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Induced Spawning of Two Major Species of Chinese Carps, Ctenopharyngodon idellus and Aristychthys nobilis in Sri Lanka

By

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Introduction

Induced spawning of three major species of Chinese carps, viz., *Ctenopharyngodon idellus* (Grass carp), *Aristychthys nobilis* (Bighead carp) and *Hypophthalmychthys* molitrix (Silver carp) has been successful in most of the Southeast Asian countries and in the tropics. This has been possible with the knowledge gained from the histological studies made on the development of oocytes of the three species. In most of these countries successful propagation by induced spawning of the three major species has been attributed to careful studies made on the selection of spawners and donors, induced ovulation and artificial fertilization.

Extensive studies made on induced spawning in the People's Republic of China and other Southeast Asian and Western countries have clearly demonstrated the importance of using pituitary hypophyseal extract, human chorionic gonadotropin and leutinizing hormone in brigning about the release of matured eggs for fertilization under artificial conditions. It is known that L H like gonadotropin present in the pituitary of the donor common carp, may be the factor which is responsible for the induction of ovulation in these three major species (Chen, Chow and Sim, 1969).

Extensive studies made in tropical countries like Malaysia by Hickling (1963) and others have conclusively shown that due to the warm climate in the tropics, more than one spawning cycle might be possible, and that food plays a very important role in making this a reality. In Sri Lanka culture and propagation of two varieties of Chinese carp, viz., grass carp and bighead carp, were effectively undertaken with aid from the People's Republic of China under whose guidance and expertise this had been possible within one-and-a-half years in the case of grass carp and three years for bighead carp.

Induced spawning of grass carp (*Ctenopharyngodon idella*) and bighead carp (*Aristychthys nobilis*) was carried out successfully in Sri Lanka for the first time in 1977 and 1978, respectively. This article describes the techniques involved in induced spawning in Sri Lanka at the Fresh water Fish Breeding and Experimental Station in Udawalawa.

TECHNIQUES OF CULTURE

Materials and Methods

Fry of grass carp and bighead carp were first airlifted to Sri Lanka from the People's Republic of China in 1975 and were cultured to fingerling size of 10-12 cm. in cement tanks. The fingerlings were then introduced into the mud ponds at the Freshwater Fish Breeding and Experimental Station

in Udawalawa in December 1975. The initial stocking density of grass carp fingerlings was 40 per pond* (No. 3) and that of bighead fingerlings was approximately 600 per pond (No. 2). The grass carps were next transferred to pond No. 1 in June, 1976, when they were between 1.5-2.0 kg. From late June and early July there was an outbreak of infection by *Synergassillus spp.* among the grass carp adults.

During this period there was heavy mortality until the number reduced to 20 individuals. The disease was arrested with application of copper sulphate and masonate and also by bathing in potassium permanganate solution as a precautionary measure. By March 1977 the parent grass carp had reached 6 kg. in weight and the females and males were ready for spawning. During the entire culture period the pond water in which grass carp were reared was fertilized only with cowdung. Inorganic fertilizers were never used. Fertilizing of water was carried out only at the initial stages of stocking in grass carp ponds and during the entire culture period the adult and parent grass carp were fed with supplementary food like poonac (coconut residue cake) at approximately 5 kg./0.13 ha and various types of grasses. In pond No. 2 where the big head fingerlings were stocked, the fingerlings reached a size of 0.25 to 0.5 kg. by June 1976. A series of thinning operations and transfers were carried out whereby the biggest adults were selected and stocked in different ponds. Up to September, 1976, this process continued spurred on by deteriorating water quality of ponds in which the bighead carps were stocked and the detection of larger size groups during netting out operations. This resulted in stocking of two ponds with approximately 90 individuals per pond from the original batch of 600 individuals; by December 1976 the size of these bigheads was 0.55 to 1.0 kg. Further thinning out and transfers carried out beyond September 1976 resulted in four ponds being stocked by the end of 1977 with 30 individuals (No. 7), 30 individuals (No. 8), 60 individuals (No. 4), and 60 individuals (No. 5). By this time the maximum size recorded for bighead carp was 8 to 10 kg. in pond No. 7 and pond No. 8. During this period only the female bighead carps were mature on examination. Since the male bighead carps failed to mature even by March 1978, water was made to flow in ponds 7 and 8 in order to bring about a lowering of temperature as it was suspected that the high warm temperature of the pond water had a slowing effect on the maturity of the gonads of the male bighead carps. Inspection of male bighead carps in May 1978 revealed the maturation of some males. During the entire culture period of bighead carp they were fed with crushed poonac at 5 kg./0.13 ha and as the bighead carps are planktophagus a rich source of planktons was maintained in each of the ponds in which bighead carp were cultured by the application of cowdung. Inorganic fertilizers were never used at any stage.

Induced Spawning

Artificial spawning or induced spawning of grass carp and bighead carp was successfully executed in 1977 and 1978, respectively. The successful maturation of males and females of both species had been possible due to careful control of stocking densities and manipulation of feeding schedules. Successful induced spawning was carried out in both species on the basis of three main factors : (1) Selection of spawners and donors ; (2) Ovulation ; (3) Artificial insemination and fertilization. In the tropics, according to Kurth and Chow (1969) grass carp oocytes undergo seven stages in development. During the seventh stage (migratory nucleus stage) the nucleus moves to the animal pole, through which the sperm enters the mature ova for fertilization after release. During this stage the eggs are almost ripe and ready for release [prematuration stage] triggered off by certain natural conditions which are found only in very large rivers. Among these natural conditions which initiate the release of eggs in large rivers are the water flow and an

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* Area of pond=0.13 ha.

optimum temperature. However, these optimum conditions will not induce the females to release the eggs ui der captive conditions. In induced spawning the females are forced to release the eggs in captivity by injecting hypophysial extract or other similar hormcnes which contain an L.H. type of gonadotropin, which is believed to be the key hormone responsible for the induction of ovulation.

Selection of Spawners and Donors

The spawners and donors were first netted and each individual was placed upside down in cloth bags. During examination for maturity, the fish were always kept under water except when feeling the abdomen and catheterization during which a part of the abdomen was raised above the water. No anaesthetics were used during selection. In mature females the abdomen is full and bulges laterally along the length of the body tapering toward the genital opening. The abdomen is also soft to the touch and gentle tapping produces a feeling similar to the effect produced by the gentle tapping of a soft leather bag filled with water. If the abdomen is firm and hard to the touch it indicates that the ova have not yet reached the tertiary or the migratory nucleus stage. In mature females another indication is that the genital opening is swollen, pink in colour and slightly bulging. Spawners of both glass carp and bighead carp have been identified to a great extent by this method, but a surer method is by catheterization. In this method a long galvanized metal rod (30 c.m. long and approximately 2 mm. in diameter) is inserted at an angle through the genital opening more towards one side of the abdomen. The rod moves freely in once the correct path through the genital opening is found in the ovary and on gentle rotation some of the eggs are released and get into the cavity of the catheter. The rod is pulled out and the eggs are tapped on to a petridish containing alcohol for examination under the microscope. In mature eggs the nucleus has migrated towards the animal pole. If the nucleus is still at the centre of the egg, it indicates that the eggs are still in the late tertiary nucleus stage.

The selection of male donor is comparatively easy. In mature males, gentle application of pressure to the abdomen produces a free flow of whitish milt through the cloaca.

Ovulation

At the Freshwater Fish Breeding and Experimental Station induced ovulation of grass carp was initiated by using three types of hormones on an experimental basis. One batch of grass carp females was injected with a combination of leutinizing release hormone and human chorionic gonadotropin and the second batch with pituitary extract and human chorinic gonadotropin. Tables 1 and 2 show the dosage of each combination of hormones used and their effect on induced ovulation, fertilization and hatching. It is clearly seen from the results of fertilization and hatching, of the combinations used, that the most effective combination was pituitary extract and human chorionic gonadotropin. Dry pituitary preserved in acetone was used for this purpose. The required amount of pituitary and human chorionic gonadotropin were mixed and crushed in a mortar to a fine powder and mixed with normal saline or fresh distilled water. The full dosage was divided into two, the first representing 10% of the total dose. The first dose (10%) was administered around 4.30 to 5.00 p.m. and the balance of 90% at 11.30 p.m. to 12.00 midnight after a 6 to 7 hour gap. The injection was administered to the body cavity at the base of the pectoral fin. Usually the first injection to the female contains only the pituitary extract and the mixture of pituitary and H. C. G. is given at the time of the second injection. The male was administered only half the dose and at the time of the second injection given to the female.

Fertilization

During artificial spawning experiments of grass carp in 1977 and 1978 rapid courtship and the oestrus was not observed 5 to 6 hours after the second injection. Although males and the females constantly followed a certain path (pursuing behaviour ?) in the spawning pond after the second injection, the violent rapid movements which characterizes oestrus and the release of eggs was not observed in the early hours of the morning. At a temperature of 28° to 30° C. and after a time interval of 6 to 7 hours since the second injection, artifiial insemination and fertilization was carried out. In 1977 during artificial fertilization, the dry method was adopted. In this method the females were made to release the eggs into a clean enamel basin by gently squeezing the belly (Stripping). Next the milt from the male was squeezed out into the same basin, effective fertilization being facilitated by gentle rotation of the contents in the basin. In 1978 due to the low number of males used, the normal saline method was adopted. Here the milt from the males was collected in normal saline in an enamel basin and this was used to fertilize the eggs of the female collected in another basin. The advantage of this method is that the milt could be kept for a comparatively longer period without losing their effectiveness, and the eggs of the female could be fertilized in batches.

Hatching and Feeding of Fry

The fertilized eggs were next placed in hatching jars made of polypropylene. In these the water was kept circulating at a certain speed by means of 5 inlets directed at angles at the periphery of the base of the hatching jar. The circulating water helped to keep the eggs floating and moving constantly, and provided continuous aeration by a constant flow mechanism. At 28° to 30° C. under these conditions the eggs of grass carp and bighead carp hatched within 15 to 17 hours after fertilization. The larvae were kept for two more days in the hatching jars until they were about 7 mm. in length before being introduced directly to the mud ponds. The hatched out larvae obtained their nutrients for growth from the yolk in the yolksac. After stocking in mud ponds the fry start feeding on rotifera and other small insects and as size increases they start feeding on larger crustacea like the copepods and cladocera. In addition to this soybean milk was also added as supplementary food. At a stocking density of 150,000 and 250,000 per 0.13 ha the fry reached a size of 4.0 cm. and 3.0 cm. respectively, from the original stocking size of 7 mm.

The induced spawning of bighead carp was carried out in June, 1978, more or less on the same lines as that described for grass carp. For the first time in Sri Lanka hybridization was done by crossing bighead carp female (*Aristychthys nobilis*) with silver carp male (*Hypophthalm ychthys molitrix*). The fertilization rate in this case was 85%.

DISCUSSION

Induced spawning of the major Chinese carps was carried out in 1958 in the People's Republic of China and since then various types of research and experimental work had been carried out on the aspects of gonadal development, the effect of various types of hormones on induced ovulation, etc., by various scientists all over the world. Hickling (1963) had first attempted induced ovulation in June 1963 by injecting grass carp with pituitary extract of *Puntius spp*. Negative results were obtained by Kurth (1963, 1964, 1966) by using Follicle Stimulating Hormone and corticotrophin. He had also found that the effective development of the adults and juvenile fish metabolism were affected badly by Follicle Stimulating Hormone and that LH had a marked positive effect on the ovarian growth in adults.

Work carried out by Chung Ling (1958) and others had shown that induced ovulation and the release of eggs for fertilization was possible by injecting grass carp spawners with pituitary extract and then subjecting the males and females to flowing water in spawning ponds at a temperature range

of 28° to 30° C. They had also conclusively shown that failure to spawn naturally under artificial conditions without the use of hormonal injections was due, not to the failure in gonadal development, but to the lack of spawning behaviour of the female, which might have resulted from the absence of certain special environmental conditions present only in large water bodies like the Yangtze and Yellow Rivers in China. These environmental conditions are believed to trigger the release of the necessary hormones which bring about the transformation of the migratory nucleus stage to the prematuration stage of the oocytes with subsequent release of eggs in the female. This transformation is brought about under artificial conditions by pituitary hormonal injections.

In all vertebrates the pituitary gland or hypophysis consists of two parts based on the embryology, structure and function. These are : (1) The Neurohypophysis, (2) The Adenohypophysis. These two parts meet and enclose between them a mesodermal rudiment which gives rise to their intrinsic blood vessels (Green and Maxwell, 1959 ; Green, 1966). Therefore this gland is a composite organ bearing different endocrine functions. The adenohypophysis is the site of synthesis, storage and release of different peptide hormones into the blood. Of these some are glycoproteins ; namely, gonadotropins, follicle stimulating hormones (FSH), leutinizing hormones (LH) and thyropin or thyroid stimulating hormones (TSH). It is believed that FSH and LH play a very important role, in Gonadal development.

The poor results obtained by LRH in induced spawning experiments of grass carp in 1977 at the Freshwater Fish Breeding and Experimental Station, Udawalawe, may have been due to its indirect action on the production of LH which is believed to be the key hormone repsonsible for artificial propagation. LRH brings about an acceleration of the growth of the pituitary gland which in turn produces the hormones, FSH and LH.

Work carried out by Hickling (1963) and others at the Tropical Fish Culture Research Institute, Malacca, have conclusively shown that in tropical countries, gonadal development was not suppressed and induced ovulation was possible. The failure to ovulate viable eggs, from grass carp in earlier experiments in the tropics had been later attributed to low gonadosomatic ratios used. According to Chen, Chow and Sim (1969), to obtain a complete understanding of induced ovulation of grass carp in the tropics focus must be made on the feeding schedules of potential spawners. According to them the recommended feed for potential spawners is an initial feed of hydrilla, for somatic growth followed by a final feed of napier grass for gonadal development.

At the Freshwater Fish Breeding and Experimental Station in Udawalawe, the grass carp from the early adult stages were fed with various types of grasses ; Eg. mana, *Cymbopogon* sp., illuk, *Imperata* sp. bata dalla-*Ifachnesp*. Brackaria and tender shoots of all these weeds.Of these the grass carp preferred grasses like Brackaria and other tender grasses which do not have a serrated margin. In this respect adults and spawners rejected grasses like mana and illuk, though they sometimes preferred the tender shoots of these varieties. On the other hand they fed voraciously on grasses like batadalla (*Ifachne sp.*), but the incidence of gill infections due to *Synergassillus sp.* was high sometime after that. This was discontinued immediately and the fish were fed with mainly land grasses. As a supplementary feed solid poonac was given once a day. A pond of about half an acre containingabout 30 grass carp parents had to be supplied with about 30 to 40 kg of grass per day. Detailed feeding schedules are discussed elsewhere and not within the scope of this article. Under these culture conditions a good mature female weighing 8 to 9 kg. produced approximately 600,000 eggs.

Growth and maturation of grass carp and bighead carp has been very fast under local climatic conditions reaching 9 to 10 kg. and 10 to 12 kg. in one and a half years and three years, respectively. Although the female grass carp matured fast the males showed low maturation and during induced

spawning in 1977 the milt produced by the males was insufficient and hence the low fertilizing and hatching rates during that year. In the following year the maturation was comparatively good and fertilizing and hatching rates have been fair.

In temperate countries like China the major carps breed in late spring and summer. Growth is inhibited during winter in which period the fish live on their reserve food. In the case of tropical countries this hazard is absent and continued growth is possible right throughout the year under the warm climate. In Udawalawa, Sri Lanka, the rapid rate of growth could be partly attributed to the warm air and water temperature. The temperature in the water ranged between 29° and 32°C while the surface temperature during the hottest months ranged between 33° and 35°C.

The main purpose of the Freshwater Fish Breeding and Experimental Station in Udawalawe is the production of sufficient fish seed for stocking the major and minor water bodies throughout the country. These two species have been selected due to their high growth rate in large water bodies and the comparative low economy in culture as size increases due to growth. Although at the initial stages of growth artificial food like soyabean milk and crushed poonac are used in feeding, according to Gidumal 'as grass carp increases in size it become less expensive to feed, so producing a cheap rich source of protein for the population.....'. In the case of bighead carp this applies too as they are planktophagus and depends on natural production in the water for growth. These factors combined with the fact that these carps may be spawned about 3 times a year would be the key to producing a better and a much larger catch per ha per year from our natural water bodies once they are stocked intensively for a period of 3 to 4 years.

Much research has to be carried out on the type of local grasses which would produce a better effect in ovulation and maturation rate of the male grass carp. As mentioned earlier comparative low fertilization rates and hatching rates and low production of milt by the males could have been due to the type of grass with which the grass carps are fed here. And according to Chen, Chow and Sim (1969) ' the manipulation of feeding regime of high protein diet for grass carp in different ponds ' and also by stocking bighead carps in different well fertilized ponds, it would be possible to stagger and control the spawning cycles in order to produce the much required fish seed for stocking the water bodies intensively at different times of the year. This could be considered the most important advantage in artificial propagation.

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TABLE I

Artificial Propagation of Grass Carp (Ctenopharyngodon Idellus)
March and June, 1977

Type of Combination	Female		Male		Results
	Dosage		Dosage		
	L.R.H.	H.C.G.	L.R.H.	H.C.G.	
L.R.H. and Human chorionic gonadotropin	22 mug/kg of fish 1st inj. 10% 2nd inj. 90%	1000 IU/ kg of fish given with 2nd inj.	11 mug/kg of fish	500 IU/kg of fish	There was no effective release of eggs in the female and the quantity of milt produced by the males was insufficient for effective fertilisation.
			Both given to the male at the time of 2nd. inj. to female		

Type of Combination	Female		Male		Results
	Dosage		Dosage		
	Pituitary	H.C.G.	Pituitary	H.C.G.	
Pituitary and Human chorionic gonadotropin	3.0 mg/kg of fish 1st inj. 10% 2and inj. 90%	1000 IU/ kg of fish given with 2nd inj.	1.5 mg/kg of fish	500/UI kg of fish	Effective release of eggs in the female and comparatively good production of milt by the males. However the number of males used was insufficient for effective fertilisation.
			Both given to the male at the time of 2nd injection to female		

A Record of Monthly Average of Morning and Evening Temperature from October, 1976 to November, 1977

Month	*Morning*			*Evening*		
	Maximum	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*
January ..	29.0 ..	28.2 ..	28.0 ..	31.0 ..	29.7 ..	29.0
February ..	20.0 ..	28.0 ..	27.5 ..	31.0 ..	28.9 ..	28.0
March ..	30.0 ..	28.5 ..	27.0 ..	33.0 ..	30.0 ..	29.0
April ..	31.5 ..	29.7 ..	28.5 ..	33.0 ..	31.1 ..	29.5
May ..	30.5 ..	28.5 ..	28.0 ..	31.5 ..	29.7 ..	28.5
June ..	29.0 ..	28.1 ..	27.0 ..	31.0 ..	29.6 ..	28.0
July ..	28.5 ..	27.3 ..	26.5 ..	29.5 ..	27.8 ..	27.0
August ..	28.5 ..	27.5 ..	27.0 ..	29.5 ..	28.6 ..	27.0
September ..	29.0 ..	27.7 ..	27.0 ..	30.0 ..	28.7 ..	28.0
October ..	28.0 ..	27.9 ..	27.5 ..	29.0 ..	28.5 ..	28.0
November ..	29.5 ..	28.1 ..	25.0 ..	30.0 ..	29.2 ..	27.0
December ..	29.0 ..	27.6 ..	26.0 ..	31.0 ..	29.6 ..	27.0

TABLE II

Artificial Propagation of Grass Carp (Ctenopharyngodon idellus), 1978

Type of Combination	*No. of females*	*Weight of females*	*Quantity of eggs produced*	*Fertilization rate*	*Hatching rate*	*Quantity of fry*	*Remarks*
Female	1	9.5 kg.	595,000	60%	33.2%	118,875	Low hatching rate would have been due to probably the low gonadosmatic ratio used as there were insufficient numbers of male
Pituitary and H.C.G.	2	9.0 kg.	455,000	56%	20.8%	63,000	
3.0 mg/kg. of fish+	3	8.5 kg.	525,000	63%	59.8%	198,000	
1000 IU/kg. of fish	4	9.0 kg.	770,000	87%	50%	333,000	
Male 500 IU/kg. of fish	5	8.0 kg.	630,000	75.7%	43.3%	207,000	

Artificial Propagation of Bighead Carp (Aristychthys nobilis), 1978

Type of Combination	*No. of females*	*Weight of females*	*Quantity of eggs produced*	*Fertilization rate*	*Hatching rate*	*Quantity of fry*	*Remarks*
Female	1	12.0 kg.	487,500	22%	10.0%	11,217	The low hatching rates in the first two cases could be attributed to the insufficient milt produced by some of the males
Pituitary and H.C.G.	2	12.0 kg.	845,000	74%	11.6%	72,914	
3.0 mg/kg. of fish–	3	12.0 kg.	1,300,000	43%	58.6%	325,307	
1000 IU/kg. of fish	4	12.0 kg.	1,267,500	82%	34.5%	358,960	
Male 500 IU/kg. of fish	—	—	—	—	—	—	