Guidelines for hatchery production of Pa Phia fingerlings in Lao PDR

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Guidelines for hatchery production of Pa Phia fingerlings in Lao PDR

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Executive Summary

Culture-based fisheries (CBF) increases the supply of fish for food in rural communities and helps alleviate poverty by providing additional income to rural farmers through sale of harvested fish. CBF requires stocking and recapture of fish in small water bodies, which are often managed by the surrounding community. The harvested fish provide a food source and income for local village farmers.

Pa Phia (*Labeo* [syn *Morulius*] *chrysophekadion*) is a much desired indigenous species of Lao PDR. Due to its popularity, the species has been selected to support CBF in the region.

The objective of this manual was to provide basic guidelines for the hatchery production of Pa Phia fingerlings. This manual drew on published information on Pa Phia; results of artificial propagation trials conducted on Pa Phia during two projects funded by the Australian Centre for International Agricultural Research (ACIAR) (Project No. FIS/2005/078 and FIS/2011/013), and the experiences of technicians at two government hatcheries.

This manual provides information on managing and spawning broodstock, genetic guidelines, egg incubation, hatching larviculture and fry rearing.

Although the guidelines were specifically developed for production of Pa Phia at government hatcheries in the Lao PDR, they may be applied to other, related, species that are produced in hatcheries for stocking purposes, and could be adopted and commercialized by the private sector hatcheries in the region.

Introduction

Culture-based fisheries (CBF) increases the supply of fish for food in rural communities and helps alleviate poverty by providing additional income to rural farmers through sale of harvested fish (De Silva *et al.* 2006). CBF is essentially a stock and recapture activity in small water bodies which are often managed by the surrounding community. The stocked fish not only provide a food source for farmers but also a potential income if excess harvested fish are sold.

For CBF to be achievable there must be sufficient production of juvenile fish, of selected species, for stocking purposes. Recent developments in CBF encourage the use of indigenous species to reduce and or minimize the dependence on exotics and enhance the dependence on indigenous species. Accordingly, Pa Phia (*Labeo* [syn *Morulius*] *chrysophekadion*), a much desired indigenous species, was chosen for CBF development in Lao PDR. This manual deals with the captive breeding techniques for Pa Phia that are being developed in government hatcheries in the Lao PDR to produce fingerlings for CBF, and potential adoption and commercialization by the private sector hatcheries.

CBF in Lao PDR are being developed through two projects funded by the Australian Centre for International Agricultural Research (ACIAR) (Project No. FIS/2005/078 and FIS/2011/013) (De Silva 2008, De Silva *et al.* 2010). These projects aim to develop best practice approaches and production models for CBF that will improve the yields and economic benefits to village communities.

Objectives

The objective of this manual is to provide guidelines for the hatchery production of Pa Phia to support CBF. This manual draws on published information on Pa Phia; results of artificial propagation trials conducted on Pa Phia during the ACIAR Projects (De Silva *et al.* 2010); and the experiences of hatchery technicians at the Vientiane Provincial Fisheries Station (VPFS), Nam Cheang and the Pak Bo Fisheries Station (PBFS), Savannakhet.

Pa Phia *(Labeo [Morulius] chrysophekadion)*

Class Actinopterygii (Ray-finned fishes)
Order Cypriniformes
Family Cyprinidae
Genus *Labeo*
Species *L. chrysophekadion* (Bleeker, 1849)

Common names: Black shark minnow, sailfin shark carp, Pa Phia, Pla ee too, Gar, Trey kaek, Cá et moi.

Pa Phia (Figure 1) is a large benthopelagic cyprinid species that is widely distributed throughout Asia, including the Mekong (Thailand, Cambodia and Vietnam) and Chao Phraya (Thailand) basins, as well as the Malay Peninsula, Sumatra, Java and Borneo (Malaysia and Indonesia) (www.fishbase.org, MRC 2003). Pa Phia has a ventral mouth and is a herbivorous algal grazer.

In many areas Pa Phia is an important commercially harvested species and in southern Lao PDR is one of the more expensive fish at the market (Singhanouvong and Phouthavong 2002; Rainboth 1996; MRC 2003). The species has good potential for aquaculture. Captive breeding and rearing of Pa Phia has been described in both Thailand and Lao PDR, but information is limited and not readily available (Pennapaporn 1970; Thavonnan and Udomkananat 1979; Thienchareon *et al*. 1989; Thienchareon *et al*. 1990; Tienchareon and Oonsrisong 1990; Unsrisong *et al*. 1990; Leelapatra *et al*. 2000; Thi et al. 2003).

Although microsatellite makers have been described for the species (Nguyen, in Aggarwal *et al.* 2011), there is little information on the genetic structure of Pa Phia.

Figure 1. Pa Phia (*Labeo chrysophekadion*).

Broodstock Management Guidelines

The goal for broodstock management of Pa Phia is to assure the sustainable supply of quality seedstock (both genetically and physically) for CBF, aquaculture and replenishing wild stocks in Lao PDR.

Procurement of new broodstock

Capture

Since the genetic structure of Pa Phia is not known, broodstock should be collected from the catchment or river system that is intended to be re-stocked. This will ensure the genetic structure of the population within the region is maintained and that potential stocking activities and escapees from aquaculture activities, including CBF, have minimal impact on that structure.

Capture and transport techniques should aim to minimise stress and physical damage to captured fish (= potential broodstock).

Microchipping

In order to effectively manage breeding programs, either for stocking, CBF or other forms of aquaculture, ideally all broodstock must be individually identifiable. This can be achieved by tagging each fish, such as with a microchip (PIT Tag).

Broodstock maintenance

Pond and tank maintenance/ management

Broodstock can be maintained in earthen ponds (or dams). Ideally, females and males should be held in separate ponds to assist stock management. Do not stock other species of fish into the ponds.

Ponds should be provided with good water quality by maintaining water level and adding freshwater regularly to replace water lost by evaporation and seepage. Providing aeration is also beneficial.

Stocking fish in tanks and ponds at high densities can expose fish to constant, elevated concentrations of ammonia and/or nitrite, and low concentrations of DO, which can stress fish, suppress growth and inhibit maturation. Since optimal stocking densities for Pa Phia broodstock have not been determined, densities used for other, related, species should be used. For example, the broodstock of Common, Chinese and Indian Major carps are maintained at densities of 1,000 to 3,000 kg/ha (Jhingran and Pullin 1985, Kumarasiri and Seneviratne 1988l, Singh *et al.* 2000).

Sedation and anaesthesia

It is important to handle broodstock as gently as possible to preserve the surface mucous coating and to prevent any physical damage. Handling of fish is always stressful, and may lead to lowered immune function and subsequent increased risk of infectious disease. Thus, for certain procedures, especially with larger fish, sedation is required to minimise stress and injury.

Pa Phia can be sedated or anaesthetised for a range of purposes, including measurement, tagging, spawning and transfer between holding facilities.

Common drugs used to sedate and anaesthetize fish include, Aqui-S, MS222 and benzocaine. These drugs are administered through the water and are taken up across the gills. In general, appropriate anaesthetic dose rates depend on the species, size of fish water temperature and level of sedation required. Use of these drugs should follow the instructions provided by the supplier/ manufacturer. Exposure time should be minimised where possible to avoid death from over exposure.

When using anesthetics, it is important that the water is well aerated or oxygenated to maintain relatively high (> 5 ppm) dissolved oxygen concentrations. Fish condition should be monitored closely while under anaesthesia, for example, opercula movement should be regular, flaring of opercula may indicate over exposure and immediate steps should be taken to recover the animal. Following anaesthesia, fish should be placed in clean, well aerated water and monitored until balance and swimming returns. Full recovery may take several minutes or longer depending on species, type of drug, dose rate and exposure time. In some cases, “swimming” of fish to increase ventilation of the gills may facilitate recovery from deep anaesthesia.

Water quality

Water is a vital component of hatchery operations as it is the medium in which the fish live and grow. Both the quantity and quality of water used are critical to the well-being of fish. Ideally the water supply should be reliable, free of pathogens, and free of pollutants (organic, industrial and urban) and consequently, regardless of the water source, some form of pre-treatment will be required before it is used in the culture system.

Poor water quality can influence fish health both directly and indirectly. Each species has a preferred range of water quality parameters and outside of this range will suffer stress, which may lead to reduced growth, disease and even death.

Water quality may be influenced by farm management, fish husbandry (including stocking density and feeding) as well as excreted wastes from fish. Culture of fish at high densities requires close monitoring of water quality, especially dissolved oxygen (DO), pH, ammonia and nitrite to ensure that critical levels are not reached or exceeded. The physical action of the fish in stirring up sediments can also have an impact on some parameters.

Nutritional requirement and feeding

Nutritional requirements, especially for protein, amino acids, lipids, minerals and vitamins, varies from one species of fish to another, and for some species the nutritional requirements will also vary with age (De Silva and Anderson 1995). There is a wide variation in the types and quality of diets used to feed farmed fish.

Broodstock nutrition and feeding can greatly affect not only egg and sperm quality but also seed production (Izquierdo *et al.* 2001). Gonadal development and fecundity in broodstock are affected by certain essential dietary nutrients. Therefore, it is important to feed the broodstock a high quality diet that meets their nutritional needs.

There are no commercially available artificial diets specifically formulated for Pa Phia. Instead, artificial feeds formulated for other species, as well as inert "natural" foods (grain products) are used to feed Pa Phia in captivity. At the VPFS and PBFS, broodstock have been fed with,

- floating catfish grow-out pellets (Centago, Thailand, 25% Protein, 3% fat) at a rate of 2-3% body weight per day
- mixture of rice bran and pig diet (29% protein, 2% fat, 9% fibre & 12% moisture) made into dough so that it sinks
- Broken rice.

Feeds should be stored under cool (<20^{o}C) and dry (<75% humidity) conditions to prevent spoilage. Feeds can become rancid when stored in damp, warm conditions and breakdown of fatty acids in feeds containing insufficient amounts of anti-oxidants can result in the production of harmful toxins. Moulds and fungi growing on the feeds may also produce toxins. Do not use feeds that have a musty or stale smell, are discoloured or appear to be "sweating". Feeds showing any signs of contamination by moulds or fungi should be discarded.

Biosecurity

Biosecurity management in a hatchery/aquaculture facility, such as the VPFS and the BVFS, should aim to protect the facility against unwanted introductions of animals and infectious diseases, and to prevent the escape of animals and pathogens from the facility to the surrounding environment. Regardless of the water source, water should be physically screened (preferably less than 0.5 mm) to prevent unwanted animals entering the facility, and ideally sterilized (ozonated, UV irradiated) to eliminate waterborne pathogens.

An important source of pathogens in hatcheries is new fish stock brought to the hatchery. To ensure that diseases are not introduced to the hatchery facilities, all new stock, regardless of the source, must be quarantined from other stock for at least 2 weeks. The Quarantine facility should be separate from culture facilities. During that time, fish should be checked regularly for signs of disease, and be given prophylactic treatments, such as salt and formalin baths, to kill pathogens.

Reduction of escape from hatcheries/aquaculture facilities is achieved by locating facilities away from flood-prone land. Physically screening discharge water will prevent escape of animals to receiving waters. Maintaining hygienic conditions on the facility and having an active health monitoring/management program will reduce the incidence of pathogens on the facility and in discharge water.

Health management

Disease outbreaks have long been recognised as a significant constraint to aquaculture production and economic viability. A wide range of pathogens (viruses, bacteria, parasites etc.), environmental factors (water quality, etc) and even husbandry factors have caused heavy losses in aquaculture facilities (Sarig 1971, Brown 1993, Noga 2000, Hoole *et al.* 2001). Often these factors are linked in disease outbreaks. For example, a decline in water quality associated with poor husbandry practices may lead to an increase in the incidence of bacterial infections. Not only can disease

outbreaks inflict heavy mortalities of stock, but **fish in poor health have considerably lower growths rates, produce poor quality seedstock, or may not mature/spawn.**

The major sources of pathogens are the water supply, especially if there are fish present in the source water, and new fish brought to the facility from other locations. Both these sources must to be appropriately screened and treated to ensure that introduction of pathogens is minimised and biosecurity maintained (see Biosecurity section).

A stringent fish health-monitoring program incorporating regular monitoring for diseases is required to maintain healthy fish stocks. Recording information about each holding facility (tank or pond) or batch of fish greatly facilitates disease diagnosis and long-term management. These records should include daily mortalities, health checks, water quality and treatments. The type of information that should be recorded and frequency of collection is summarised in Appendix II.

Stress is considered as an environmental externality that reduces the ability or capacity of a fish to maintain health and well-being. More importantly, stress can reduce growth and illicit poor fish health. Stressed fish suffer from depressed immune systems and consequently lowered resistance to disease or parasite infestation. Stressors in fish farming include handling, transport, territorial behaviour, predation pressure, confinement and overstocking, chemicals and water quality and parasitism/disease.

There is little published information on the diseases of Pa Phia. Parasites that have been recorded from Pa Phia include *Epistylis* sp., *Stephanostomum* sp. metacercaria, *Senga* sp. (Syn.: *Polyonchobothrium* sp.), *Philometra* sp. (Arthur and Te 2006), and *Opisthorchis viverrine,* an important parasite than can infect humans (Sohn *et al.* 2012). General disease text are recommended for disease diagnosis and treatment (Schlotfield and Alderman 1995, Iwama *et al.* 1997, Noga 2000, Hoole *et al.* 2001).

Fish health management strategies aim to maintain the health and well-being of stock, while optimising production. Key actions, which are critical to achieving this, are:

- Take an active approach to managing the health of stock - monitor health of stock regularly
- Maintain hygienic conditions
- Maintain biosecurity (sterilise inlet water and quarantine all new stock)
- Guard against poor water quality. Monitor water quality regularly
- Minimise unnecessary stress
- Feed fish an appropriate diet (nutritional composition, size and amount).

Data management

In order to effectively manage the breeding program, data on each broodstock should be maintained in a database which should include information on: source of fish (where collected from in the wild), location of fish on the farm, length and weight data, sex, diet and spawning data. The latter should include dates and times of spawning induction, hormone treatments, volume of gametes (eggs and milt) stripped, number eggs stripped, individuals used in matings, fertilisation rates and hatch rates.

Artificial Propagation

Genetic considerations

Some fish produced by hatchery production of Pa Phia, may eventually breed with wild fish, or become future broodstock. Therefore, an important goal in managing hatchery-based breeding programs is to avoid loss in genetic diversity or change in genetic structure of populations. This can be achieved by ensuring genetically sound management strategies are incorporated into breeding plans. Selective breeding programs, in which particular traits of interest are selected for, must only be undertaken with the guidance of a geneticist and will not be dealt with in this manual.

Genetic guidelines for captive breeding programs for fish stocking purposes have been developed (see Miller and Kapuscinski 2003, Bert 2007, Kapuscinski and Miller 2007). The following guidelines aim to minimise loss of genetic diversity in the progeny produced, and should be included in breeding plans for Pa Phia.

- Spawn as many broodstock as possible each year to increase the effective breeding number (N*e*). As a minimum spawn at least 10 fish (5 females and 5 males) each year (Figure 2)
- Spawn a different group of broodstock each year. Do not re-use fish that have been spawned for at least 2 years, and do not spawn the same fish any more than twice
- Spawn an equal number of female and male fish each year. An unbalanced sex ratio in the breeding population reduces the N*e* and increases the rate of inbreeding and increases the chance of losing diversity
- Undertake single pair matings (1 male and 1 female) (Figure 2)
- Retain equal numbers of eggs/larvae from each spawning for rearing (Figure 2). The number retained should be enough to meet annual production requirements. Excess eggs /larvae should be discarded as soon as possible to minimise the cost of their maintenance
- Replace at least 10% of mature broodstock with new stock each year. Preferably new fish should be wild-born rather than hatchery-born stock. These fish should be caught locally to avoid transfer of genetic material between genetically different populations.

Spawning

Females

In captivity Pa Phia is known to reach sexual maturity within a year at a body weight of 600 g when cultured in earthen ponds (Thienchareon *et al*. 1989). Spawning at the VPFS has been induced in captive female Pa Phia broodstock weighing from 700 g weight and 350 mm fork length (Table 1). Pa Phia maintained in 600 m^2 have been reported to spawn twice within two months in the one spawning season (Thienchareon *et al*. 1990).

Fecundity of spawned Pa Phia at the VPFS was 21,000 – 260,000 (mean 88,300), which represents 22,100-180,500 egg/kg (mean 82,200 eggs/kg) (Figure 3). Since the number of eggs produced varies according to the size of broodstock, larger fish can produce considerably more eggs; Kamonrat *et al*. (1972) reported in excess of 1 million eggs from a 49 cm fish.

Mature oocytes of mature fish are 1.08-1.33 mm dia. (mean 1.23 mm dia.). Based on 5 spawnings at the VPFS, the relationship between volume of eggs stripped and umber of eggs is (Figure 4):

No. eggs = 16,819 + 467 x Vol. eggs (mL).

Males

Male Pa Phia mature at a smaller size than females, with running ripe males being from 400 g (Table 1). Males are usually running ripe at the time spawning is undertaken.

Spawning season

Pa Phia is a semi-migratory species that undergoes upstream migrations to spawn during the monsoon season (Boonmon and Kantejit 1977, Baird *et al.* 1999). Fish in reservoirs tend to spawn later and over a longer period than fish in rivers (July-October) (Boonmon and Kantejit 1977, Chabjinda *et al.* 1992) (Table 2).

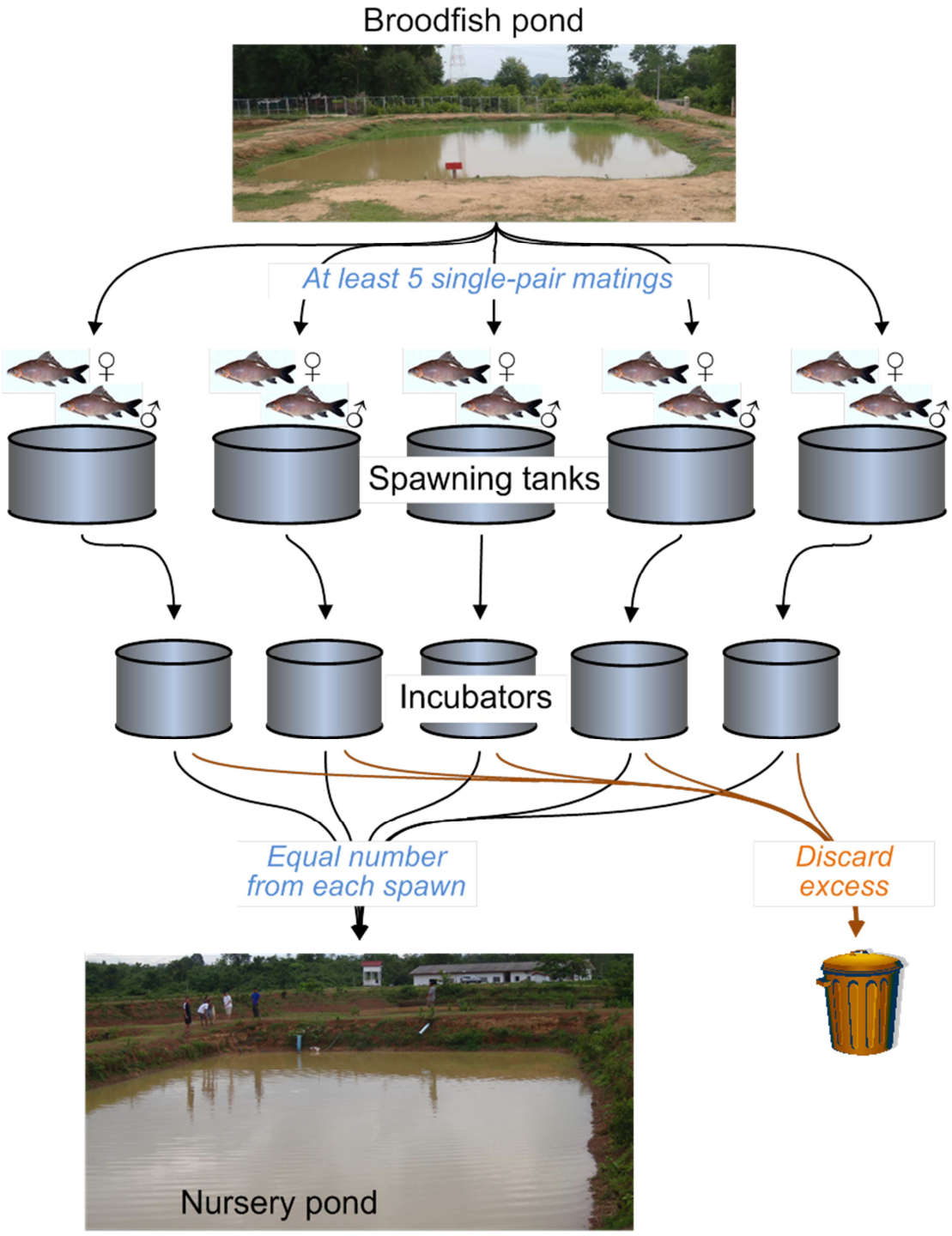

Figure 2. Breeding plan for Pa Phia.

Table 1. Fork length and weight of Pa Phia broodstock held at the Vientiane Provincial Fisheries Station, Nam Cheang. Values represent range with mean in brackets.

Parameter	Overall		Mature fish*	
	Females	***Males***	***Females***	***Males***
Fork Length (mm)	280-480 (372 ± 7)	300-470 (373 ± 6)	350-420 (382 ± 7)	360-470 (397 ± 11)
Weight (g)	300-2,000 (913 ± 48)	300-1,900 (874 ± 51)	700-1,700 (974 ± 62)	400-1,600 (941 ± 91)

*** Mature females produced ovulated eggs following hormone injection. Mature males were running ripe.**

Table 2. Spawning season of Pa Phia.

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Country	Habitat	Source
					onwards							Thailand	Pond	Laoobuth *et al.* 1993
												Cambodia	River	Bardach 1959, Sokheng *et al.* 1999
												Lao PDR	Pond	Vientiane Provincial Fisheries Station, Nam Cheang
												Lao PDR	Pond	Pak Bo Fisheries Station, Savannakhet
												Thailand	River	Boonmon and Kantejit 1977
												Lao PDR	River	Baird *et al.* 1999
												Thailand	Reservoir	Boonmon and Kantejit 1977, Chabjinda *et al.* 1992
												Thailand	Ponds	Thienchareon *et al.* 1989, Tienchareon and Oonsrisong 1990

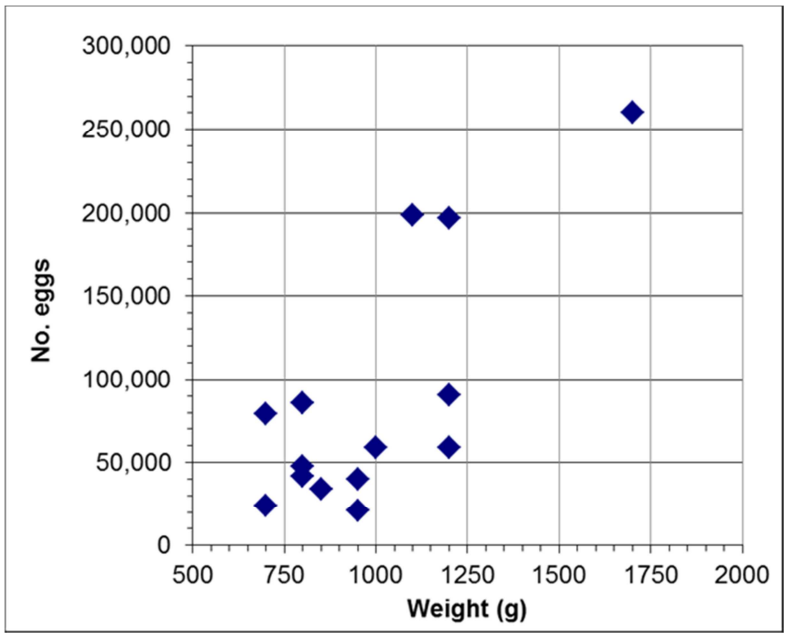

Figure 3. Relationship between female weight and no eggs spawned at the VPFS.

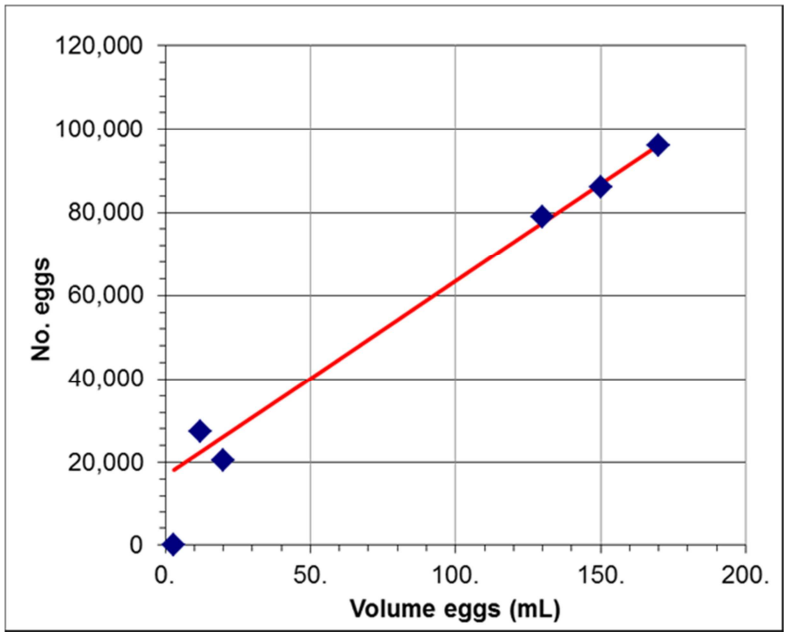

Figure 4. Relationship between volume of eggs stripped from Pa Phia and number of eggs Equation (red line): No. eggs = 16,819 + 467 x Vol. eggs (mL).

Hormone injection

Pa Phia has been induced to spawn using a range of hormone treatments (Table 3). However, the preferred method is use of Luteinizing Hormone Releasing Hormone analogues (LHRHa), which are highly effective (and affordable) in stimulating gonadotropin secretion and inducing ovulation in freshwater fish.

One LHRHa in particular, Buserelin Acetate (Suprefact®, Sanofi-Aventis), is combined with the dopamine antagonist Motilium® (Janssen-Cilag) to further enhance its effectiveness. This treatment has been successfully used to induce spawning in Pa Phia at both the VPFS and PBFS, as well as at other hatcheries (Table 3, Table 4). A single injection of Ovaprim (Syndel Laboratories Ltd., Canada) (0.5 mL/kg) has also induced spawning in broodstock at the VPFS (Table 4).

Males are typically injected with a lower dose rate of hormones than that given to females, or are not injected (Table 3).

Table 3. Summary of hormone treatments for induction of spawning in Pa Phia (modified from Ingram and Lasasimma 2008).

Treatment	First injection	Second Injection	Hours between injections	Source
PG[1] ("dose")	♀ 0.5-0.7	♀ 1.5-2.0	6	Thienchareon *et al.* 1989, Tienchareon and Oonsrisong 1990
PG[1] (mg/kg) + HCG[2] (IU/kg)	♀ 2.3+500 ♂ 1.2+800	♀ 3.5+2,000 ♂ Nil	6-8	Thi *et al.* 2003
PG[1] (mg/kg) + LHRHa[3] (µg/kg) + DOM[4] (mg/kg)	♀ 2.0+0+0	♀ 4.0+150+15		Trinh Quoc Trong *et al.* 2005
Suprefact® (µg/kg) + Motilium® (mg/kg)	♀ 5-8+5-10 ♂ 3-4+3-5	♀ 10-15+5-10 ♂ Nil	6	Tienchareon and Oonsrisong 1990, Leelapatra *et al.* 2000
Suprefact® (µg/kg) + Motilium® (mg/kg)	♀ 18-25+10 ♂ 12-15+5-10	♀ Nil ♂ Nil		Spawning trials conducted at VPFS and the PBFS
Ovaprim® (mL/kg)	♀ 0.5 ♂ 0.25	♀ Nil ♂ Nil		Spawning trials conducted at VPFS

1. PG = pituitary gland of either common carp or mrigal.
2. HCG = Human chorionic gonadotrophin
3. LHRHa = Luteinizing Hormone Releasing Hormone analogue
4. DOM = Domperidone

Table 4. Comparison of spawning results for Pa Phia injected with either Suprefact + motilium or Ovaprim (trials conducted at the VPFS between 2008 and 2010).

Dosage	Number. injected	Number spawned naturally	Number stripped	Number Hatched	Hatch rate (%)
Ovaprim (0.5ml/kg)	18	2	6	3	<1 – 27 (mean 5.1)
Suprefact (18µg/kg) + Motilium (10 mg/kg)	7	2	1	2	Not determined
Suprefact (25 µg/kg) + Motilium (10 mg/kg)	14	3	7	2	<1 – 17 (mean 7.1)
Summary					
Total	39	7	14		
No. hatched		6	1	7	

Hormones injections can be administered into either the peritoneal cavity (behind the pectoral fin) or the dorsal musculature (Figure 5).

Fish are generally injected late evening (6:00 – 8:00 pm) so that spawning occurs on the following morning.

Following injection, fish may be placed communally in one tank and allowed to spawn naturally, or placed separately into individual tanks and artificially spawned by hand stripping of gametes following ovulation.

Figure 5. Injecting sedated broodstock at the VPFS.

Latency Period

The latency period is defined as the time between injection and ovulation. At the VPFS, the latency period in fish that either spawned naturally or were stripped ranged from 7.9 – 10.3 hours post first injection (at 27-28^{o}C).

Natural spawning

After injection with hormones (See Section Hormone injection), 1 - 3 females and 1 -3 males are placed together in a tank and are allowed to spawn naturally. Tanks should be covered to prevent fish from jumping out and fine screens placed over outlets to prevent eggs from being flushed out.

At the VPFS fish are held in a 5,000 L rectangular (2.9 m x 2.9 m x 0.6 m) concrete tank that is lightly aerated to keep eggs in suspension (Figure 6a). After spawning, the broodstock are removed and the eggs allowed to hatch in the tank.

At the PBFS broodstock are placed into a 1,800 L rectangular (0.3 m x 0.2 m x 0.3 m) concrete tank, which is supplied with a constant flow of fresh water and lightly aerated to ensure that spawned eggs remain in suspension (Figure 6b). After spawning, eggs are dispersed to 1,800 L rectangular concrete tanks for incubation and hatching (se Section Egg incubation and hatching).

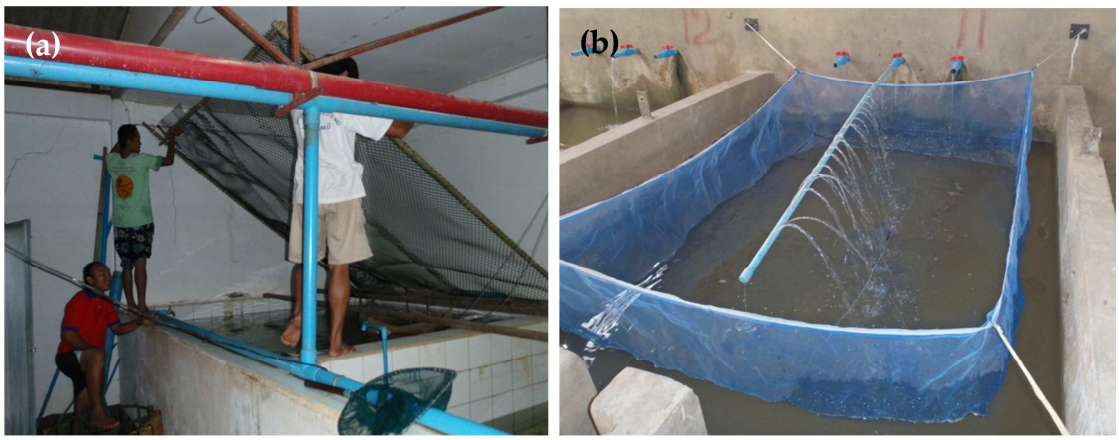

Figure 6. Broodstock holding tanks at, (a) the VPFS and (b) the PBFS.

Artificial spawning (hand-stripping)

Ovulated eggs and milt can be hand-stripped from females and running-ripe males. Fish must be anaesthetised during handling and stripping. To determine if ovulation has occurred, female Pa Phia are anaesthetised and pressure applied to the abdomen to determine whether eggs could be easily stripped from the fish. The vent of ovulated females will be swollen and pinkish in colour (Figure 7).

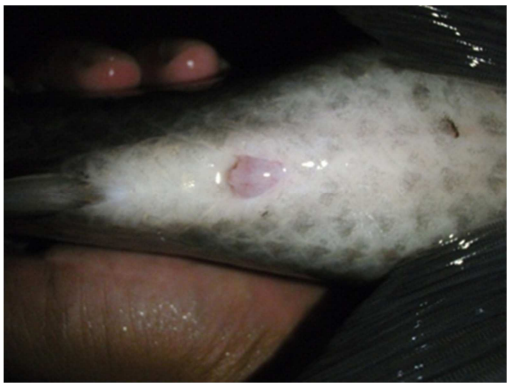

Figure 7. Vent of ovulated Pa Phia.

Since spawning occurs 7-10 hours after hormone injection (see section Latency period), fish should be first checked for signs of ovulation at around 7.5 hours post-injection. Fish that have not ovulated should be returned to the holding tank and checked every 30-60 minutes thereafter.

The gametes are hand-stripped from the broodstock and combined using a dry fertilisation method (see Tienchareon and Oonsrisong 1990). Ovulated eggs are stripped into a clean dry bowl (Figure 8a). The stripped eggs are then immediately fertilised with milt freshly stripped from an anaesthetized male (Figure 8b). **Prevent fish mucus, urine and faecal matter from entering the bowl while stripping. The steps that follow are:**

- The eggs and milt are then mixed together by swirling, or stirring with a clean dry feather (Figure 8c)
- After allowing up to 3-5 minutes for eggs to be fertilised, the eggs are rinsed with fresh hatchery water to remove excess milt and ovarian fluid (Figure 8d)
- The cleaned eggs are then decanted into a clean 50 mL, 100 mL or 250 mL measuring cylinder (depending on volume of eggs stripped) and the settled volume measured before transfer to a tank for incubation
- **Eggs from each stripped female should be incubated separately so that the hatch rate for each can be determined for each spawn**
- **After stripping all broodstock should be treated with 5% salt solution to reduce stress and potential infection**
- The relationship between stripped egg volume and number of eggs is presented in Figure 4.

Figure 8. Artificial spawning of Pa Phia. (a). Stripping eggs. (b) Stripping milt. (c) Mixing eggs and milt together. (c). Rinsing fertilised eggs.

Egg incubation and hatching

Eggs may be incubated in the tanks where broodstock spawned, or are transferred to other tanks for incubation. Since the eggs of Pa Phia are semi-buoyant, incubation tanks that prevent eggs from settling onto the bottom should be used. Tanks are supplied with a constant flow of water (1-2.5mL/min.), and are lightly aerated to avoid excess turbulence, which may damage eggs and larvae.

At the PBFS eggs are incubated in 1,800 L rectangular (0.3 m x 0.2 m x 0.3 m) concrete tanks at a density of 5 L eggs/tank (Approx. 140,000 eggs). The eggs are placed inside a coarse screen hapa which sits within a fine screen hapa (Figure 9). In this system, hatched larvae move into the outer hapa while un-hatched eggs are retained in the inner hapa. The tank is provided with a constant flow of freshwater via a series of perforated pipelines on the bottom of the tank. Aeration is provided by air stones located in the corners of the tank (Figure 9).

Upwelling incubators may also be used (Figure 10). Eggs are kept in suspension by a constant flow of water entering the bottom of the incubator. A screen prevents the eggs and larvae from being washed from the incubator.

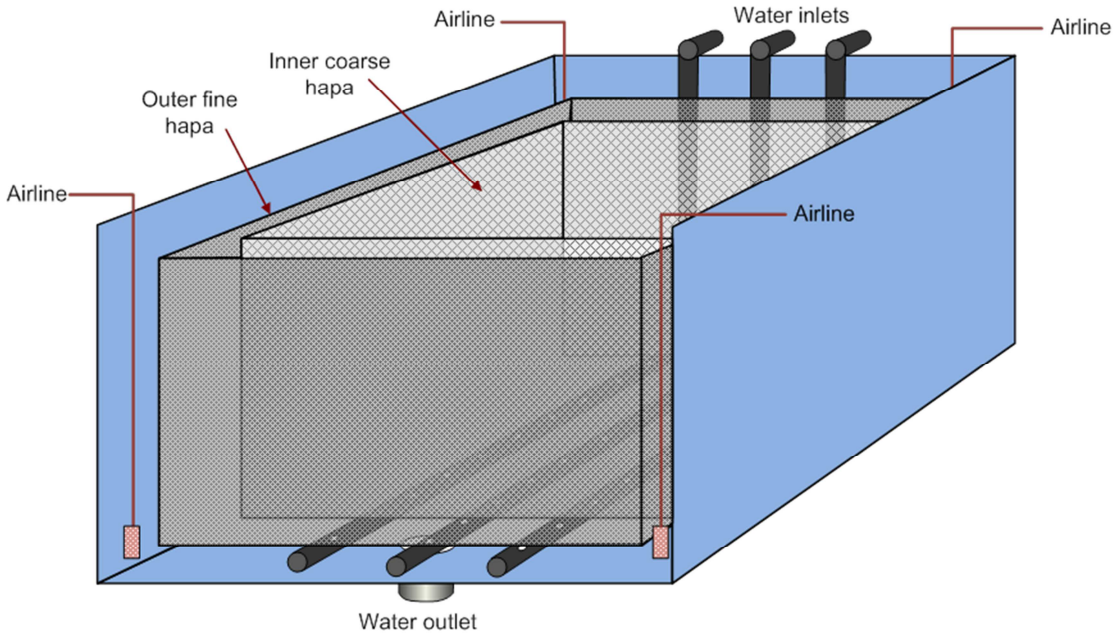

Figure 9. Design of 1,800 L egg incubation tank at the Pak Bo Fisheries Station, Savannakhet.

Figure 10. Upwelling egg incubators.

Embryonic development

Embryonic development of Pa Phia eggs is presented in Figure 11. fertilised eggs swell to a diameter of 1.92-4.49 mm (mean 3.62 mm) within 1 hr post-fertilisation (PF). The blastula is obvious within 2 hrs PF and by 2.5 hrs PF it has extended over 1/3-1/2 of yolk cell, which is oval in shape. By 5 hrs PF the trunk of the embryo has extended around most of the yolk cell and somites are clearly visible. Just prior to hatching eggs reach a 4.4-4.71 mm dia. (mean 4.54 mm dia) and larvae are very active in a now softening shell.

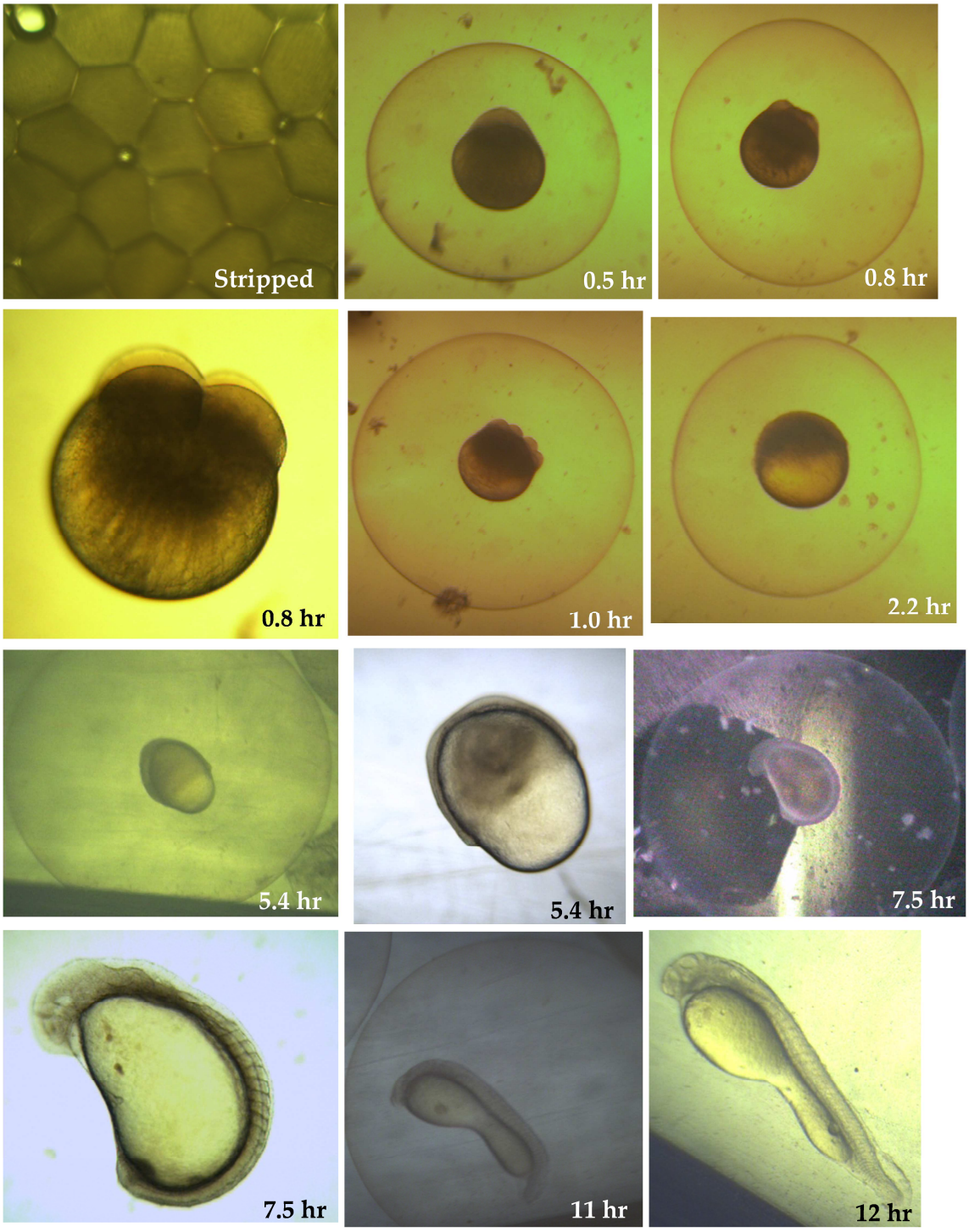

Figure 11. Embryonic development of Pa Phia eggs (time in hours post fertilisation).

Time to hatching depends on water temperature. At lower temperatures hatching occurs later, and is more protracted, than at higher temperatures. At VPFS, the water temperature of hatching commences from 12 hrs PF at 28-30^{o}C. Published data indicates hatching occurs 14-16 hours after fertilisation at 28°C (Watanadirokul *et al.* 1983, Leelapatra *et al.* 2000), 18-22 hrs at 20-30ºC (Tienchareon and Oonsrisong 1990) and approximately 12 hrs at 29-30°C (Thi *et al.* 2003).

Newly hatched larvae are poorly developed, elongate, 3.05-3.9 mm in length (mean 3.56 mm), with an elongated yolk sac that tapers posteriorly, and lacking pigmentation (Figure 12). The pectoral fin buds and undifferentiated dorsal and anal finfolds are present, yet the mouth is not open.

The hatching rates at the PBFS and VPFS are variable, ranging from 0 to 80%, though higher rates may be achieved for good quality eggs.

Figure 12. Newly hatched Pa Phia larva (25.2 hrs post-fertilisation).

Larvae and fry rearing

Larviculture

Hatched larvae require good water quality to survive. Ensure larval rearing tanks are provided with a constant flow of fresh water. Care should be taken in aerating tanks as excessive turbulence from air bubbles may stress or injure injury larvae. For this reason, air stones are located outside the hapas used for rearing larvae (Figure 9).

Tanks should be checked daily. Remove uneaten food and dead fish to reduce fouling of the water, and check water quality, growth and health of fish (see Appendix II).

Newly hatched larvae are active swimmers. By day 3 post-hatch, some larvae commence feeding. As the larvae grow, energy reserves in the yolk sac will diminish and their appetite will increase.

There are no larval feed rates published for Pa Phia, however, during the early days of exogenous feeding, larvae should be fed to excess to ensure that their energy needs are met. At one private hatchery, larvae are fed soy bean for the first 7 days of feeding, then rice bran thereafter.

Nursery phase (fry rearing)

During this nursery phase fish are typically reared in a fertilised earthen fry rearing (nursery) pond using greenwater pond culture methods. Growing juvenile fish in earthen ponds that have been fertilised to encourage the development of plankton blooms on which the fish feed, is a widely used and cost effective means of mass rearing juvenile fish species in aquaculture worldwide (e.g. Jana and Chakrabarti 1993, Opuszynski and Shireman 1993, Feldlite and Milstein 1999). During this phase larvae undergo metamorphosis and are grown through to a fingerling stage (2-3 cm in length).

The principal objective of rearing juvenile fish in fertilised fry ponds is to provide an environment in which the fish survive and grow rapidly. This relies on the maintenance of an appropriate size and abundance of food organisms (phytoplankton, zooplankton and macroinvertebrates), while simultaneously maintaining water quality suitable for survival and growth.

It is important to time preparation and filling of ponds to coincide with fish spawnings to ensure that when larvae are ready to be stocked into the ponds, there has been sufficient time for the ponds to develop suitable blooms of plankton. Ponds should be prepared for stocking before spawning of Pa Phia to allow plankton blooms to develop. Ponds may be limed, filled and fertilised 3-7 days before the anticipated stocking date.

Pond Preparation

At the VPFS fry ponds are limed at a rate of 0.05 kg/m^2 before filling. A wide range of fertiliser regimes made be applied to ponds (see Lin *et al.* 1997, Knud-Hansen *et al.* 1998) Fertilizer regimes for ponds depend on availability of fertilizers available. For example, at the VPFS, ponds are fertilised with cow dung (250 kg/ha) and Urea (25 kg/ha) once per week.

Since Pa Phia is thought to be a benthic grazer, Rocks are added to the fry rearing ponds at the PBFS to provide a substrate for growth of plants (algae) and animals, on which the larvae and fry will graze. This approach has been applied for rearing the fry of other herbivorous cyprinids (Keshavanath *et al.* 2001, Keshavanath *et al.* 2002, van Dam *et al.* 2002, Gangadhara *et al.* 2004).

Stocking density

Fish may be stocked into ponds at the onset of feeding (3-4 days after hatching, approx.. 1 cm in length), or after feeding in the hatchery for a few days.

Stocking densities for cyprinids larvae are typically between 50 and 500 larvae/m^2 (e.g. Feldlite and Milstein 1999, Chakraborty and Mirza 2007, Rahman *et al.* 2008). It has been observed that lower densities result in higher growth rates and survival rates (Chakraborty and Mirza 2007).

Water quality

Water quality should be monitored at least weekly (see Appendix II and IV). Due to diurnal variations in some parameters, a monitoring program should ensure that samples are routinely collected at the same time each day. Additional samples should be collected at times when critical levels in key water quality parameters occur, such as early morning (low dissolved oxygen) and late afternoon (high pH). Supplementary aeration (such as by paddlewheels) may be required to increase dissolved oxygen, circulate water and prevent pond stratification. Ponds should be flushed regularly (at least weekly) with fresh water to replace water lost through evaporation and seepage, and to reduce the

effects of poor water quality events such as high pH or high ammonia. Regular (weekly) sampling of plankton concentrations will provide a guide to pond productivity and food availability. Fish should be sampled weekly, to monitor, growth and condition and to check for diseases (see Appendix II). Water quality variables recorded in a fry pond at the VPFS are provided in Table 5.

Table 5. Water quality variables recorded in an earthen fry rearing pond at the Vientiane Provincial Fisheries Station, Nam Cheang.

Parameter	Mean ± s.e.	Range
Temperature (^{o}C)	31.3 ± 0.8	28 - 36.6
pH	7.88 ± 0.23	6.16 - 9.23
Dissolved oxygen (mg/L)	2.48 ± 0.26	1.3 - 3.8
Ammonia (mg/L)	1.08 ± 0.25	0.09 - 1.88
Secchi disk depth (cm)	17 + 1.4	12 - 24

Supplementary feeding

Pa Phia larvae and fry will feed on planktonic and benthic plants (algae) and animals that grow in the pond. For example, cladocerans and midge (chironomid) have been found in the stomachs of larvae stocked into a fry pond at the VPFS.

During the nursery phase, both the VPFS and the PBFS provide supplementary food to Pa Phia larvae and fry, particularly in ponds that have been stocked at high densities and when natural food is limiting. Food includes powdered egg, rice flour, rice bran and foods for other animals (eg. freshwater catfish). Supplementary feeding regimes used at the VPFS and the PBFS are provided in Table 6.

Table 6. Supplementary feeding regime for Pa Phia fry rearing ponds (VPFS).

Period (days)	No. of larvae/fry		
	< 100,000	250,000	500,000
1-10	1/3 bag rice flour + 2 eggs (powdered)	1 bag rice flour + 2 eggs (powdered)	1 bag rice flour + 2 eggs (powdered)
10-25	125 g rice bran + 125 g pig feed*	500 g rice bran + 500 g pig feed*	1.25 kg rice bran + 1.25 kg pig feed*
25-45	250 g rice bran + 250 g pig feed*	750 g rice bran + 750 g pig feed*	2 kg rice bran + 2 kg pig feed*

* Pig feed: 29% protein, 2% fat, 9% fibre & 12% moisture.

Fish growth and recovery

Pa Phia reared in a pond at the VPFS grew at a rate of 0.78 mm/day (Figure 13). At this rate fish will reach 3 cm at 32 days of age.

Ponds are harvested after fish reach 2-3 cm in length (35-45 days after stocking). Recovery rates recorded at the VPFS and the PBFS are usually between 15% and 20% (exceptionally up to 40%).

Partial harvesting, to remove small numbers of fish as required, may be done by netting the pond with a fine net. Ponds may be completely harvested by draining to recover the fingerlings.

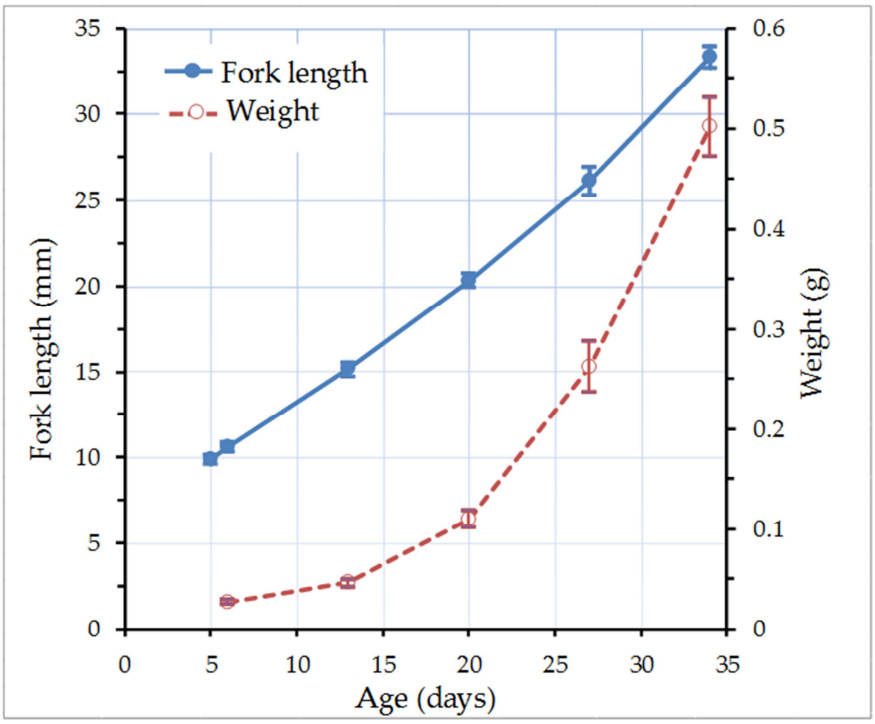

Figure 13. Growth of Pa Phia fry in a nursery pond at the VPFS (values = mean ± standard error).

Conclusions

The guidelines presented in this document will assist in the hatchery production of Pa Phia fingerlings which can be used by rural communities to develop culture-based fisheries.

Although the guidelines were specifically developed for production of Pa Phia at government hatcheries in the Lao PDR, they may be applied to other, related, species that are produced in hatcheries for stocking purposes, as well as be adopted and commercialised by the private sector hatcheries in the region.

References

Aggarwal, R.K., Nguyen, T. and et al (2011). Permanent Genetic Resources added to Molecular Ecology Resources Database 1 August 2010 – 30 September 2010. *Molecular Ecology* 11: 219-222.

Arthur, J.R. and Te, B.Q. (2006). *Checklist of the parasites of fishes of Viet Nam.* . FAO Fisheries Technical Paper No. 369/2. FAO, Rome. 133 pp.

Baird, I.G., Inthaphaisy, V., Kisouvannalath, P., Phylavanth, B. and Mounsouphom, B. (1999). *The Fishes of Southern Lao*. Lao Community Fisheries and Dolphin Protection Project. Ministry of Agriculture and Forestry, Lao PDR. 161 pp.

Bardach, J. (1959). *Report of Fisheries in Cambodia*. USOM, Phnom Penh, Cambodia.

Bert, T.M. ed. (2007). *Ecological and Genetic Implications of Aquaculture Activities*. Springer, Dordrecht, The Netherlands. 548 pp.

Boonmon, V. and Kantejit, J. (1977). *Life history of black shark.* . Annual report 1977, Ubonratchathani Inland Fisheries Station, Inland Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives. 19-22 pp.

Brown, L. ed. (1993). *Aquaculture for Veterinarians. Fish Husbandry and Medicine*. Pergamon Press Ltd., Oxford. 447 pp.

Chabjinda, K., Tienchareon, P. and Jittakorn, A. (1992). *Monitoring of stocking of black shark, Morulius chrysophekadion (Bleeker) in Mae Ngad Somboonchon Reservoir, Chiang Mai Province*. Technical Paper No. 10/1992. Inland Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives. 14 pp.

Chakraborty, B.K. and Mirza, M.J.A. (2007). Effect of stocking density on survival and growth of endangered bata, Labeo bata (Hamilton-Buchanan) in nursery ponds. *Aquaculture* 265 (1-4): 156-162.

De Silva, S.S. (2008). First culture-based fisheries growth cycle in Lao PDR is overwhelmingly encouraging. *Aquaculture Asia* 13 (4): 9-10.

De Silva, S.S., Amarasinghe, U.S. and Nguyen, T.T.T. eds. (2006). *Better-practice approaches for culture-based fisheries development in Asia,* Monograph 120. ACIAR, Canberra. 96 pp.

De Silva, S.S. and Anderson, T.A. (1995). *Fish Nutrition in Aquaculture*. Chapman and Hall, Melbourne. 319 pp.

De Silva, S.S., Saphakdy, B., Ingram, B.A., Nguyen, T.T.T. and A., P. (2010). *Culture-Based Fisheries Development in Lao PDR. Final report for ACIAR Project FIS/2005/078*. 37 pp.

Feldlite, M. and Milstein, A. (1999). Effect of density on survival and growth of cyprinid fish fry. *Aquaculture International* 7 (6): 399-411.

Gangadhara, B., Keshavanath, P., Ramesha, T.J. and Priyadarshini, M. (2004). Digestibility of Bamboo-Grown Periphyton by Carps (*Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Tor khudree*) and Hybrid Red Tilapia (*Oreochromis mossambicus* x *O. niloticus*). *Journal of Applied Aquaculture* 15 (3-4): 151-162.

Hoole, D., Bucke, D., Burgess, P. and Wellby, I. (2001). *Diseases of Carp and other Cyprinid Fishes*. Fishing News books, Oxford. 264 pp.

Ingram, B.A. and Lasasimma, O. (2008). Production of *Cirrhinus molitorella* and *Labeo chrysophekadion* for culture based fisheries development in Lao PDR. Part 1. Captive spawning. *Aquaculture Asia* 13 (2): 24-31.

Iwama, G.K., Pickering, A.D., Sumpter, J.P. and Schreck, C.B. eds. (1997). *Fish Stress and Health in Aquaculture*. Cambridge University Press, Cambridge. 278 pp.

Izquierdo, M.S., Fernandez-Palacios, H. and Tacon, A.G.J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* 197: 25-42.

Jana, B.B. and Chakrabarti, R. (1993). The effect of management protocols for juvenile carp (*Cyprinus carpio*) culture on life history responses of a zooplankton food source, *Moina micrura* (Kurz.). *Aquaculture* 110: 285-300.

Jhingran, V.G. and Pullin, R.S.V. (1985). *A Hatchery Manual for the Common, Chinese and Indian Major Carps* ICLARM Studies and Reviews 11, Asian Development Bank, Manila, Philippines and International Center for Living Aquatic Resources Management, Manila, Philippines 191 pp.

Kapuscinski, A.R. and Miller, L.M. (2007). *Genetic Guidelines for Fisheries Management*. University of Minnesota Sea Grant Program, Duluth, MN. 113 pp.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M. and Verdegem, M.C.J. (2002). The effect of periphyton and supplemental feeding on the production of the indigenous carps *Tor khudree* and *Labeo fimbriatus*. *Aquaculture* 213 (1-4): 207-218.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Rooij, J.M., Beveridge, M.C.M., Baird, D.J., Verdegem, M.C.J. and van Dam, A.A. (2001). Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. *Aquaculture Research* 32: 189-197.

Knud-Hansen, C.F., McElwee, K., Baker, J. and Clair, D. (1998). *Pond fertilization: ecological approach and practical application*. Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University Corvallis, Oregon.

Kumarasiri, W. and Seneviratne, P. (1988). Induced multiple spawnings of Chinese carps in Sri Lanka. *Aquaculture* 74 (1): 57-62.

Laoobuth, P., Pooruxkiati and Tienchareon, P. (1993). *Annual spawning potential of black shark, Morulius chrysophekadion (Bleeker)*. Technical Report No. 9/1993, Inland Fisheries Division, department of Fisheries, ministry of Agriculture and Cooperatives. 8 pp.

Leelapatra, W.P., Srisakultiew, P. and Sukumasavin, N. (2000). *Biology and Breeding of Indigenous Mekong Fish Species in Thailand*. Management of Reservoir Fisheries in the Mekong Basin II, Vientiane.

Lin, C.W., Teichert-Coddington, D.R., Green, B.W. and Veverica, K.L. (1997). Fertilization regimes. In: *Dynamics of Pond Aquaculture* (Egna, H.S. and Boyd, C.E. eds.), pp. 73-107. CRC Press, New York.

Miller, L.M. and Kapuscinski, A.R. (2003). Genetic guidelines for hatchery supplementation programs. In: *Population Genetics Principles and Applications for Fisheries Scientists* (Hallerman, E.M. ed.), pp. 329-355. American Fisheries Society, Bethesda.

MRC (2003). *Mekong Fish Database version 2003. CD-ROM*. Mekong River Commission, Phnom Penh, Cambodia.

Noga, E.J. (2000). *Fish Disease Diagnosis and Treatment*. Iowa State University, Ames, Iowa. 367 pp.

Opuszynski, K.K. and Shireman, J.V. (1993). Strategies and tactics for larval culture of commercially important carp. *Journal of Applied Aquaculture* 2(3/4): 189-219.

Rahman, M.A., Zaher, M. and Azimuddin, K. (2008). Evaluation of growth, survival, and production of an endangered fish, Labeo gonius (Hamilton) fingerlings in earthen nursery ponds. *Journal of Applied Aquaculture* 20 (1): 62-78.

Sarig, S. (1971). *Diseases of Fishes. Book 3: The Prevention and Treatment of Diseases of Warmwater Fishes under Subtropical Conditions, with Special Emphasis on Intensive Fish Farming*. TFH Publications, Inc. Ltd., London.

Schlotfield, H.-J. and Alderman, D.J. (1995). What Should I do? A Practical Guide for the Fresh Water Fish Farmer. *Supplement to Bulletin of The European Association of Fish Pathologists* 15 (4): 60 pp.

Singh, B., Das, R., Sahu, A., Kanungo, G., Sarkar, M., Sahoo, G., Nayak, P. and Pandey, A. (2000). Balanced diet for the broodstock of *Catla catla* and *Labeo rohita* and induced breeding performance using ovaprim. *Journal of Advanced Zoology* 21 (2): 92-97.

Sohn, W.-M., Yong, T.-S., Eom, K.S., Pyo, K.-H., Lee, M.Y., Lim, H., Choe, S., Jeong, H.-G., Sinuon, M. and Socheat, D. (2012). Prevalence of *Opisthorchis viverrini* infection in humans and fish in Kratie Province, Cambodia. *Acta tropica* 124 (3): 215-220.

Sokheng, C., Chhea, C.K., Viravong, S., Bouakhamvongsa, K., Suntornratana, U., Yoorong, N., Tung, N.T., Bao, T.Q., Poulsen, A.F. and Jørgensen, J.V. (1999). *Fish migrations and spawning habits in the Mekong mainstream: a survey using local knowledge (basin-wide). Assessment of Mekong fisheries: Fish Migrations and Spawning and the Impact of Water Management Project (AMFC)* Vientiane, Lao PDR. .

Thi, T.V., Dang, V.T., Dinh, H., Hoang, Q.B., Pham, D.K. and Nguyen, M.T. (2003). Preliminary artificial propagation results of greater black carp (*Morulius chrysophekadion*). In: *Proceedings of the Fifth Technical Symposium on Mekong Fisheries, 11-12 December 2002* (Poulsen, A. ed.), pp. 143-149. Mekong River Commission Phnom Penh.

Thienchareon, P., Unsrisong, K. and Jittakorn, A. (1989). *Breeding and rearing of Pla Ka Dum*. Annual Report 1989. Chiangmai Inland Fisheries Research Centr. Inland Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives. 108-115 pp.

Tienchareon, P. and Oonsrisong, K. (1990). Breeding of Pla Kar Dum (*Morulius chrysophekadion*). *Thai Fisheries Gazette* 43 (1): 47-50.

Trinh Quoc Trong, Dang Van Truong, Thi Thanh Vinh, Dinh Hung, Hoang Quang Bao, Khoi, P.D. and Thanh, N.M. (2005). Artificial propagation of indigenous fish *Cirrhinus microlepis*, *Leptobarbus hoevenii*, *Labeo chrysophekadion* and *Osteochilus melanopleurus* in the Mekong delta of Vietnam. *In World Aquaculture 2005 (9-13 May 2005, Bali, Indonesia)*, pp. 379.

van Dam, A.A., Beveridge, M.C., Azim, M.E. and Verdegem, M.C. (2002). The potential of fish production based on periphyton. *Reviews in Fish Biology and Fisheries* 12 (1): 1-31.

Watanadirokul, K., Kitisuwan, P. and Boonpeo, L. (1983). *Life history of Morulius chrysophekadion (Bleeker) in Ubolratana Reservoir*. Annual Report 1983. Ubolratana Reservoir Fisheries Development Station, Inland Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives. 14 pp.

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Appendix 1 - Glossary

ACIAR	Australian Centre for Agricultural Research
Aquaculture	Growing of water plants and animals
Blastula	Ball of cells, formed by dividing cells of zygote
Biodiversity	The variety of life in all its forms, levels and combinations, encompassing genetic diversity, species diversity and ecosystem diversity
Broodstock	A group of fish, generally from the same population, that are held and eventually artificially spawned to provide a source of fertilized eggs for hatchery programs.
CBF	Culture-based fisheries
DOM	Domperidone
Effective breeding number (*Ne*)	The breeding (genetic) size of a finite population. It is determined by the number of breeding individuals, the sex ratio, the breeding program, the variance of family size, and previous inbreeding
Fish hatchery	A facility at which adult broodstock are held, or where eggs are collected and incubated, or where eggs are hatched, or where fish are reared.
Gametes	Reproductive cells of male (sperm) and female (egg).
Genetic diversity	The heritable variation within and among populations which is created, enhanced or maintained by evolutionary forces
HCG	Human chorionic gonadotrophin
Inbreeding	Matings between individuals that have one or more ancestors in common
LHRH	Luteinizing Hormone Releasing Hormone
LHRHa	Luteinizing Hormone Releasing Hormone analogue
PBFS	Pak Bo Fisheries Station, Savannakhet, Lao PDR
PF	Post-fertilisation
PG	Pituitary gland
PIT	Passive integrated transponder
Somites	Body segments
UV	Ultra-violet
VPFS	Vientiane Provincial Fisheries Station, Nam Cheang, Lao PDR
Zygote	Cell formed by union of male and female reproductive cells (gametes)

Appendix 2 – Monitoring guidelines

Parameter	Frequency of sampling	Comments
General (all data sheets)		
Pond/tank number	Every sample period	
Date and time	Every sample period	
Primary water quality parameters		
Temperature	Daily	May fluctuate diurnally
Dissolved oxygen	1-2 daily (tanks) 1-3 weekly (ponds)	Marked diurnal fluctuations can occur in ponds
pH	1-2 daily (tanks) 1-3 weekly (ponds)	Marked diurnal fluctuations can occur in ponds
Ammonia	Daily (tanks) Weekly (ponds)	Amount of ammonia in toxic form influenced by temperature and pH
Other water quality parameters		
Alkalinity	Weekly (ponds)	Where water source is low in alkalinity
Nitrite	Daily (tanks) Weekly (ponds)	
Nitrate	Daily (tanks) Weekly (ponds)	
Phosphorus or phosphate	Weekly (ponds)	
Secchi disk depth	Daily to weekly (ponds)	Indicator of plankton density
Turbidity	As required	
Suspended solids	As required	
Salinity	As required	
Fish health		
Sample collection	1-2 weekly	
Behaviour	Each sampling	Feeding activity, swimming behaviour etc.
Appearance	Each sampling	Skin, eyes, fins, gills, colouration, markings, fin condition, shape of body etc.
No. fish affected	Each sampling	No. of moribund fish, No. of dead fish, etc.
No. fish sampled	Each sampling	
Length of fish	Each sampling	
Weight of fish	Each sampling	
Location of parasites on/in fish	Each sampling	body surface, gills, fins, internal organs
Parasite species observed	Each sampling	If cannot be identified, photograph and/or draw and describe size, appearance and behaviour.
No. of parasites present	Each sampling	No. observed per field of view
Action taken	Each sampling	What action was taken following inspection of fish (ie, treatments applied et.)
Additional useful information		
Tank/pond volume, date filled, date stocked		
Source/Species/strain of fish stocked		
Length, weight and age at stocking, and Number fish stocked		
Feeding information (Feed type and feed rate etc)		
Chemicals added (type, amount and reason for addition)		

Appendix 3 – Useful conversions and formulae

Volumes

1 m^3 = 1,000 L

1,000 mL = 1 L (litre)

1,000,000 L = 1 ML

Weight

1,000 ug = 1 mg

1,000 mg = 1 g

1,000 g = 1 kg

1,000 kg = 1 tonne

Conversions

1 ppm = 1 mg/L

1 ppm = 1 L in 1,000,000 L

1 ppm = 1 mL in 1,000 L

1ppt = 1 g/L = 0.1%

1 ppt = 1,000 ppm

1% = 10 ppt = 10 g/L

1 mg/L = 1 g in 1,000 L

Specific growth rate (SGR), is expressed as the percentage increase in body weight per day (%/day) and is determined by using the formula:

$$\text{Specific Growth Rate (SGR)} = \frac{(\ln Wt_2 - \ln Wt_1)}{(t_2 - t_1)} \times 100\ \%$$

where: t = time in days.
lnWt_2 = natural logarithm of the average weight at time t_2.
lnWt_1 = natural logarithm of the average weight at time t_1.

Food Conversion Ratio (FCR) is determined by the formula:

$$\text{Food Conversion ratio (FCR)} = \frac{\text{Food consumed (g dry } Wt) \text{ between } t_1 \text{ and } t_2}{\text{Increase in fish biomass (g wet } Wt) \text{ between } t_1 \text{ and } t_2}$$

where: t_1 = Initial time.
t_2 = Final time.
Wt_2 = Final fish weight (g) (at time t_2).
Wt_1 = Initial fish weight (g) (at time t_1).

Appendix 4 – Datasheet templates

Breeding data

Location/Hatchery:	***Species*:**	**Trial No.**	**Tank No.**

FEMALE 1	**FEMALE 2**	**MALE 1**	**MALE 2**
Source:	Source:	Source:	Source:
Tag:	Tag:	Tag:	Tag:
Tot. Lth (mm): Weight (g):	Tot. Lth (mm): Weight (g):	Tot. Lth (mm): Weight (g):	Tot. Lth (mm): Weight (g):
Gamete status:	Gamete status:	Gamete status:	Gamete status:
1st Injection	***1st Injection***	***1st Injection***	***1st Injection***
Date: / / Time:	Date: / / Time:	Date: / / Time:	Date: / / Time:
Hormone 1: Dose /kg	Hormone 1: Dose /kg	Hormone 1: Dose /kg	Hormone 1: Dose /kg
Hormone 2: Dose /kg	Hormone 2: Dose /kg	Hormone 2: Dose /kg	Hormone 2: Dose /kg
2nd Injection	***2nd Injection***	***2nd Injection***	***2nd Injection***
Date: / / Time:	Date: / / Time:	Date: / / Time:	Date: / / Time:
Hormone 1: Dose /kg	Hormone 1: Dose /kg	Hormone 1: Dose /kg	Hormone 1: Dose /kg
Hormone 2: Dose /kg	Hormone 2: Dose /kg	Hormone 2: Dose /kg	Hormone 2: Dose /kg

Spawning information		***Fertilisation information***
☐ **Natural spawning**	☐ **Hand-stripped (per female)**	**Date: / / Time: Fertilisation rate: %**
Date:____/___/____ Time:_____	Date: ____/____/____ Time: _____	***Hatching information***
No. eggs spawned:_____	No. eggs stripped:_____	Start hatch: Date: _____/_____/_____ Time:_____
No. females:____ No. males:____	No. males used:_____ Milt(ml): _____	End hatch: Date: _____/_____/_____ Time:_____
Incubator: _____	Incubator: _____	Hatch rate: _____% Deformed larvae:_____%
Temperature (^{o}C):_____	Temperature (^{o}C): _____	Total No. Larvae: _____ Larvae to?:_____

Comments:

Source of broodstock = Pond No or Tank No. or Cage No. *Gamete status* = oocyte stage, diameter, males running ripe etc. *. Lth (mm)* = Total length (mm).

Fry Pond sampling - Fish

- Collect data one day per week, every week until the pond is harvested.
- Collect and measure at least 10 fish.
- Check the gut contents of 2 fish.
- Check plankton species in the pond
- Check 2 fish for diseases.
- At harvest measure 20 fish, and record total number of fish harvested.

DATE	FISH		Diet information (2 fish)	Plankton information	Disease information
	Fork length (mm)	Weight (g)			
	1.				
	2.				
	3.				
	4.				
	5.				
	6.				
	7.				
	8.				
	9.				
	10				
	11.				
	12.				
	13.				
	14.				
	15.				
	16.				
	17.				
	18.				
	19.				
	20.				
Comments:					

Water quality

- Measure temperature, pH, dissolved oxygen and secchi depth daily. Please do this at the same time each day (at the first feeding)
- Measure Ammonia one day per week.
- Record fertilizer added and food added

DATE	Time	Temperature	pH	Secchi depth	DO	Ammonia	Fertilizer added		Food added	Comments
		(oC)		(cm)	(mg/L)	Mg/L	Cow dung (kg)	Urea (kg)		

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