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Further Trials on Induced Breeding of *Pangasius pangasius* (Hamilton) in Bangladesh

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Abstract

The endangered riverine catfish, locally known as Pangas (*Pangasius pangasius* Hamilton; Pangasiidae), is a very important catfish of Bangladesh. Though it is also distributed in India, Myanmar and Pakistan the induced breeding technique of the species has not been established. To save this fish from extinction, the experiment was conducted with 23 broods of *P. pangasius* (10 females and 13 males) weighing 4.2 kg on an average to establish a suitable breeding technique. Three breeding trials were conducted. Four different doses viz. 9, 10, 11 and 12 mg·kg^{-1} body weight of acetone dried carp pituitary gland (PG) were tested. Though all the four doses resulted in 100% ovulation in females, the dose of 10 mg·kg^{-1} body weight of PG demonstrated the best result in consideration to fertilization and hatching rates of eggs. Hatching of fertilized eggs occurred between 28 and 32 hours of incubation at 26 to 28°C. Hatching rate was highest (65%) in the second trial. Newly hatched larvae were planktonic and transparent. Feeding of larvae started between the third and fourth days after hatching. A number of larvae died at the free swimming stage and many died at the first feeding stage. The majority died within 10 days of hatching. Only 3 larvae survived after 10 days out of the thousands that hatched. These larvae preferred tubificid worms.

Introduction

Pangasius pangasius locally known as pangas, is an important riverine catfish and considered as an endangered species of Bangladesh (IUCN Bangladesh 1998) and is also found in India, Pakistan and Myanmar (Roberts and Vidthayanon 1991). It commands a high price because of its delicate taste. Rahman (1992) reported that *P. pangasius* attains maturity at the fourth year of its life. David (1963) reported that it breeds between June

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and August while Rahman (1992) reported that breeding season occurs between July and October in Bangladesh. Khan and Mollah (1998) observed that both male and female broods of *P. pangasius* mature in captivity. Though the fish was abundant in the rivers and estuaries of Bangladesh in the past, there is an ever-declining trend in this fishery in recent years apparently due to deterioration of the habitat, over-exploitation and lack of proper management. Further, increasing water pollution and destruction of breeding grounds for various reasons have restricted natural breeding of pangas resulting in the threat of extinction of this important fishery. Pangas has been proven to be a suitable candidate for both mono- and polyculture with carps either in ponds or in net cages (Hannan et al. 1988, Sarder and Mollah 1991, Rahman 1992). However, unavailability of fry is the main constraint towards culture of this species as breeding techniques have not been established in Bangladesh. Boonbrahm et al. (1966 and 1968) reported success in breeding this species in Thailand, which has recently been shown to be a misinformation by Roberts and Vidthayanon (1991) since *P. pangasius* inhabits only in India, Pakistan, Bangladesh and Myanmar. In Bangladesh, Rahman (1992) first attempted to induce breeding of *P. pangasius* but all his efforts resulted in failure. In 1997, Mollah and Khan (1999) were partially successful for the first time in Bangladesh in inducing ovulation (50%) of *P. pangasius* by administering carp pituitary gland (PG) extract injection. However, the larvae died very shortly after hatching. This article reports the first success in inducing ovulation in females and larval rearing of *P. pangasius* and discusses important information vital to larval rearing.

Materials and Methods

Sources of brood fish

The brood fish of *P. pangasius* used in the breeding trials were obtained from two rearing ponds (18 m x 14 m each) managed by the Department of Fisheries Biology and Genetics, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. The fish were first transported from the Meghna River at Chandpur, Bangladesh in 1989 and since then they have been reared in the ponds of the department. For this experiment, 23 brood fish of *P. pangasius* with a mean weight of 4.2 (±0.4) kg were used. Ten females were stocked in one pond and 13 males were stocked in a second pond on 15 January 1999.

Rearing of brood fish

The brood fish were provided with supplemental feed at the rate of 4% of their body weight per day. The feed was formulated with 20% fish meal, 16% rice bran, 16% wheat bran, 18% mustard oil cake, 12% sesame oil cake, 12% soybean oil cake, 5% wheat flour and 1% vitamin-mineral-premix. The feed was given twice daily- at 0800 hours and 1600 hours, respectively. They

were also supplied with poultry viscera at the rate of 1% of their body weight at 1200 hours daily. The weight of each fish was taken on the 15th of each month from February to August 1999 using a spring balance. The fish were treated with acetone dried carp pituitary gland (PG) at the rate of 1 $mg \cdot kg^{-1}$ on the 15th of March, April and May 1999.

Selection of brood fish

Three breeding trials were conducted which started on the 4 July (First trial), 15 July (Second trial) and 3 August (Third trial) 1999. Usually both males and females of *P. pangasius* were selected in the morning of the starting day of a trial and they were kept in a breeding pool with continuous water flow (water exchange rate: 35 $l \cdot min^{-1}$). But, in the first trial, though females were selected in the morning of the starting day, males were selected after the occurrence of ovulation of females just prior to stripping i.e., at 0800, 1200, 1600 and 1800 hours on 5 July 1999. In trials 1 and 2, mature males were selected based on the availability of milt following gentle pressure in the abdominal region. Though milt flowed easily upon stripping the untreated males in the first trial, treatment with pituitary gland extract of the males produced sufficient milt in the second trial. In mid July milt was available upon stripping but, by August, milt flow had stopped and as such, males for third trial conducted in August were selected based on their physical appearance of a comparatively slender and short body. In all trials, mature females were randomly selected based on the display of a swollen and soft abdomen with a pinkish and protruding genital opening.

Administration of carp pituitary gland extract

Detailed experimental design is shown in table 1. In the first trial, conducted on 4 July 1999, two females were selected while for the other trials, one female was selected for each trial. The fish were treated twice with acetone dried carp pituitary gland extract. Distilled water was used as solvent for preparation of pituitary gland extract. A dose of 3 $mg \cdot kg^{-1}$ body weight was injected as first injection at 1400 hours on the day the trial started while the second dose ranged between 6 and 9 $mg \cdot kg^{-1}$ body weight was administered 8 hours apart of the first injection i.e., at 2200 hours on the day the trial started. In the first trial, five untreated males were used and they were caught soon after ovulation of the females. In the second and third trials, one and four males were used, respectively, and were injected with acetone dried carp pituitary extract at the rate of 2 $mg \cdot kg^{-1}$ body weight during the second injection of females, i.e., at 2200 hours on the day the trial started.

Ovulation and fertilization of eggs

Since the treated females did not spontaneously release any eggs nor show any difference in behavior as a sign of ovulation, stripping of the

females were performed periodically by pressing the abdomen to detect ovulation. The females were first checked for ovulation 8 hours after the second injection. The release of eggs through the genital pore following gentle pressure on the abdomen was considered as commencement of ovulation. Ovulation rate was calculated as the percentage of spawners ovulated out of the total number of spawners used in the trial. Eggs from ovulated females were stripped into fertilization trays (29 cm x 19 cm x 4 cm) made of aluminum. Following deposition of a good number (100 g to 125 g) of eggs on the tray, milt was quickly stripped from the males into the tray. Fertilization of eggs was done by mixing the eggs and milt, and allowing the eggs to stand for 3 to 5 mins.

In the first trial, eggs from both females were first stripped 10 hours after the second injection. After stripping eggs from the larger female, it became very exhausted and stripping was discontinued. The smaller female was stripped three times up to 20 hours after the second injection. In the second trial, eggs were first stripped at 11.5 hours after the second injection and continued for another half an hour. In the third trial, though the female released eggs following stripping, the males did not produce any milt upon pressing on the abdomen for up to 22 hours after the second injection.

Incubation and hatching of eggs

After fertilization, the eggs were immediately transported to the Wet Laboratory of the department and spread on steel trays (101.6 cm x 40.6 cm x 12.7 cm) for incubation and continuous water flow (water exchange rate: 3 $l \cdot min^{-1} \cdot tray^{-1}$) was maintained to the trays. The eggs were examined under the microscope 10 to 15 min after mixing the eggs and milt to see wheather the blastodisc had formed as an indication of successful fertilization. Two hours after mixing the eggs and milt, the unfertilized eggs turned whitish while the fertilized eggs remained transluscent. The fertilization rate was determined 2 hours after fertilization by counting the proportion of fertilized eggs in several egg samples. The incubation trays were kept under room temperature ranging between 26 and 28°C.

In the first trial, when hatching started, the hatchlings were collected in another tray that was set in such a manner that the water from the

Table 1. Experimental design of the study on induced breeding of *P. pangasius* in Bangladesh

Trial No.	Females			Males		
	No. of fishes treated (n)	Dose of PG ($mg \cdot kg^{-1}$ body weight)		No. of males	No. of treated (+) or untreated (-) males	Dose of PG ($mg \cdot kg^{-1}$ body weight)
		First injection	Second injection			
1	n=2			n=5	5 (-,-,-,-,-)	—-
	Fish[1]	3	6			
	Fish[2]	3	9			
2	n=1	3	7	n=1	1 (+)	2
3	n=1	3	8	n=4	4 (+, +, +, +)	2

hatching tray (Fig. 1) containing eggs could smoothly flow into another tray. In this way most of the active and strong hatchlings were collected in the second tray. Continuous water flow (water exchange rate: 3 $l \cdot min^{-1} \cdot tray^{-1}$) was maintained to the trays in which the larvae were collected. The hatching rate was determined by counting the number of hatchlings from the total number of fertilized eggs. The newly hatched larvae were measured under a compound microscope fitted with an eye piece micrometer.

Treatment and release of brood fish

The brood fish used in breeding trials were injected with tetracycline injection at a dose of 2 $mg \cdot kg^{-1}$ body weight and released in the respective ponds from which they were sampled. The ponds were intensively watched for the next few days. When any dead body was observed, it was immediately captured and then buried under soil. Seven days after release of the brood fish, they were captured by netting and the lesions which developed on their body were washed with 0.1 ppm malachite green solution and were injected again with tetracycline at a dose of 2 $mg \cdot kg^{-1}$ body weight and then released again in the same pond. On the 14th day of release they were again captured and lesions (if any) of each fish were again washed with 0.1 ppm malachite green solution and released in the pond.

Rearing of larvae

The larvae were given a combination of live zooplankton, hard-boiled chicken egg yolk, rotifer powder, newly hatched *Artemia* nauplii and finely chopped tubificid worms as first food 24 h after hatching i.e., on the second day of hatching. Zooplankton was collected from a nursery pond managed under the Department of Fisheries Biology and Genetics in BAU campus with a zooplankton net. Zooplankton was the mixture of crustacea, rotifer, etc and supplied as live. Hard boiled chicken egg yolk was crushed and mixed well in water and the mixture was sieved with a piece of cloth having

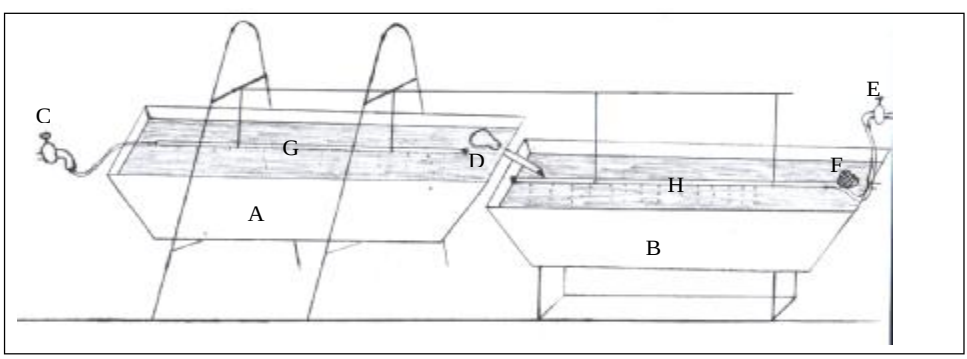

Fig. 1. Collection of hatchling of *P. pangasius* from first tray to second tray (A = 1st tray, B= 2nd tray, C = Inlet of 1st tray, D = Outlet of 1st tray, E = Inlet of 2nd tray, F = Outlet of 2nd tray (with screen), G = Porous PVC pipe for continuous water supply in 1st tray, H = Porous PVC pipe for continuous water supply in 2nd tray).

fine meshes before given to the larvae. Rotifer powder was prepared by Ocean Star International Inc., U.S.A. It was first mixed with water and then sieved by the same piece of cloth used to sieve the mixture of egg yolk. *Artemia* cysts (manufactured by Sanders Brine Shrimp Company, U.S.A.) were incubated in 70 ppt saline solution at room temperature (26 to 28°C) with vigorous aeration for 24 hours and newly hatched *Artemia* nauplii were supplied to the trays containing larvae. From the third day, the larvae were provided with finely chopped tubificid worms, live zooplankton and newly hatched *Artemia* nauplii twice daily at 0900 hours and at 1700 hours and this was continued up to 20 days of age. After this period, the larvae were provided with finely chopped tubificid worms only at 0900 hours and at 1700 hours daily. Feeding schedule of larvae is shown in table 2. The larvae were taken into an aquarium from the rearing tray at 18 days of age and they were reared in the aquarium up to 65 days of age. The washed and left over feeds were siphoned from the trays or aquarium twice daily at 0800 hours and 1600 hours before feeding. Continuous aeration was maintained in the water of the aquarium. At the termination of the study, the length and weight of fry were determined by using a milimeter graphpaper and an electronic balance (Mettler Toledo B204-S), respectively.

Results

Brood fish

The brood fish used in breeding trials were in the same age group and their average body weight was 4.2 (± 0.4) kg on 15 January, 4.3 (± 0.4) kg on 15 February, 4.5 (± 0.4) kg on 15 March, 4.6 (± 0.4) kg on 15 April, 4.8 (± 0.5) kg on 15 May, 5.0 (± 0.5) kg on 15 June, 5.2 (± 0.4) kg on 15 July and 5.1 (± 0.3) kg on 15 August 1999. Females were larger than males.

Injection and doses of PG

Detailed doses of pituitary gland used in the different trials are shown in table 3. Though all the doses trialled resulted in ovulation, the total dose

Table 2. Feeding schedule of larvae of *P. pangasius* from second day to 65th day under laboratory condition

Feed	Day		
	2	3-20	21-65
Chicken egg yolk	√	-	-
Rotifer powder	√	-	-
Zooplankton	√	√	-
Artemia nauplii	√	√	-
Tubificid worms	√	√	√

Note: √ = Feed given

Table 3. Summary of results on induced breeding trials of *P. pangasius* in Bangladesh

Trial No.	Weight (kg) of fish		Date time, Number of injection or doses of PG ($mg \cdot kg^{-1}$ body weight)			Ovulation response of females (+) or or (-)	Ovulation rate of females (%)	Reponse time (hr.)	Date and time of stripping of females	Fertilization rate (%)	Incubation temperature (°C)	Incubation period (hr.)	Hatching rate (%)
	Female	Male	Female		Male								
			1st injection	2nd injection									
1	6.6 and 5.4n=2	4.6 4.7 5.2 5.2 and 5.4 n=5	04.07.99 1400 hrs 3	04.07.99 2200 hrs 6 and 9	—-	Fish 1(+) Fish 2(++)	100	10.0	Stripping 1: 05.07.99 0800 hrs Stripping 2: 05.07.99 1200 hrs Stripping 3: 05.07.99 1600 hrs Stripping 4: 05.07.99 1800 hrs	Fish 1 Stripping 1: 23.0±2.1 Fish 2 Stripping 1: 17.0±1.3 Stripping 2: 1.6±0.6 Stripping 3: 0 Stripping 4: 0	26-28	28-32	Fish 1 Stripping 1: 8.2±1.3 Fish 2 Stripping 1: 3.8±0.8 Stripping 2: 1.3±0.4 Stripping 3: 0 Stripping 4: 0
2	6.0 n=1	5.0 n=1	15.07.99 1400 hrs 3	15.07.99 2200 hrs 7	15.07.99 2200 hrs 2	(++)	100	11.5	16.07.99 0930-1000 hrs	40.1±3.1	26-28	28-32	65.0±2.3
3	5.5 n=1	4.3,4.6 4.8 and 5.0 n=4	03.08.99 1400 hrs 3	03.08.99 2200 hrs 8	03.08.99 2200 hrs 2	(++)	100	12.0	—-	—-	—	—-	—-

Note : + - Ovulated
- - Not ovulated
n : Number of fishes used

of 10 mg·kg^{-1} body weight of PG produced the best results in relation to fertilization and hatching rates.

Ovulation, fertilization and hatching of eggs

Ovulation occurred between 10 and 12 h after the second injection at 26 to 28°C and ovulation rate of females was 100%. In the first trial, the first sign of ovulation was observed 10 h after the second injection and eggs were collected at 10, 14, 18 and 20 h after second injection. Fertilized eggs were light yellowish in color and very sticky. Fertilization rates of eggs collected at 10, 14, 18 and 20 h after the second injection was 20 (±4.2) % (average of two females), 1.6 (±0.6)%, 0% and 0%, respectively. In the second trial, ovulation took place at 11.5 hours after the second injection and eggs were stripped within half an hour post-ovulation and the fertilization rate was 70.1 (±3.1)%. In the third trial, none of the males produced milt upon stripping although the female ovulated. Hatching of fertilized eggs occurred between 28 to 32 h of incubation at 26 to 28°C. In the first trial, only 6 (± 2.5)% of the fertilized eggs, which were stripped at 10 h after the second injection hatched. In the second trial, hatching rate was 65 (±1.3)%.

Rearing of larvae

The newly hatched larvae were planktonic and transluscent and the total length of newly hatched larvae ranged from 6.5 to 8.0 mm. Pigmentation of the eyes started on the first day while differentiation of the heart and four barbels appeared on the second day of hatching. The distribution of melanophores was visible over and below the yolk sac on the third day. The yolk sac was greatly reduced by the fourth day. Biforkation of caudal fin was clearly visible on the fifth day. The transparent body of larvae became opaque as time progressed and after 15 days of hatching, they appeared like the adults.

The newly hatched larvae often surfaced at an angle and they spiralled down to the bottom of the trays where they rested for a while and then repeated such movements. On the second day, they swam horizontally rather than in an angular manner. Healthy larvae appeared rather restless, moving from one corner to another of the trays or aquarium probably foraging for food and they did not show any response to light at that stage. Even though feeds were supplied to the larvae from the second day onwards, feeding only commenced between the third and fourth days post-hatching. Since a large number of larvae died before reaching the free-swimming stage the most critical stage of larvae was the first feeding stage. This critical stage lasted for 10 days. Only 3 out of all that hatched out in the first trial, lived up to 10 days post-hatch. Even though thousands of larvae hatched in the second trial, all of them died within 10 days post-hatch. One of the three larvae in the first trial lived up to 17 days post-hatch while the remaining two larvae were

reared until 65 days post-hatch in an aquarium. By 15 days post-hatch, larvae showed preference to finely chopped tubificid worms than zooplankton and *Artemia* nauplii and they consumed the tubificid worms voraciously. The length and weight of two surviving fry 65 days post-hatch were 7.9 and 6.8 cm; and 4.1 and 3.6 g, respectively. While the dorsal part of fry was shiny blue in color, the ventral part of the body was silvery.

Discussion

P. pangasius attains maturity in the fourth year of its life in Bangladesh (Rahman 1992), however, the brood fish used in the breeding trials were 9-years-old. Pantulu (1962) and David (1963) reported that it breeds between June and August while Rahman (1992) observed that breeding season occurs early in the monsoon season-between July and October. Rahman also reported that *P. pangasius* brood fish do not attain final maturity when reared in captivity. In contrast, Khan and Mollah (1998) observed that both males and females of the species attained final maturity in ponds. They also reported that in captivity, males become mature earlier than the females in the breeding season. The results of this experiment indicated that the peak breeding season of the species was July.

In the first trial, the untreated males were caught from the pond following ovulation of females. Even though milt was flowing during stripping, the amount was considered not adequate. After discharging of several drops of milt, blood was observed. This may be attributed to the peculiar anatomy of the testes of *P. pangasius* that comprised of a large number of dentations on the testes (Khan and Mollah 1998) for which the pressure imposed during stripping could hardly result to increased milt flow. As such, injection of males with carp PG was essential to obtain milt as per requirement from one or two males to avoid handling of a large number of males. While selecting males for the second trial in mid July, several fish were found milting, however, no milt were observed upon stripping the males in the third trial although all of them were treated with PG dose of 2 $mg \cdot kg^{-1}$ body weight. In general, milt was available upon stripping of the males from 15 March till 15 July 1999. From the preliminary observations, it could be said that males became ripe and spent earlier than females.

The females used in the first and second trials died within 24 h of release in pond and may be attributed to stress as a result of repeated capture and release for detection of the actual time of ovulation as they did not show any changes in behavior i.e., mating behavior prior to ovulation. As they were also stripped several times for collection of eggs in these two trials- a) stripping and b) repeated handling may have caused internal injury resulting death in consequence. The female used in the third trial was handled with extreme care to prevent mortality. The fish survived and subsequently, recovered from several lesions on body. As

the fish has very reduced scale, the skin got injured easily during stripping due to repeated handling that subsequently developed lesions.

Rahman (1992) failed to induce ovulation when the females were treated 24 mg·kg^{-1} of carp pituitary gland extract, 3000 i.u. kg^{-1} of human chorionic gonadotropin or 0.55 mg·kg^{-1} of ovaprim. Mollah and Khan (1999) reported the first success in inducing ovulation of *P. pangasius* in Bangladesh by treating the females with 8 mg·kg^{-1} body weight of PG and in that study, partial ovulation occurred. They had treated seven females at different dosages between 8-12 mg·kg^{-1} body weight of acetone dried carp pituitary gland and only fish in one treatment partially ovulated. During the present experiment the ovulation rate was 100% in all the trials i.e., all the selected females ovulated when treated with acetone dried carp pituitary glands at the rate of 9, 10, 11 and 12 mg·kg^{-1} body weight. The success in inducing ovulation in all females could be considered as a major breakthrough in establishing the propagation techniques of this species.

Even though all the treated females ovulated, the total dose of 10 mg·kg^{-1} body weight of PG produced the best results in relation to fertilization and hatching rates of eggs. The time interval between the two injections was 8 h in all trials, indicated that this time period was acceptable at 26 to 28°C. Mollah and Khan (1999) reported that the effective time interval between the first and second injections of the females was 8 h. Accurate timing for stripping of female fish is very important to achieve success in induced breeding and in the present study, ovulation occurred between 10 to 12 h after the second injection. As such, females should be first checked for ovulation 10 h after the second injection to reduce handling stress of females and eggs should be stripped when the female had fully ovulated.

In the first trial, one female was stripped 4 times. The fertilization rate of eggs during first trial was very low (20% - average of eggs stripped at first attempt of 2 females) in eggs collected 10 h after the second injection. Fertilization rates of eggs collected at 14, 18 and 20 h were 1.3%, 0% and 0% respectively, in the same trial. The number of eggs ovulated was less at first attempt of stripping and more eggs were coming out following stripping afterwards. But, the fertilization and hatching rates of eggs produced in the first attempt of stripping was higher than those produced in subsequent attempts. So, one is tempted to conclude that the viability of *P. pangasius* eggs was rather short. Hatching of fertilized eggs in this study occurred within 28 to 32 hours of incubation at 26 to 28°C and this finding was consistent with that of Mollah and Khan (1999) that reported the incubation period of *P. pangasius* eggs to be between 29 to 33 hours at 26 to 28°C. The hatching rate of eggs was the highest (65%) in the second trial, however, all the hatched larvae died within 10 days of hatching. Only 3 larvae of first trial survived more than 10 days. Most of the larvae died at the first swimming and first feeding stages. This might be due to stress imposed on the females for repeated checking during the breeding trials. Liao (1975) reported that repeated checkings were often detrimental to the ovary and eggs of grey mullet. Nash and Kuo (1976) also reported that the ovarian develop-

ment of females was seriously affected by stress. Further research needs to be done to find out the inherent reason behind mass mortality of larvae.

Conclusion

This is the first major success in induced ovulation of *P. pangasius* in Bangladesh. The carp pituitary gland dose at the rate of 10 $mg \cdot kg^{-1}$ body weight, produced the best results. Fertilization and hatching rates were high when the females were treated with a total dose of 10 $mg \cdot kg^{-1}$ of carp pituitary gland. This is considered as a landmark in the strategy of saving this endangered species by establishing a technique for mass production of fry. The suitable time interval between the two injections should be eight hours and fishes of both sexes should be treated with pituitary extract. The females should be first checked 10 h after the second injection. As the larvae preferred finely chopped tubificid worms, they could be reared with a minimum cost. Based on the information generated during this research program, causes of mass mortality of the larvae needs to be undertaken so that a reliable technique of fry production can be established.

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