Production of *Cirrhinus molitorella* and *Labeo chrysophekadion* for culture based fisheries development in Lao PDR 2: Nursery culture and grow-out

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Introduction

This article is the second of a two part series that aims to review current, readily available, information on these two indigenous species, *Cirrhinus molitorella* (mud carp, "Pa Keng") and *Labeo* (syn *Morulius*) *chrysophekadion* (black sharkminnow, "Pa Phia"), which are being used to improve and refine artificial propagation and culture techniques to support Culture Based Fisheries (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). The first part of this review was published in the previous issue of Aquaculture Asia.

Nursery culture

Larviculture

Gorda (2001) described the larviculture of *C. molitorella*. Initially larvae are held in incubation tanks in the hatchery for 4-5 days and fed boiled chicken egg yolk, finely smashed, twice each day, before being placed into fertilised earthen ponds for rearing. Nuanthavong and Vilayphone (2005) reared *C. molitorella* larvae at 19 larvae/L in a 3,000 L tank for 5-7 days. The water was aerated and 1/3 of the volume replaced daily. On the third day after hatching, larvae commenced feeding on boiled egg yolk and green plankton (fed once-twice/day).

Fry rearing

Fry are transferred to nursery facilities shortly after feeding commences. There are many forms of nursery facilities, including earthen ponds, concrete tanks, fiberglass tanks and 'Orlon' cages, that have been used successfully to rear fish fry in Lao PDR (Meenakarn and Funge-Smith 1998). Good pond preparation requires the following steps to be observed (Meenakarn and Funge-Smith 1998):

- Empty the water out of the pond and dry the pond.
- Add lime to the pond at a rate of 1 kg for every 25 m^2 of pond area. Lime assists soil fertility and reduces the amount of fertilizer required to produce an algal bloom in the nursery pond.
- Add organic fertilizer (manure) to the pond at the rate of 1.5 kilos per 10 m^2. Fertilizers that can be used include buffalo, cow, chicken and pig manures. After the manure is applied the pond can be filled to a depth of 5 - 10 cm to allow the breakdown of the manure. After 3 - 5 days, the pond should be filled to a depth of 30 - 50 cm and is then ready for stocking.
- Fry are stocked into the nursery pond at a rate of 125 - 500 individuals/m^2.
- After stocking the fry into the pond (usually done in the early evening) the water level is maintained for one week and then increased to 80 cm.

C. molitorella

Earthen ponds (usually 0.1-0.2 ha and 1.5-2.0 m deep) are used for the nursing of *C. molitorella*. Dry ponds are quick-limed at 900-1,125 kg/ha, and fertilised (animal manures 3,000 kg/ha and/or plant wastes 4,500 kg/ha) to increase the natural biomass of algae and zooplankton, 5-10 days before stocking, according to water temperature (FAO 2007). Fry are stocked at a rate of 450-600 fish/m^2, depending on targeted fish size at harvest. The nursery phase usually takes 4-5 weeks in China. Organic fertilizer is applied at a rate of 1,500-3,000 kg/ha once every 4-5 days to maintain and enhance plankton (FAO 2007). Occasionally other food sources (both direct and indirect to supplement or replace organic fertilizers) are added to the ponds, including soybean milk, soybean cake or other by-products from grain processing, water lettuce and water hyacinth (FAO 2007). After 4-5 weeks fish are about 30 mm (called summer fingerlings in China).

Growth rates are generally high in nursery ponds, but survival rates can vary greatly. Survival rates of *C. molitorella* fry during this period are typically 70-80%, but can exceed 90% under good management (FishBase 2007). In contrast, survival rates of *C. molitorella* fry reared in Lao PDR are considerably lower than this; 30-45% (Gorda 2001, Somboon et al. 2003). One of the principle problems affecting survival of fry stocked into nursery ponds in Lao PDR is predation by carnivorous dragon fly nymphs, tadpoles and fish that enter the ponds (Meenakarn and Funge-Smith 1998).

Stocking density will affect growth and survival in nursery ponds. Somboon et al. (2003) reared *C. molitorella* larvae (0.004 g, 5.0 mm) in fertilised earthen ponds at different stocking densities from 100 fish/m^2 to 1,000 fish/m^2, and after one month final mean lengths and weights varied from 14-36.6 mm and 0.01-0.37 g, respectively. Not surprising growth rates were greatest (14.9%/day) at the lowest stocking density. Somboon et al. (2003) concluded that a stocking density of 500 fish/m^2 was optimal in terms of growth and yield (fish produced). Harvested fish were sold at an average price of 100 Kip/fish (9,000 Lao Kip ≡ US$1).

Gorda (2001) described the rearing of *C. molitorella* larvae in fertilised earthen ponds, which had been dried, limed (200-300 kg/ha), applied with cut grass

Table 1. Polyculture of *C. molitorella* as a primary species with other fish species (Source: FAO 2007).

Type of polyculture	Size of fish (g)	Stocking rate (fish/ha)	Fish production after one year (kg/ha)
C. molitorella[1]	25-50	15,000-25,000	2,000-3,000
Ctenopharyngodon idella (grass carp)[1]	250	1,200-1,800	1,800
Aristichthys nobilis (bighead carp)[1]	500	450-2,250	2,700
Hypophthalmichthys molitrix (silver carp)[2]	250	375-750	700
Tilapia[2]	15-20	3,000-6,000	560
Cyprinus carpio (common carp)[2]	100	375	270
1 = Primary species and 2 = Secondary species.			

Table 2. Published information on the grow-out of *L. chrysophekadion*.

Stocking and culture details	Duration (mths)	Size and yield at harvest	Source
1 fish/m^2	9	166 kg/rai (1,038 kg/ha)	Thavonnan and Udomkananat 1979
2 fish/m^2	9	290 kg/rai (1,813 kg/ha)	
4 fish/m^2	9	249 kg/rai (1,556 kg/ha)	
3 fish/m^2 in 1,200m^2 earthen pond (initial size 1.8 g). 192 kg/pond (1,600 kg/ha)	12	60.7 g, 192 kg/pond (1,600 kg/ha)	Thienchareon *et al.* 1990
0.5 fish/m^2 in polyculture with *Cyprinus carpio* at 1:10 (*L. c* : carp).		71.6 kg/1,600m^2 (447.5 kg/ha)	Pennapaporn 1970
0.6 fish/m^2 in polyculture with *Pangasius sutchi* at 1:1 ratio. Fed 5% body weight/day	11	94.63 g (21.9 cm), 590 kg yield	U-domkananat 1983
1.2 fish/m^2 in polyculture with *Pangasius sutchi* at 1:1 ratio. Fed 5% body weight/day	11	94.63 g (21.9 cm), 714 kg yield	
0.17 fish/m^2 earthen pond (initial size 1.8 g). Fed 20-30% protein diet	12	428.7 g (32.9 cm)	Unsrisong *et al.* 1990

(780-1,200 kg/ha), chicken manure (5,000 kg/ha), buffalo manure (10,000 kg/ha) and urea (100-150 kg/ha), and then filled. Manure was continually added throughout the culture period. Larvae were stocked at a rate of 400 fish/m^2, 5-6 days after filling. By the 1st week fish were 11 mm in length and 4th week were 30.2 mm in length, which represented a growth rate of 0.91 mm/day. According to Gorda (2001), in 2000 the Nah Luang Hatchery Station sold 15,000 larvae and 62,060 fry (1 month old) at 5 Kip/fish and 100 Kip/fish, respectively

Nuanthavong and Vilayphone (2005) described the nursing of *C. molitorella* fry at a small private farm in the Luang Prabang Province, undertaken in hapas situated in a 800 m^2 pond. Initially, fry (5-7 days old) were stocked into hapas made from plankton net (1,800 L; 1.5 x 2.0 x 0.8 m) at a rate of 2.8 larvae/L. After two weeks fry were transferred to larger hapas made from 3 mm mesh (9.4 m^3, 3.5 x 3 x 1 m) (stockings density 0.7 fry/L) and reared for a further three weeks. During the culture period fish were fed plankton, rice bran and floating pellets. The survival rate 6 weeks after hatching was 20%.

L. chrysophekadion

The larvae of *L. chrysophekadion* have been reared in fertilised earthen ponds by Thienchareon et al. (1990). The ponds were dried for 5-7 days, limed at a rate of 100 kg/rai (625 kg/ha; 1 rai = 1,600 m^2), fertilised with chicken or cattle manure at a rate of 400-600 kg/rai^2 (2,500-3750 kg/ha), then filled to a depth of 80 cm. After 3-5 days, when the water has turned green due to the increase in phytoplankton, 3 day old fry were stocked at a rate of 200 fish/m^2. The fish were fed as follows:

- 3-4 day old fry, fed with chicken egg yolk.
- 5-6 day old fry, fed with Artemia or Moina and a mixture of fish meal and rice bran.
- 10-30 days old fry, fed with meal and rice bran.

After one month, fry were to 3-4 cm in length (Thienchareon et al. 1990).

Grow-out

C. molitorella

The most commonly adopted method for on-growing *C. molitorella* is in large earthen ponds in polyculture with other fish species, as both the primary and secondary species, at various densities depending on the species mix (FAO 2007). As the primary species, *C. molitorella* is stocked at 15,000-25,000/ha (25-50 g fish) along with a range of other species including grass carp and bighead carp also as primary

Pond-reared *L. chrysophekadion*.

The fry of *L. chrysophekadion*.

Pond reared *C. molitorella*.

species, and silver carp, common carp, black carp, tilapia and bream as secondary species (FAO 2007) (Table 1). In monoculture, fingerling *C. molitorella* (30-60 mm in size) are on-grown in earthen ponds at a stocking density of 3.5-4.5 million/ha. During the fingerling growing phase, which takes 4-8 months in China, fish are mainly fed commercially manufactured feeds and ponds are fertilised to encourage the proliferation of plankton (FAO 2007).

C. molitorella grows slowly and will not reach a large size, but can be reared at a high density and has a high production rate (FAO 2007). After one year in polyculture as the primary species, *C. molitorella* typically reach 125-200 g, with a production level of 2,000-3,000 kg/ha, accounting for about 24% of total production, which ranges from 7,500 to 10,000 kg/ha (FAO 2007). As a secondary species *C. molitorella* is stocked at 7,500-9,000/ha (25-50 g fish), and after one year production can achieve 1,000-1,500 kg/ha, which may account for 10-15% of total production (FAO 2007).

In Lao PDR grow-out of *C. molitorella* is concentrated in the Luang Prabang Province (Ounidate et al. 1993, Souksavath 2001, Nuanthavong and Vilayphone 2005).

L. chrysophekadion

Several studies have examined the monoculture and polyculture of *L. chrysophekadion* in ponds and although information is limited, yields of 447.5-1,813 kg/ha are reported (Table 2). One study indicated that fish grew from 1.8 g to 60.7 g in 12 months (0.96%/day, 0.16g/day) (Thienchareon et al. 1990), while in another, fish grew from 1.8 g to 428 g (32.9 cm) in 12 months (1.5%/day, 1.17g/day) (Unsrisong et al. 1990). The species may also be suitable for culture in net cages and pens (Warren 2000).

L. chrysophekadion grows well when stocked into reservoirs (Chabjinda et al. 1992a, Leelapatra et al. 2000). In Mae Ngad Somboonchon Reservoir (1,040 ha), Chiang Mai Province, Thailand, L. chrysophekadion grew at a rate of 4.09 g/day, and reached maturity at 2 years of age (62 cm, 2,960 g) (Chabjinda et al. 1992b).

Research and development needs

Based on this review (Parts 1 and 2), a number of key areas of research and development have been identified to improve production of *C. molitorella* and *L. chrysophekadion*, which in turn will facilitate and augment culture based fisheries development in Lao PDR. These needs are:

(a) Captive breeding:

- Develop and refine captive spawning techniques for *C. molitorella* and *L. chrysophekadion* to support culture based fisheries development.

- Establish baseline information on the reproductive performance of *C. molitorella* and *L. chrysophekadion* under various husbandry conditions.

Monitor influence of broodstock holding conditions (ponds and tanks, monoculture and polyculture, stocking density, feeding regime, nutritional profile of diet, etc.) and performance of individually tagged broodstock (e.g. temporal changes in weight, length and condition, fecundity, spawning frequency, gamete quality, etc.).

(b) Incubation and larviculture stage

- Improve the growth and survival of hatchery produced *C. molitorella* and *L. chrysophekadion* eggs and larvae, determine the best food types and feeding regimes, determine the optimal stocking densities for this period of development, and undertake research on weaning larvae to suitable dry feeds.

(c) Fry rearing (nursery) stage

- Improve the growth and survival of hatchery produced *C. molitorella* and *L. chrysophekadion* fry, determine the best fry culture method (pond, hapas, tanks, etc.), determine the best food types and feeding regimes, and determine optimal stocking densities for nursery ponds, hapas and tanks.

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stocking sites prior to stocking so that all infectious stages die due to lack of hosts, quarantine prior to stocking and introduction to the rearing system and frequent cleaning of holding tank/nets. The parasites can be controlled by fresh water bath for 10-15 min or by chemical treatment using 1000 ppm hydrogen peroxide or 100-200 ppm formalin for 30-60 min. Some of the treatments commonly applied in brackishwater fishes are shown in Table 2. Strong aeration must be provided during treatment. Drying of unused tanks also helps to destroy the developing stages. Treated fish should be transferred to clean parasite free facility.

It is well recognized that parasites act as mechanical vectors of the pathogens though they are not an obligatory host. It is likely that any fluid or tissue feeding parasites could potentially act as a vector for bacteria, fungus, virus etc. It has been speculated that parasitic copepods may serve as vectors of viral and bacterial diseases of fish due to their feeding activities on host mucous, tissues and blood.

Parasitic copepods with relatively narrow host ranges such as Ergasilus are easier to control especially, where there are few wild hosts present. Species with broad host ranges and / or abundant wild hosts (eg. *Caligus* sp.) in the vicinity of aquaculture sites are generally difficult to control because of recurrent infestations from carrier hosts. *Lernaea* sp. is very difficult to control due to different stages of life cycle showing different susceptibility to chemicals. Further the concentration of these chemicals required to kill the developmental stages are toxic to fish. Temperature dependant development of larval stages and the lethal effects of even low salinity on larval stages etc. can be utilized for the control of fresh infections in the system. Eradication of copepods using freshwater bath is also suggested.

Conclusions

Crustacean parasites are numerous and have a worldwide distribution in marine and brackishwater aquaculture systems. Copepods comprise the largest group of crustacean parasites on fish causing economical loss. Disease outbreaks and subsequent mortalities are rare under effective broodstock management systems due to effective treatment methods. However, increasing incidence of copepod parasitism is becoming a regular phenomenon in culture conditions. The only sure way to prevent parasitic infection is to deny the parasite access to the protected habitat. Although it is well established that parasitic crustaceans have a major impact on brackish water aquaculture there are relatively few published reports of disease and / or disease treatments and economic losses associated with these infections in India. We need to study the ecology of the parasite, including seasonality, maturation and the population dynamics and transmission mechanisms vis-à-vis physicochemical parameters of the rearing water in order to prevent and control the outbreak of the species in brackish water aquaculture.

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