic Gonadotropin (HCG) has also been successfully used to induce fish spawning and this can be obtained as the dry hormone and can be used directly. This is more convenient and dosages for fish are more accurate.

The induction of spawning of fish using Luteinizing Hormone Releasing Hormone analogue (LHRHa) has now become standardised through repeated experimentation and can be used with certainty for the spawning of many fish species. LHRHa has an additional advantage in that only one dose is required to induce spawning, in contrast to the two doses previously required with carp pituitary extract. The chemical name of LHRHa is ‘buserilin acetate’ and this is available either as LHRHa or as the human drug ‘Suprefact’. The effectiveness of LHRHa is further enhanced when used together with domperidone maleate. This is sold as a drug for human use under the trade name ‘Motilium’ or ‘Mirax’ .

Since both ‘Suprefact’ and ‘Motilium’ are now widely available and used in Thailand, this method is favoured for hatcheries in Lao PDR. Initially, it is expected that the Department of Livestock and Fisheries will facilitate the availability of ‘Suprefact’ and ‘Motilium’ through the Provincial Livestock offices and Provincial hatcheries
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It is important that hormones for use in fish spawning are stored in a cool place to prevent deterioration. Typically, storage temperature should be between 4 - 20 ^{o}C.

5.3 Method for using LHRHa

LHRHa is typically supplied as a standard weight of hormone given as micrograms (ug). The dosages of LHRHa used for female fish are also expressed as micrograms of hormone per kilo of broodstock fish.

The most common form of LHRHa currently available is the chemical known as 'buserelin acetate'. This is sold as a drug for human usage under the trade name 'Suprefact'. 'Suprefact' has been used successfully in the spawning of a wide range of fish species in many countries. Suprefact is sold in bottles containing 10 cm^3 of dissolved hormone. This volume of dissolved hormone contains 10,000 ug of 'buserelin acetate', therefore 1 cm^3 contains 1,000 ug.

Since the dosages of 'Suprefact' are very low (typically 0.2 ug per kilo of fish), the hormone must be further diluted so that it is easier to handle. It is usual to dilute 'Suprefact' to $^1/_{10}$ of its concentration with distilled water (*i.e.* 1 cm^3 'Suprefact' + 9 cm^3 distilled water). Thus 1 cm^3 of the diluted 'Suprefact' contains 100 ug of 'buserelin acetate'.

When using 'Suprefact' to induce fish spawning it is necessary to mix it with domperidone maleate. domperidone maleate enhances the effect of the Suprefact and improves spawning success. It is sold as a drug for human use under the trade names of 'Motilium' and 'Mirax'. 'Motilium' comes in tablet form, with each tablet containing 10 mg of domperidone maleate.

When using 'Motilium', it is necessary to crush the tablet into a powder and dissolve with distilled water so that it can be mixed with 'Suprefact' for injection into the fish. The dosage rate for the female fish is determined by the weight of the fish and only one dose (one injection) is necessary to induce spawning. This makes the use of 'Suprefact' and 'Motilium' extremely reliable and successful in the spawning of fish. Comparisons with other methods of hormonal spawning induction also show that egg production and fry survival are consistently higher using this method than using HCG or carp pituitary extract. Table 4 below gives the required dosages of 'Suprefact' and 'Motilium' required for induction of fish spawning, together with the timings for injection and egg stripping.

Table 4. Dosage rates, time to spawning and time to hatching for a range of aquaculture fish species.

Species	Dosage of hormone 'Suprefact' (ug/Kg) and 'Motilium' (mg/Kg.)	Time until stripping after injection (hours)	Hatching time after fertilization (hours)	Optimal temperature range (^oC)
Common silver barb	(10 - 15) + (5 - 10)	4 - 8	8 - 12	25 - 30
Red cheek barb	(5 - 10) + (5 - 10)	5 - 6	18 - 19	28 - 30
Tinfoil barb	(10 - 15) + (5 - 10)	6 - 8	18 - 20	28 - 30
Rohu	(10 - 30) + (5 - 10)	5.5 - 11	14 - 18	28 - 32
Mrigal	(10 - 30) + (5 - 10)	5.5 - 11	14 - 18	28 - 32
Big head carp	(10 - 30) + (5 - 10)	7 - 12	14 - 18	28 - 32
Silver carp	(10 - 30) + (5 - 10)	7.5 - 12.5	14 - 18	28 - 32
Grass carp	(10 - 20) + (5 - 10)	8 - 12	14 - 18	18 - 30
Walking catfish	(10 - 30) + (5 - 10)	14.5 - 17.5	22	28 - 33
Giant carp	(20 - 25) + (5 - 10)	10 - 12	2	29 - 30

Golden shark	(20) + (5 - 10)	8 - 10	22	27 - 28
Striped catfish	(10 - 30) + (10)	12 - 18	24 - 26	28 – 32

5.4 Equipment required for the induction of fish spawning

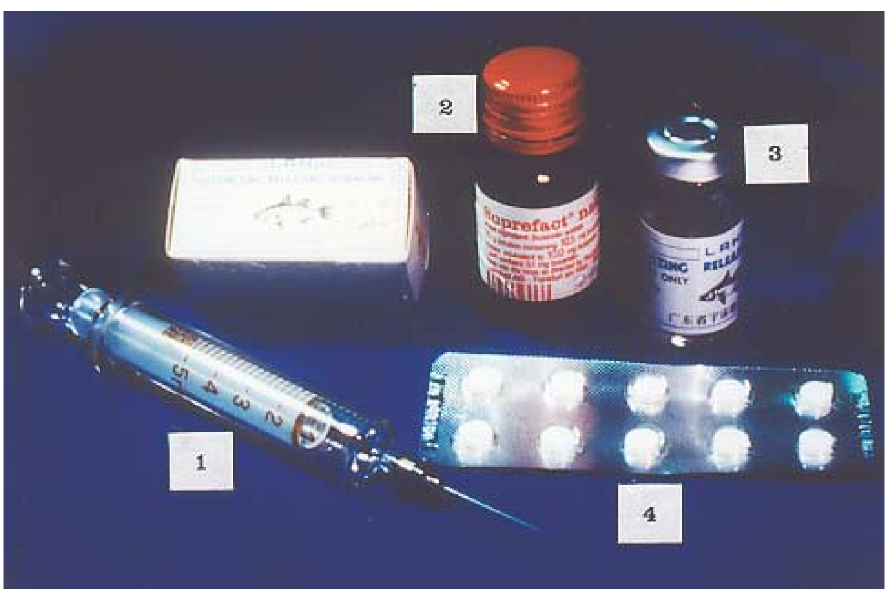
During the late afternoon the female fish to be injected must be weighed so that the correct hormone dosage can be injected.

Equipment used in hormone induction of spawning

Injection of broodstock to induce spawning is relatively simple and requires the following equipment.

- Syringe (1 -2 cc size depending on dosage/size of fish)
- Needle for injection (18 -27 gauge, depending on size of fish)
- Pestle and mortar or glass homogeniser (to crush and mix hormones)
- Weighing scales (to weigh broodstock to assess appropriate dosage)
- 'Suprefact' (Luteinizing Hormone Releasing Hormone analogue, LHRHa, chemical name -buserilin acetate), diluted to $^{1}/_{10}$ of original concentration.
- 'Motilium' (chemical name - domperidone maleate)
- Distilled water (obtained in ampoules, boiled water or distilled water for car batteries have also been used successfully)

Figure 10. Equipment required for induction of fish spawning.



Equipment used for egg stripping and fertilisation.

- Bowl (2 - 5 litres volume) - used for collecting the eggs stripped from the female and the milt stripped from the male fish.
- Bird feather - used for mixing the eggs and milt so that fertilisation is achieved efficiently. The feather does not damage the eggs.
- Clean cloth - used to wipe the fish down to remove excess water and mucus before stripping.
- Jug or suitable container - for holding water for washing eggs
- Clean water - water is added to the eggs after mixing to trigger fertilisation. The clean water is also used to wash the eggs after fertilisation.

Egg hatching equipment

- Net cage made of 'Orlon' - used for holding eggs during incubation - can be used for sticky eggs - (*e.g.* common carp, catfish species)
- Upwelling hatching cone made of 'Orlon' - used for hatching eggs that are not sticky (*e.g. Puntius gonionotus*, Chinese carp, Indian carp)

5.5 Method for hormone injection

Successful spawning of fish requires selection of mature broodstock and also the correct administration of the hormone. This involves the use of the correct dosage (for the species and size of fish) and also careful timing of the process so that maximum spawning efficiency is achieved.

Volume of hormone dosage for injection

Since the size of fish used for spawning varies according to species and age of the fish, the total volume of the dosage required also varies. Ideally the volume of the mixed hormone dosage should be approximately 1 cm^3 per kilo of female fish. Since the volume of 'Suprefact' used is quite low, the extra volume of the dose is made up using distilled water. Using correct volume for injection insures that the fish absorbs the hormone efficiently and that the hormone has the required effect.

Table 5. Dosage volume and needle size for female broodstock according to weight

Weight of female fish (Kg)	Total volume to be injected (cm^3)	Needle size (number)
0.2 - 0.5	0.3 - 0.7	25
0.5 - 2	0.4 - 1.0	24
2 - 5	1.0 - 2.5	22
5 or more	2.5 - 10	18

Hormone preparation

After weighing the female fish the correct dose of hormone can be prepared. When using ‘Suprefact’ that has been diluted to $^{1}/_{10}$ of its original concentration the concentration of hormone is 100 µg / cm^3

Worked example:

Three female *Puntius gonionotus* broodstock females need to be injected and they have a total combined weight of 900 grams (the fish weight 300, 300 and 300 grams).

1. The dosage rate is 15 µg per kg (1,000g) of broodstock female, therefore the quantity required is:

$$900 / 1000 \times 15 = 13.5\ \mu g$$

1 cm^3 of diluted ‘Suprefact’ ($^{1}/_{10}$) contains 100 µg of hormone, therefore the total volume of hormone required is:

$$13.5/100 \times 1 = 0.135\ cm^3$$

2. The dosage rate of ‘Motilium’ required is 10 mg per kg of broodstock female, therefore the quantity required is :

$$900 / 1000 \times 10 = 9\ mg$$

One tablet of ‘Motilium’ contains 10 mg of active chemical so just less than a whole tablet could be used.

3. The volume of the total dose should be approximately 0.4 cm^3 for each of the 300g fish giving a total volume of 1.2 cm^3.

Since the volume of 'Suprefact used is only 0.135 cm^3 the rest of the volume should be made up with distilled water (approximately 1.1 cm^3).

4. The 'Suprefact' and 'Motilium' are combined and ground into a paste by adding some of the distilled water. When they two are fully mixed the rest of the distilled water is added to make the final volume. In this situation, the fish are given equal volumes of the hormone mixture since they are the same size.

Injection location

The hormone mixture must be injected into the muscle of the fish. Suitable locations for injection are illustrated in Diagram 9

- 2.1 Behind the dorsal fin
- 2.2 Into the muscle below the dorsal fin on the side of the fish
- 2.3 Behind the pectoral fin
- 2.4 In front of the tail fin on the side of the fish
- 2.5 Behind the pelvic fin

Diagram 9. Suitable locations for hormone injection

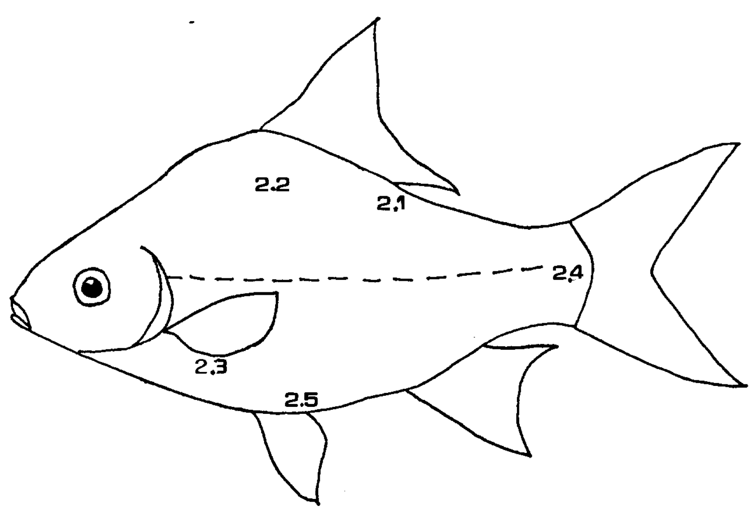


Figure 11. Injection of female Puntius gonionotus into muscle below dorsal fin

Female fish are isolated in a net and injected. After injection of the female fish, they should be held together with male fish, in a tank or an 'Orlon' net cage fixed in a pond that contains well-oxygenated water. The male and the female should be held together so that if they spawn before they can be stripped, the males can fertilise the eggs in the net cage, and the eggs will not be lost. If a large mesh net cage is used the eggs will be released into the pond and cannot be transferred into the hatching container. Oxygenation of the 'Orlon' cage or tank can be achieved by flowing water into the cage or tank and allowing the water to splash to increase aeration.

Fertilisation of the eggs

From Table 4 , the time after injection of hormone until female *Puntius* are ready to be stripped is between 4 - 8 hours. This time is variable depending upon temperature and the dosage of hormone. The female fish should be checked every 30 minutes to one hour to see when the eggs begin to be released into the water. This can be checked by sampling the water using a glass and looking for the first signs of egg release. When eggs are first observed, the female fish should be removed quickly for egg stripping.
There are two methods for fertilising the eggs using the artificial method

- **Wet method** - eggs are stripped into a bowl (2 -5 litres) of water from a female fish that is ready to spawn. The milt from the male is stripped simultaneously if possible to ensure that the eggs are fertilised. This method can result in low fertilisation rates if not done quickly and the eggs and milt are not mixed well. The only advantage of this method is in cases where the amount of milt from the males is low such as the Climbing perch (*Anabas anabas*) and small sized Common Silver barb (*Puntius gonionotus*).

- **Dry method** - this is the best method since high fertilisation rates are guaranteed. The milt and eggs are stripped from the male and female into a dry bowl and then mixed together using a feather. After mixing, clean water is added to fill the bowl. The sperm in the milt is only activated once the water is added and so efficient fertilisation is obtained. It is important that once the water is added to the eggs that they are not disturbed for about 5 - 15 minutes to allow the eggs to swell. If the eggs are disturbed before swelling they can be easily broken and survival will be low as a result. Once the eggs are swollen they should be washed in clean water 2 -3 times to remove mucus that is discharged with the eggs from the female (this will encourage bacterial growth if left with the eggs and may affect hatching). This method is suitable for most species of aquaculture fish (especially, *Puntius spp.,* Chinese carp, Indian carp, catfish species)

Figure 12. Stripping eggs from a female Puntius gonionotus using the dry method

Figure 13. Stripping milt from a male Puntius gonionotus for dry fertilisation method

Figure 14. Mixing the eggs and milt with a chicken feather using the dry method.