Artificial propagation of Hoeven's slender carp (*Leptobarbus hoevenii*)

Dang Van Truong*, Nguyen Minh Thanh, Hoang Quang Bao, Thi Thanh Vinh, Pham Dinh Khoi , Nguyen Thi Hong Van and Trinh Quoc Trong

Aquaculture of Indigenous Mekong Fish Species Component, MRC Fisheries Programme

ABSTRACT

The artificial propagation of Hoeven's slender carp (*Leptobarbus hoevenii*) was studied under trial conditions at Research Centre for Aquaculture in the Mekong Delta, Research Institute for Aquaculture No. 2, within the framework of Aquaculture of Indigenous Fish Species (AIMS) component. Trials showed that females required two injections of hormone, preparatory and resolving, to induce spawning; males however required just a single resolving dose. Following injection females and males were kept in spawning tanks with flow-through water. Females were stripped or spawned naturally 5.5 – 8.0 hrs after the resolving injection. Relative fecundity of female ranged between 84,043 and 92,907 eggs/kg. Fertilised eggs were incubated in Zuger jars or aerated fibreglass tanks with flow-through water. Fertilisation and hatching rates ranged from 47.0 to 72.5% and 64.7 to 87.8%, respectively. Hatching period took approximately 13 hrs at water temperature of 28 – 29°C.Two-stage nursing technique in concrete tanks and thereafter in earthen ponds was tested. Survival rate of *L. hoevenii* fingerlings at 50 – 60 days old was found to be at 50 – 60%.

KEY WORDS: Viet Nam, Hoeven's slender carp, artificial propagation

INTRODUCTION

Hoeven's slender carp (*Leptobarbus hoevenii*) is an indigenous freshwater fish found naturally in rivers and springs in Viet Nam, Lao PDR, Thailand and Cambodia. It feeds on larva, worms, and zooplankton during its larval stage and phytoplankton and forest fruits when it reaches maturity (Rainboth 1996). The species is economically important to fisheries in the Mekong Delta where it is native. Here production is mainly through natural capture as efforts to culture it by artificial means to date have been relatively unsuccessful due to a lack of seed and ineffective culture techniques. However, the successful production of seed of sufficient quality and quantity for aquaculture needs further research. As well as being commercially beneficial, this seed would help to reduce the demands on the already limited numbers of this species in the wild.

METHODS

Brood-stock conditioning

Study of the artificial propagation of *L. hoevinii* was carried out from June 2001 to October 2003 at the Research Centre for Aquaculture in the Mekong Delta (Cai Be Centre), Research Institute for Aquaculture No. 2 (Cai Be district, Tien Giang province, Vietnam), under AIMS Vietnam Sub-component.

Fish were collected from the wild in June-July 2001. A further 240 individuals were collected in 2003

* Research Centre for Aquaculture in the Mekong Delta, Research Institute for Aquaculture No. 2, An Thai Trung village, Cai Be district, Tien Giang province, Viet Nam.

and stocked at a density of 24 kg/100m^2 in two separate earthen ponds (700 m^2 each) at the Cai Be Centre.

In order to monitor water quality in the brood-stock ponds, temperature and levels of dissolved oxygen (DO) were measured daily at 07:00 and 14:00 and pH and levels of dissolved carbon dioxide (COD) were measured weekly at these same times of day.

Daily aeration of the brood-stock ponds (23:00 to 06:00) maintained DO at minimum 2.5 mg/l. Water level was kept constant at between 1 to 1.2 m and approximately 30-30% of the pond water was exchanged once or twice every month. In order to create a current, water was pumped every morning for two hours during the period from April to October.

Conditioning of the brood stock took place in two stages. During the first, from December to April, fish were fed an equivalent of 4% of their body weight. This was reduced to 2% body weight during the second stage (May to October). Local-made feed consisted of fishmeal, blood powder, rice bran, fish oil and premix (containing approx. 35% of crude protein). In addition, fruits such as guava and plum were given at a rate of 2% of body weight.

Egg samples were periodically collected from the brood stock to determine stage of maturity based on histology. Samples of eggs were also collected before and after the primary hormone injection; these were kept in Davidson solution. The diameter of each egg was measured under microscope to determine egg transformation after injection. Based on this information, technicians decided when would be the best time to apply the resolving injection.

Records of spawning seasons, maturation ratio, maturation coefficient, absolute fecundity, relative fecundity, real relative fecundity, ratio of brooders releasing eggs, fertilization and hatching rates were made.

Induced spawning

Spawning was induced by injecting Luteinising Hormone-Releasing Hormone analogue (LH-RHa) plus

Table 1. *Hormone doses used to stimulate artificial propagation in Hoeven's slender carp.*

Year	Treatment	Hormone	Preparatory dose/kg		Resolving dose/kg	
			Female	Male	Female	Male
2002	1	Pituitary (mg)	2.0		4.0	3.0
		LHRHa (μg)	80.0		200.0	80.0
		DOM (mg)	10.0		20.0	10.0
	2	Pituitary (mg)	2.0	1.0	4.0	2.0
		LHRHa (μg)	50.0	25.0	140.0	50.0
		DOM (mg)	5.0	2.5	15.0	5.0
2003	1	Pituitary(mg)	1.0		1.2	0.6
		LHRHa (μg)			130.0	65.0
		DOM (mg)			13.0	6.5

domperidone (DOM) and carp pituitary gland extract (PG). The doses used were different in 2002 and 2003 (Table 1). In the 2002 spawning season, two treatments were carried out to find the most suitable dosage. This was achieved by gradually reducing the amount of each hormone used.

Dissemination was accomplished using the 'dry' method, i.e. eggs and milt were mixed before adding water. Fertilized eggs were incubated in Zuger jars or in aerated fibre tanks with flow-through water.

Nursing

Once the yolk sac was absorbed, fry were nursed by two separately treatments:

Treatment 1 (two-stage nursing):

Stage 1: fry were nursed in 12.5 m^2 concrete tanks at a density of about 1,000-1,200 indiv/m^2 (in 2002) and 2,000 indiv/m^2 (in 2003) until they were 20 days old.

Stage 2: thereafter fish were moved to 200 m^2 earthen pond. Density reduced to 50-100 indiv/m^2. This stage last to 40 days.

Treatment 2: fry were moved into 700 m^2 earthen ponds after the yolk sac was exhausted. Density was 200-300 indiv/m^2. Nursing period lasted for 60 days.

Table 2. *Type of feed used to nurse fry*

Age (day)	Type of feed
1-10	Milk powder + moina
11-40	Fish meal + rice bran
41-60	Fish meal + rice bran + pellet feed

RESULTS AND DISCUSSION

Environmental parameters of the brood stock ponds

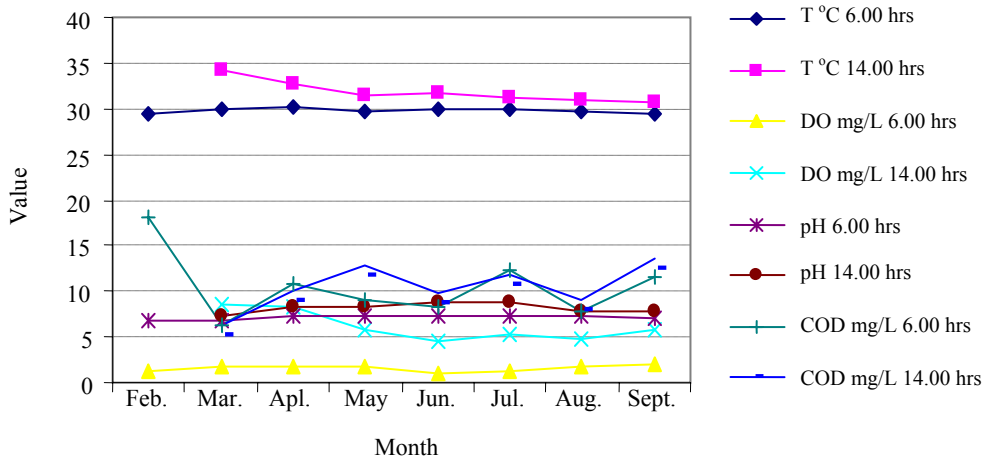

Figure 1. Water quality parameters in brood-stock ponds

Brood-stock maturation

In the wild Hoeven's carp normally take two years to teach maturity. In pond culture, the 34% of brood-stock collected in June and July 2001 reached maturity in 2002. This low percentage may be because the fish had not yet fully adapted to pond environments.

In 2003, the ratio of mature fish increased to 88% (females) and 73% (males), showing that *L. hoevinii*, can thrive well in captivity once they have adapted to artificial feed and the environmental conditions in the brood ponds.

Figure 2 illustrates the maturation ratio of females to males during the spawning season. Males matured earlier than females; while 13% of males were mature by mid-March most females did not reach phases III and IV until April. Some females in phase V could reproduce in May. By June, the maturation ratio had reached 30% in females and 20% in males.

The maturation ratio of both females and males reached a peak in June; thereafter the ratio dropped dramatically in males but remained relatively high in females. Successful spawning requires a large numbers of males to females as males produce only a small amount of sperm. As result, the reproductive season was short. Females are able to reach maturity again 90-120 days after spawning and some of the females in this study were able to reproduce twice in one spawning season.

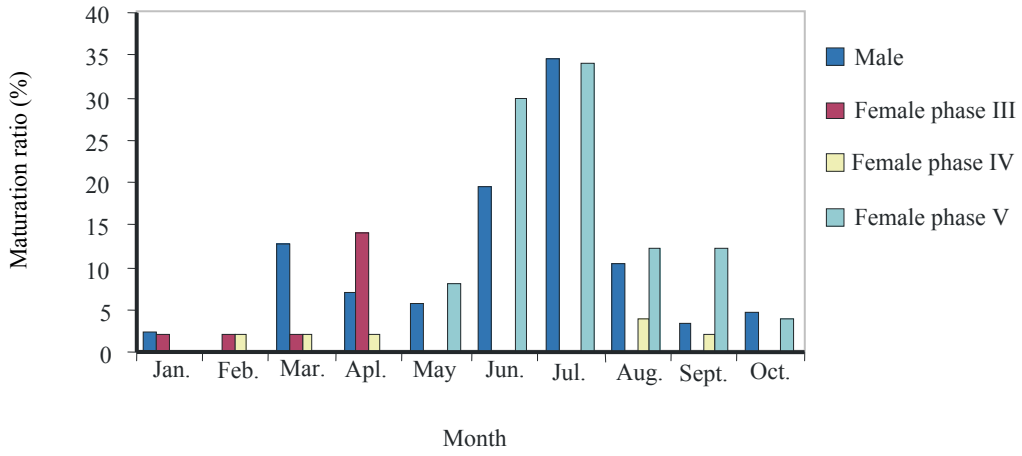

Figure 2. Maturation of brood stock in confined culture conditions

Some reproductive characteristics of L. hoevinii vs. other cyprinids

The reproductive biology of *L. hoevinii* was studied in 2002, along with other three target species (also cyprinids) of AIMS: *O. melanopleura, L. chrysophekadion* and *C. microlepis*. The GSI (Gonadal Somatic Index) of female *L. hoevinii* was 5.3 - 6.7%. Absolute fecundity ranged from 139,360 - 158,000 eggs and relative fecundity from 84,043 - 92,907 eggs/kg. The mean diameter of eggs was 0.98 mm.

L. hoevinii and *O. melanopleura* have similar GSI, which is much higher than that of *L. chrysophekadion* and *C. microlepis*, but much lower than other well-adapted cyprinids like silver barb *Barbodes gonionotus* or red-tail tinfoil barb *Barbodes altus*.

Absolute and relative fecundity of *L. hoevinii* and *O. melanopleura* were similar but were two to three times higher than that of *L. chrysophekadion* or *C. microlepis*. Relative fecundity of *L. hoevinii* was higher than the 50,000 - 70,000 eggs/kg reported by Meanakarn (1985, cited by Leelapatra *et al.*, 2000).

However, the study took place in 2002 after just one year of adaptation and conditioning; at that time, the brood-stock may not have been well matured. Feasibly, GSI, absolute and relative fecundity may be higher in fish fully adapted to artificial conditions.

Table 3. *A comparison of the reproductive characteristics of some indigenous species*

Species	GSI (%)	Fecundity		Diameter of egg (mm)
		Absolute (egg)	Relative (eggs/kg)	
Barbodes gonionotus	15-18	282,377-800,620	250,000-400,000	0.5-0.6
Barbodes altus	15-21	18,250-207,100	228,000-721,000	0.40-0.55
L. hoevinii	5.3-6.7	139,360-158,000	84,043-92,907	0.98 ± 0.03
L. chrysophekadion	1.4-1.7	42,306-51,408	28,204-27,057	1.01 ± 0.12
O. melanopleura	4.3-6.8	106,700-177,975	73,586-114,823	1.07 ± 0.01
C. microlepis	3.1-3.5	30,036-37,592	19,378-22,113	1.30 ± 0.04

Induced spawning

Table 4 gives the results of artificially propagating *L. hoevinii* in the 2002 and 2003 spawning seasons. The results in 2003 show a marked improvement over 2002. The ratio of fully ovulated females increased from 50-75% to 83-100% and fertilization and spawning rates were 47.0-72.5% and 64.1-

Table 4. *Reproductive characteristics Hoeven's slender carp propagated artificially during 2002 and 2003*

Year	Date	No. of females	Ratio of females ovulated completely (%)	Total weight of females[1] (kg)	Real relative fecundity[2] (eggs/kg)	Fertilization rates (%)	Spawning ratio (%)
2002	02/04/02	4	50	5.2	84,663	5.0	63.8
	06/05/02	4	75	4.1	30,366	16.5	64.1
	17/06/02	7	57	7.7	41,208	67.3	61.8
2003	12/05/03	4	100	6.75	41,760	10.0	23.0
	04/06/03	5	80	4.8	104,244	59.0	80.0
	05/06/03	2	100	2.5	151,740	60.0	78.5
	23/06/03	7	57	4.3	96,573	10.8	86.5
	07/07/03	7	86	7.0	88,458	72.5	87.8
	21/07/03	6	83	4.35	133,218	69.6	64.1
	11/08/03	5	100	6.7	107,185	47.0	75.2

Notes: [1]Total weight of females ovulated completely (A) (excluding the weight of females ovulated partially)
[2]Real relative fecundity = total number of eggs of females ovulated completely/A (excluding the number of eggs of females ovulated partially)

87.8% respectively. This improvement is a consequence of more *L. hoevinii* reaching maturity following induced spawning.

In 2003, the brood stock matured well considerably reducing the need for hormone injection. A consequential reduction the amount of hormone used (PG alone for the primary dose, PG and LH-RHa+DOM for the resolving dose) improved the economics of induced spawning.

Average size of eggs before and after resolving injection was 0.94-1.02 mm and 1.01-1.04 mm, respectively. The eggs did not show significant increase in size (only 0.04-0.08 mm) after the primary dose, though this varied in each spawning. The results show that the resolving injection can be given once eggs reach 1 mm in diameter. Latency time is 5-7 hrs after the resolving injection. Eggs began to hatch after 13 hours at water temperature of 29°C -30°C. The rate of hatching rate depends largely on water quality in incubation medium.

L. hoevinii spawn from April to August but mainly in June and July. According to Meanakarn (1985) and Watanadirokul and Kongship (1987) (cited by Leelapatra *et al.*, 2000) the natural spawning season of *L. hoevinii* is from May to November and for the most part from May to September. Although females in phase V were still fertile in September and October, gonad development in most of the males had declined by this time, as had the quantity and quality of sperm. Males usually mature earlier than females but deteriorate quickly making the spawning season short.

In this study, while the quality of ovulation was good, in some cycles fertilization rates were low because of a shortage of milt to fertilize the available eggs. Males matured in ponds produced little sperm and therefore successful spawning required more males than females. According to Leelapatra *et al.* (2000), the ideal spawning ratio of females to males is 1:3 although a ratio 1:2 may be adequate.

Fingerlings

Fingerlings reared under a two-stage regime reached an average weight of 2.54 g and an average length of 6.26 cm by day 60 (Table 5). This growth rate was lower than that seen in the greater bony lipped barb (weight 2.82 g and length of 6.86 cm) and the greater black shark (weight 3.91 g and length of 6.86 cm) at 60 days (Khanh *et al.* 2000).

Table 5. *Results of fingerling nursing in cement tanks and earthen ponds*

Growth rate			Survival rate (%)	
Age (day)	**Weight (g)**	**Length (cm)**	**2002**	**2003**
10	0.02 ± 0.01	1.33 ± 0.04		
20	0.15 ± 0.09	2.02 ± 0.21	70.2-91.4	72.0-76.8
30	0.74 ± 0.20	4.06 ± 0.35		
40	1.39 ± 0.47	5.10 ± 0.49		
50	1.56 ± 0.42	5.41 ± 0.83		
60	2.54 ± 0.56	6.26 ± 0.41	64.1-86.6	53.8-66.7

Two-stage nursing technology produced a high and stable survival rate in Hoeven's slender carp. Survival rates for juveniles at 20 and 60 days were 70.2-91.4% and 64.1-86.6% respectively in 2002 and 72.0-76.8% and 53.8-66.7% in 2003. The results in both these years compared favourably with rates achieved employing one-stage nursing technology. Rearing under this later regime achieved survival rates of only 58.7%.

Maintenance of water quality in the confines of the cement tank more efficient and moreover fry are able to find prey in addition to their regular feed. A clean, predator-free environment and a plentiful supply of feed at this earlier stage in the fish's life cycle promoted survival through to the next stage and beyond.

CONCLUSIONS

1. Nursing *L. hoevinii* in captivity realised maturation rates of 88% in females and 73% in males. Brood stock took two years to mature and three years to spawn efficiently. Females re-matured 90-120 days after the first reproduction of the spawning season.

2. The spawning season of *L. hoevinii* is from April to August and mainly between June and July. The spawning season is short because males and females do not mature synchronously. Males usually mature earlier and decline faster than females.

3. The maturation rate of females ranged from 5.3 to 6.7%. Absolute fecundity ranged from 139,360 to 158,000 eggs and relative fecundity 84,043 to 92,907 eggs/kg. The mean diameter of eggs was 0.98 mm.

4. Induced spawning of *L. hoevinii* using PG, LH-RHa+DOM gave good results. The ratio of females that fully ovulated was 83 to 100%. Real relative fecundity ranged from 88,458 to 51,740 eggs/kg. Fertilization and spawning rates for the 2003 spawning season were 47.0 to 72.5% and 64.1 to 87.8%, respectively. Results of induced spawning depend largely on quality of sperm.

5. *L. hoevinii* fingerlings reached 2.54 g in weight and 6.26 cm in length after nursing for 60 days. Two-stage nursing achieved a high and stable survival rate, between 53.8 and 86.6% fingerlings survived to 60 days.

REFERENCES

Khanh, P.V., Tuan, N., Hao, N.V., Jeney, Z., Trong, T.Q. and Thanh, N.M. (2000) Review of biology and breeding of some indigenous fish species in the Mekong Delta of Viet Nam. *Management of Reservoir Fisheries in the Mekong Basin Component Report* No 3. MRC Fisheries Programme, Mekong River Commission, Vientiane.

Leelapatra, W., Srisakultiew, P. and Sukumasavin, N. (2002) Biology and breeding of indigenous Mekong fish species in Thailand. *Management of Reservoir Fisheries in the Mekong Basin Component Report* No. 2, MRC Fisheries Programme, Mekong River Commission, Vientiane. 119 pp.

Rainboth, W.J. (1996) *Fishes of the Cambodian Mekong*. FAO. Rome 265 pp.

Truong, D.V., Vinh, T.T., Hung, D., Bao, H.Q., Khoi, P.D. and Thanh, N.M. (2002) Preliminary artificial propagation results of Hoeven's slender carp (*Leptobarbus hoevenii*). In: *Proceedings of the Fifth Technical Symposium on Mekong Fisheries, 11th-12th December 2002* (The 5th Technical Symposium on Mekong Fisheries, Khon Kaen, Thailand, 11 December-12 December 2002). A. Poulsen, ed. Mekong River Commission, Phnom Penh, pp. 297-300.

Truong, D.V., Vinh, T.T., Hung, D., Bao, H.Q., Khoi, P.D. and Thanh, N.M. (2002) Preliminary artificial propagation results of *Osteochilus melanopleurus*. In: *Proceedings of the Fifth Technical Symposium on Mekong Fisheries, 11th-12th December 2002* (The 5th Technical Symposium on Mekong Fisheries, Khon Kaen, Thailand, 11 December-12 December 2002). A. Poulsen, ed. Mekong River Commission, Phnom Penh, pp. 293-396.

Vinh, T.T., Truong, D.V., Hung, D., Bao, H.Q., Khoi, P.D. and Thanh, N.M. (2002) Preliminary artificial propagation results of Greater black carp (*Morulius chrysophekadion*). In: *Proceedings of the Fifth Technical Symposium on Mekong Fisheries, 11th-12th December 2002* (The 5th Technical Symposium on Mekong Fisheries, Khon Kaen, Thailand, 11 December-12 December 2002). A. Poulsen, ed. Mekong River Commission, Phnom Penh, pp. 143-150.