Production of *Cirrhinus molitorella* and *Labeo chrysophekadion* for culture based fisheries development in Lao PDR Part I: Captive spawning

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Project team inspecting one of the project study sites in Bolikhamxay Province.

The Government of Lao PDR is targeting an increase in fish consumption to 23 kg caput/year by the year 2020, almost a doubling of the current level of consumption. In view of the unlikely increase in capture fisheries, the envisaged increase in per caput consumption can only be realized through increasing aquaculture related production. A substantial increase in fish production could be obtained through effective and optimal utilisation of seasonal water bodies, such as flood plain depressions and reservoir coves for culture-based fisheries (CBF), a practice that requires little or no capital inputs and harnesses natural productivity of these water bodies for augmenting fish production (De Silva et al. 2006). The practice also is environmentally non-perturbing, compared to conventional aquaculture. Through village community participation, CBF augment current food fish availability, particularly in the lean period of the traditional wild capture fisheries (March to August), and raise the income of village communities.

The Australian Centre for International Agricultural Research (ACIAR) has recently instigated a project, Culture-Based Fisheries Development in Lao PDR (Project No. FIS/2005/078), which aims to develop best practice approaches and production models for CBF in Lao PDR that will improve the yields and economic benefits to village communities (Figure 1). This project involves collaboration amongst six institutions, two each in Australia (Deakin University and DPI, Victoria), two in Lao PDR (Department of Livestock and Fisheries - DoLF and Living Aquatic Resources Research Center - LARReC) and Thailand (Network of Aquaculture Centers in Asia-Pacific - NACA and Fisheries Faculty, Kasetsart University).

An important aspect of CBF is the production and provision of sufficient numbers of juvenile fish of selected species for stocking purposes. However, in Lao PDR fry/ fingerling production is low even though demand for stocking into both pond and rice-fish systems is high. Most fry produced in Lao PDR originate from the Provincial government hatcheries that have an overall production level of less than 15 million (Meenakarn and Funge-Smith 1998), which is insufficient to meet the needs of CBF.

The emphasis on aquaculture and CBF development calls for an increase in the quantity and quality of seedstock of a variety of species suitable for different forms of aquaculture practices. The Culture-Based Fisheries Development in Lao PDR project has identified two popular indigenous fish species, Cirrhinus molitorella (mud carp, “Pa Keng”) and *Labeo* (syn *Morulius*) *chrysophekadion* (black sharkminnow, “Pa Phia”) for use in CBF, based on their popularity and existing on-going culture. Their selection was also in accordance with the increasing trend in the region to lay emphasis on the culture of indigenous species in preference to exotics and or alien species.

This article is the first part of a two part series that aim to review current, readily available, information on these two indigenous species which will be used to improve and refine artificial propagation and culture techniques to support CBF development in Lao PDR. In particular, the review focuses on information within Lao PDR, as well information from the Mekong Fish Database (MRC 2003). See the next edition of Aquaculture Asia for Part 2 of this series.

Cirrhinus molitorella and *Labeo chrysophekadion*

Cirrhinus molitorella is a freshwater cyprinid native to Asia that inhabits lakes, rivers and reservoirs from the Mekong (Thailand, Cambodia and Vietnam) and Chao Phraya (Thailand) basins, to the Pearl River deltas (China) (MRC 2003, FishBase 2007) *C. molitorella*, which has fine meat texture, good meat quality and high nutritional value, is mainly sold live or fresh locally (Figure 2), but some is also canned, dried and salted, or minced to form a fish cakes or dumplings (FAO 2007a). *C. molitorella* has a long history in aquaculture being first undertaken in the Pearl River region of southern China during the Tang Dynasty (618-904 A.D.), and today is a well-established aquaculture species particularly in this region (FAO 2007b). Between 1990 and 2001, aquaculture production of *C. molitorella* was between 80,000 and 220,000 tonnes per year, the vast majority (>99%) was produced in China (FAO 2007b). *C. molitorella* is a popular aquaculture species in the Luang Probang Province of Lao PDR where it has been propagated and farmed since 1991 (Pinthip et al. 2001, Souksavath 2001, Somboon et al. 2003). In 2005 the Lao PDR produced just 3,100 tonnes (FAO 2007b).

Labeo chrysophekadion is one of the larger Asian cyprinids that is widely distributed throughout the Mekong (Thailand, Cambodia and Vietnam) and Chao Phraya (Thailand) basins, as well as the Malay Peninsula, Sumatra, Java and Borneo (Malaysia and Indonesia) (FishBase 2007). *L. chrysophekadion* is a very important commercial species in the Mekong River basin and in southern Lao PDR is one of the more expensive fish at the market (Singhanouvong and Phouthavong 2002). The species is caught with small to large scale fishing gear and sold in markets fresh (Figure 2), dried and salted, used widely in the aquarium trade, and has very good potential for aquaculture (Rainboth 1996, MRC 2003). However, capture fishery and aquaculture production statistics for this species are not readily available (FAO 2007a). Captive breeding and rearing of *L. chrysophekadion* has been described in both

> This article is the first part of a two part series that aims to review current, readily available, information on two indigenous species *Cirrhinus molitorella* (mud carp, "Pa Keng") and *Labeo* (syn *Morulius*) *chrysophekadion* (black sharkminnow, "Pa Phia") for use in Culture Based Fisheries (CBF) development in Lao PDR. These two species are consumer preferred and are currently cultured to some extent. In this part the existing knowledge on the artificial propagation of these two species is reviewed, and these will be further improved and refined to ensure supplies of good quality seed stock to support CBF development in Lao PDR.

Fresh Cirrhinus molitorella (left) and L. chrysophekadion (right) at a provincial fish market in Lao PDR.

Thailand and Lao PDR (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).

Spawning seasonality

Most freshwater tropical fish species spawn during the monsoon season, and this is also the case for *C. molitorella* and *L. chrysophekadion* (1), thought to be primarily because rainfall increases the available habitats and nutrients for the hatchlings. The water quality of many water bodies usually improves, with increased oxygen and cooler temperatures. Hardness and pH of the water also change with the inputs of rainwater runoff (Meenakarn and Funge-Smith 1998).

Table 1. Spawning seasons of *C. molitorella* and *L. chrysophekadion*

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Country	Habitat	Source
C. molitorella														
				■	■	■	■					Lao PDR	Pond	Nuanthavong and Vilayphone 2005
					■	■						Lao PDR	Pond	Gorda 2001
					■	■	■					Lao PDR	River	Singhanouvong *et al.* 1996
L. chrysophekadion														
				■	onwards							Thailand	Pond	Laoobuth *et al.* 1993
					■	■						Cambodia	River	Bardach 1959, Sokheng *et al.* 1999
					■	■						Cambodia	River	Bardach 1959, Sokheng *et al.* 1999
						■	■					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

Table 2. Summary of hormone treatments for induction of spawning in *C. molitorella* and *L. chrysophekadion*

Species	Treatment	First injection	Second injection	Hours between injections	Source
C. molitorella	Suprefact® (ml/kg) + Motilium® (tablet)	♀ 0.2+one ♂ 0.1+?	Nil		Gorda 2001
	Suprefact® (µg/kg) + Motilium® (mg/kg)	♀ 15-18+10 ♂ 7-9+5	Nil		Nuanthavong and Vilayphone 2005
L. chrysophekadion	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 (IU/kg)	♀ 2.3+500 ♂ 1.2+800	♀ 3.5+2,000 ♂ Nil	6-8	Thi *et al.* 2003
	PG[1] (mg/kg) + LHRHa (µg/kg) + DOM[2] (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

1. PG = pituitary gland of either common carp or mrigal.
2. DOM = Domperidone

Injecting a L. chrysophekadion broodstock with a hormone to induce spawning.

Both *C. molitorella* and *L. chrysophekadion* are semi-migratory species that undergo upstream migrations to spawn. *C. molitorella* migrate upstream from December to March to spawn in the floodplains of large rivers during the rainy season, before returning downstream from June to August (MRC 2003, Nuanthavong and Vilayphone 2005). Riverine *L. chrysophekadion* migrate upstream from March to spawn early in the monsoonal flood season (June-July) (Boonmon and Kantejit 1977, Baird et al. 1999), whereas fish in reservoirs tend to spawn later and over a longer period (July-October) (Boonmon and Kantejit 1977, Chabjinda et al. 1992) (Table 1).

Captive spawning

The first hormone to be widely and successfully used for induction of fish spawning was carp pituitary gland (PG) extract. But in Lao PDR this method is not suitable due to the shortage of large sized cyprinid broodstock for extracting pituitary glands (Meenakarn and Funge-Smith 1998). Human Chorionic Gonadotropin (HCG) has also been successfully used to induce fish spawning and this can be obtained commercially as a "ready-to-use" dry hormone. This is more convenient to use than CPG and dosages for fish can be applied more accurately (De Silva et al. 2007). Luteinizing Hormone Releasing Hormone analogues (LHRHa) are highly effective (and affordable) in stimulating gonadotropin secretion and inducing ovulation in freshwater fish. One LHRHa in particular, Buserelin Acetate, is widely used to induce spawning in *C. molitorella* and *L. chrysophekadion*. Buserelin Acetate is readily available in commercial form, such as Suprefact® (Sanofi-Aventis) which is used primarily to treat humans with hormone-dependent advanced carcinoma of the prostate gland. The effectiveness of LHRHa is further enhanced when used in combination with a dopamine antagonist. Suprefact® is often combined with the domperidone Motilium® (Janssen-Cilag), an antiematic medicine used to relieve nausea and vomiting, and discomfort caused by gastroparesis in humans (Table 2).

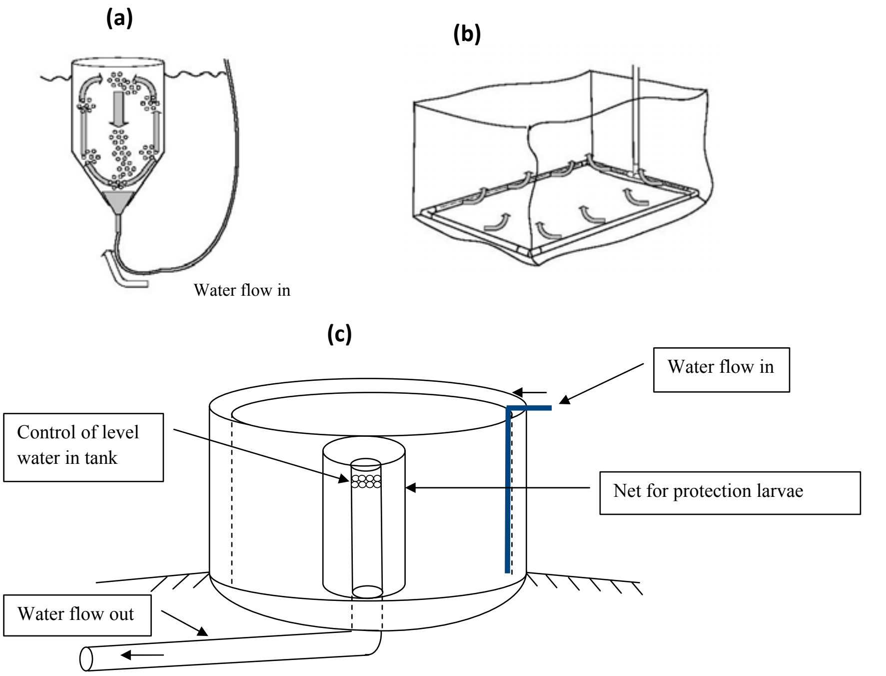
C. molitorella

C. molitorella production has been limited by dependence on use of wild-caught seed, until success in induced breeding technology for captive broodstock significantly promoted its farming (FAO 2007a). At present artificial propagation is the major source of *C. molitorella* seedstock, though seedstock may still be collected from the wild and used for grow-out and maintenance of genetic quality of broodstock (FAO 2007a). In Lao PDR, propagation of *C. molitorella* was initially achieved by collecting mature breeders from the wild, but since 1996 propagation of captive reared broodstock has occurred at the Naluang Station, and two private farms in the Luang Prabang Province (Nuanthavong and Vilayphone 2005).

In captivity *C. molitorella* reach sexual maturity from 300-500 g weight after 2-3 years (Gorda 2001), but will not spawn unless induced by hormone injection and environmental stimuli, such as flowing water (FAO 2007a). Female *C. molitorella* are induced to spawn with a single injection of Suprefact® (15-18 µg/kg) combined with the Motilium® (10 mg/kg), while a half dose is applied to males (Nuanthavong and Vilayphone 2005) (Table 2). Gorda (2001) injected females with 0.2ml/kg Suprefact® and one Motilium® tablet to induce ovulation, and males with 0.1 ml/kg Suprefact® and Motilium® to promote spermiation. Following injection, one female and two males are placed together in one tank and allowed to spawn naturally (Gorda 2001, Nuanthavong and Vilayphone 2005). Injections are given at 6:00 hours and spawning occurs 6 hours later at 26-27ºC (Nuanthavong and Vilayphone 2005), or at 21-22ºC, (Gorda 2001). *C. molitorella* often make a low mating call 'ku ku', with many bubbles coming up to the surface of water (FAO 2007a). Gorda (2001) reported fertilisation rates of 70-80% following spawning. The fecundity of C. molitorella is 80,000-100,000 eggs/kg (Gorda 2001, Nuanthavong and Vilayphone 2005). There is no information on the sperm of *C. molitorella*, though Gorda (2001) estimated there were 8-10 million spermatozoa per mL of milt.

L. chrysophekadion

In captivity *L. chrysophekadion* will reach sexual maturity within a year at a body weight of 600 g weight when cultured in earthen ponds



Egg incubators used for L. chrysophekadion and C. molitorella. (a) Jar for hatching eggs (source: Meenakarn and Funge-Smith 1998). (b) Hapa for hatching eggs (source: Meenakarn and Funge-Smith 1998). (c) Circular tank (Chinese model).

(Thienchareon et al. 1989), and can be induced to spawn from 800 g (Leelapatra et al. 2000) (Figure 3).

L. chrysophekadion have been induced to spawn by hypophysation using PG from either common carp (*Cyprinus carpio*) or mrigal (*Cirrhinus cirrhosus*) (Table 2). Thienchareon et al. (1989) and Tienchareon and Oonsrisong (1990) found that two injections of 0.5-0.75 "dose" and 1.5-2.0 "dose" (actual concentration of "dose", ie active ingredient per kg fish, unknown) given 6 hours apart resulted in spawning 4-5 hours after injection. Thi et al. (2003) induced spawning in *L. chrysophekadion* with PG in combination with HCG; females were initially injected with 2.3 mg/kg PG and 500 IU/kg HCG, then re-injected with 3.5 mg/kg PG and 2,000 IU/kg HCG 6-8 hours later. Males required a single injection with 1.2 mg/kg PG with 800 IU HCG applied at the same time as the second injection of females (Table 2). Tienchareon and Oonsrisong (1990) and Leelapatra et al. (2000) induced spawning in female *L. chrysophekadion* with two injections of Suprefact® in combination with Motilium®; 5-8 µg/kg Suprefact® with 5-10 mg/kg Motilium® and 10-15 µg/kg Suprefact® with 5-10 mg/kg Motilium® 6 hours later. These fish spawned 4-5 hours after the second injection. The gametes are hand-stripped from the broodstock and combined using a dry fertilisation method (Tienchareon and

Small hapas used to incubate the eggs of C. molitorella.

C. molitorella eggs.

Oonsrisong 1990). Male fish require a single injection equivalent to half that of females, or are not injected (Thienchareon et al. 1989).

The fecundity *L. chrysophekadion* ranges from 10,000 to 300,000 eggs (Boonmon and Kantejit 1977) to 1.09 million eggs (in a 49 cm fish) (Kamonrat et al. 1972). L. chrysophekadion maintained in 600 m^2 have been reported to spawn twice within two months in the one spawning season (Thienchareon et al. 1990). In contrast, female *C. molitorella* apparently spawn once per season.

Egg incubation

Several methods have been used to incubate pelagic or semi-buoyant eggs of fish such as those of *C. molitorella* and *L. chrysophekadion* in Lao PDR. First, fertilised eggs can be incubated in hatching jars (up to 1million eggs/m^3) supplied with a constant flow of water and aerated (Figure 4a). Second, eggs can be incubated in an aerated hapa (Figure 4b) made from plankton netting that is suspended in a tank or pond (Figure 5). Alternatively, large numbers of eggs may be incubated in circular concrete or fiberglass tanks (eg. Chinese model) that are aerated and provided with a constant flow of water (Figure 4c) (Meenakarn and Funge-Smith 1998). One disadvantage of this latter technique is that a large quantity of fresh, filtered, water is required.

The eggs of *C. molitorella* (Figure 6) swell rapidly after fertilisation and placing in freshwater (Gorda 2001). In Naluang Station (Lao PDR), large circular tanks (Chinese model) are mainly used for incubating *C. motitorella* eggs. Nuanthavong and Vilayphone (2005) incubated eggs in hapas (100,000-150,000 eggs/hapa) held in a small concrete tank. The incubation period is temperature dependent; eggs hatch 12-14 hours after fertilisation at a water temperature of 23-24ºC (Gorda 2001), to 16-17 hours at 26-28ºC (Nuanthavong and Vilayphone 2005). Hatch rates are 60-90% (Gorda 2001, Nuanthavong and Vilayphone 2005).

The semi-buoyant eggs of *L. chrysophekadion* hatch 14-16 hours after fertilisation at 28ºC (Watanadirokul et al. 1983, Leelapatra et al. 2000), 18-22 hours at 20-30ºC (Tienchareon and Oonsrisong 1990) and approximately 12 hrs at 29-30ºC (Thi et al. 2003). Newly hatched *L. chrysophekadion* larvae are 6-7 mm in length (Watanadirokul et al. 1983). The fertilisation and hatching rates of *L. chrysophekadion* were 35-88% and 35-77%, respectively (Thi et al. 2003).

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Application of ipil-ipil leaf meal as feed Ingredient for monosex tilapia fry (*Oreochronis niloticus*) in terms of growth and economics

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Background

By production volume, tilapia (*Oreochronis niioticus*) culture is one of the largest freshwater aquaculture species worldwide and is mostly produced using semi-intensive systems in developing countries (FAO, 2000). Tilapia was introduced first in Bangladesh in 1954 from Thailand (Ahmed, 1956). Legumes such as Ipil-ipil (*Leucaena leucocephala*) are potentially a valuable feed resource for aquaculture in the tropics because of their widespread distribution in those areas. Most varieties have not been evaluated as feed for fish. Ipil-ipil and leaf material of *Leucaena* compares favourably with lucerne in terms of crude protein (CP), calcium (Ca) and phosphorus (P), and it is also a good source of b-carotene. Under optimum growing conditions, *Leucaena* can yield large amounts of high quality forage (NAP 1984). Legumes provide high-quality protein and energy, the nutritive value and digestibility of tropical legumes is higher than that of tropical grasses and the quality of herbage from grasses rapidly declines with increasing maturity.

Methodology

As in most fish culture systems, balanced feed is one the most significant inputs in tilapia culture, and accounts for 30 to 60% of production costs (El-Sayed, 1990; Goddard, 1996; Tudor et al., 1996). Tilapia, however, are also able to feed on vegetables, grains, algae, zooplankton, etc., which has been proven in a number of nutrition studies (Belal and Al-Jasser, 1997). Substitution of fish meal in balanced feed has been vital to reducing feed costs, and overall tilapia production costs. The ipil-ipil tree was cultivated in the dyke and roadside of the research conducted in the Allahwala