Further Observations on the Induced Spawning of the Phytophagous Chinese Carp Species *Ctenopharyngodon idella* (Val.) and *Hypophthalmichthys molitrix* (Val.)

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Abstract

A combination of mammalian gonadotrophins containing oestrogen, follicle stimulating hormone (FSH) and luteinising hormone (LH) were employed, either with or without common carp pituitary gland extract (PGE) in induced spawning trials with the Chinese silver and grass carp species. Where combinations of FSH and LH were followed up with various dosages of PGE, only partial spawning success was achieved with the silver carp, whereas the results were generally much better for the grass carp. Despite gonadal hydration, ovulation could not be effected in both species when only PGE was used.

Introduction

The Chinese carp species *Ctenopharyngodon idella* (Val.) and *Hypophthalmichthys molitrix* (Val.) were successfully spawned during the early summer of 1977 at the hatcheries of the Lowveld Fisheries Research Station, Marble Hall, Transvaal, and their larvae successfully reared under controlled conditions before being transferred to outside ponds (Schoonbee *et al.*, 1978). Some difficulty was, however, experienced in the use of mammalian hormones and/or crude extract of common carp pituitary gland (PGE) particularly in the spawning of the grass carp. Although a satisfactory number of the grass carp fry was obtained during the 1977 spawning experiments, it was decided to investigate further combinations of certain mammalian hormones, either without or in combination with the common carp PGE to determine whether an even better gonadal hydration, and subsequent release of eggs, could be achieved. In the 1977 experiments, spawning was effected in both Chinese carp species by an initial low dosage starter injection of human chorionic gonadotrophin (HCG) followed by two main fractional injections — the first of which consisted of HCG, then followed by a final dosage of crude extract of PGE.

In view of the research worker's inability to assess the potency of PGE (Shehadeh, 1976), it was also felt necessary to find out if spawning could be achieved by using mammalian hormones containing follicle stimulating hormone (FSH) and luteinising hormone (LH), without the aid of PGE. For this reason the silver carp was again included in the spawning trails of 1978

even though the combination of hormones and PGE as used by Schoonbee *et al.* (1978) had appeared to be effective in inducing this species to spawn under laboratory conditions.

Experimental

Pond conditions and selection of spawners

Physical and chemical conditions of the water were similar to those described by Schoonbee *et al.* (1978). Water temperature ranged between 22°C and 36°C.

Based on the experience of research workers elsewhere in the world (Bardach, *et al,* 1972; Chen, 1976) and also from own experience, the male and female spawners of both Chinese carp species were kept as far as possible under favourable pond conditions. Silver carp were kept in fertilized ponds where a programme of chicken manuring was applied to promote phytoplankton growth, while grass carp spawners fed on the aquatic plants *Leersia hexandra* Sw. and *Lagarosiplon muscoides* Harv., both of which occur naturally in the ponds. This was supplemented in winter with 25% protein carp pellets. In this way the spawners of both species were found to be in a good condition at the onset of summer.

Only fish of which the gonads were already soft and the bellies visibly enlarged, were selected at the ponds and transferred to tanks in the hatchery. Three 1 000 dm^3 tanks, provided with flowing water and aeration, were used to hold the spawners. Due to a lack of available tank space, two spawners of each species were kept together in each tank. Procedures identical to those described by Schoonbee *et al.* (1978) for the removal of possible external parasites and for measuring and weighing the spawners were again used during the present spawning trials. Instead of tagging the fish by tying coloured woollen strands around the caudal peduncles (which during the 1977 experiments sometimes slipped off or cut into their skins), the woollen strands were now inserted through the dorsal part of the tail fin before tying up. This resulted in a minimal irritation of the fish and no tags were lost.

In order to calm the fish down after capture and to enable the spawners to empty their stomachs, all fish were allowed a period of 12 h in the tanks before commencing with the injection programme on 31 October, 1978.

Injection Programme

The combination of hormones used during the injection programme as well as the injection times are indicated in Table 1. Apart from HCG Pregnyl* (which largely consists of LH and was used with PGE in the 1977 experiments), stilboestrol dipropionate**, pregnant mare serum (PMS)*** and Seragon (SRGN)**** were used in the experiments. Stilboestrol dipropionate stimulates LH release in animals, while both PMS and SRGN contain FSH and are normally used to stimulate the development of the ovarian follicle.

From Table 1 it follows that in 50 per cent of both species, the spawners received a first (starter) injection (10h00, day 1, Table 1), which was with the exception of the grass carp female in tank 3 mainly intended to promote follicle development. This was followed by two main fractional injections consisting of a combination of SRGN and HCG and stipulated dosages of PGE. In the remaining 50 per cent of the fish, attempts were made to induce spawning without a starter injection. This was done by a combination of SRGN and HCG as the first injection (22h00, day 1, Table 1) followed approximately 8 h later by either a combination of SRGN and HCG or PGE. In the first injection the SRGN concentration exceeded that of the second, whereas the HCG concentration was increased in the second of the series of two injections. The main reason for the decrease in SRGN and the increase in HCG was that initially more FSH, and during the later stages of gonadal development, more LH might have been required by the fish, to provide for the proper hydration of the gonads prior to release of the eggs. The major aim of the present study was to establish if sufficient hydration of the gonads could be effected to result in the release of eggs by the use of a certain combination of mammalian FSH and LH and without the use of PGE

Since it was found in the 1977 studies that higher dosages of HCG and PGE were generally required to spawn the grass than the silver carp (Schoonbee *et al,* 1978), it was decided to follow a similar procedure with the mammalian hormones and PGE used during the present series of experiments.

Use of body girth to measure gonadal hydration

From the results obtained by Antalfi and Tölg (1971) and Rothbard *et al.* (1978) it is clear that increases in the body circumference of the Chinese carp species in the region anterior to the dorsal fin, can serve as a measure of gonadal hydration in the spawners. The extent of hydration also indicates the effectiveness of the hormones administered, especially to the female spawners. By using the ratio of the body circumference (= body girth) and body length, a body girth index (BGI) can be used to ascertain the actual increase in body girth and thereby gonadal hydration in a certain period (Table 1).

Discussion

An analysis of the BGI of the silver carp and the grass carp for the various hormonal treatments, reveals that in virtually all cases the silver carp responded better to the treatments than the grass carp. This was particularly noticeable in fish from tank 1. The only exception was that of the grass carp female spawner in tank 3 where the best spawn, namely 2 200 cm^3 eggs, was obtained with a body girth increase of more than 1 cm.

Despite the extremely good gonadal hydration recorded for the silver carp, its spawning performance in the present series of experiments compared less favourable with results obtained for the grass carp. Of the four partial spawns that were obtained from the silver carp, only two could be considered as reasonably successful, namely those from tanks 1 and 3. (Table 1) In the first case the development of all the embryos in the breeding jars ceased after 18 h of development.

Although it was hoped for better spawning results with the silver carp, the outcome of the present study for this particular species, was not entirely unexpected. The marked gonadal hydration in this fish species, resulted either in an incomplete spawn or, in a low survival rate of the fertilized eggs following induced spawning with hormones and pituitary gland extract. This was also observed by the second author during trials with the common, grass and silver carps in Israel in 1977 and also

* HCG Pregnyl — Produced by Nobilis Laboratory
** Stilboestrol dipropionate — M&B Product
*** PMS — Upjohn Project
****SRGN — Ferring AB Product

TABLE 1

RESULTS ON INDUCED SPAWNING EXPERIMENTS WITH THE CHINESE SILVER CARP *HYPOPTHALMICHTHYS MOLITRIX* AND GRASS CARP, *CTENOPHARYNGODON IDELLA* AT THE TRANSVAAL LOWVELD FISHERIES RESEARCH STATION, MARBLE HALL: 31 OCTOBER — 1 NOVEMBER, 1978

Tank no and Females of Silver and Grasscarp	Body Mass (g)	Body Lgth (cm)	DAY 1 22h00 BG	DAY 1 22h00 BG/BL	DAY 2 6h00 BG	DAY 2 6h00 BG/BL	DAY 2 7h15 BG	DAY 2 7h15 BG/BL	DAY 2 15h00 BG	DAY 2 15h00 BG/BL	DAY 1 Starter Dosage Irrespective of Spawner Biomass 10h00	DAY 1 Dosage/kg Spawner 22h00	DAY 2 Dosage/kg Spawner 06h00	DAY 2 Dosage/kg Spawner 07h15	DAY 2 Dosage/kg Spawner 15h00	Results
TANK 1																
H. molitrix	4 000	61,1					44,0	0,72	47,0	0,77	600 I.U. PMS + 5 mg stilboestrol			250 I.U. SRGN + 500 I.U. HCG	2,0 mg gland	Partial spawn at 01h00 on day 3. Blood clots in gonads. 500 cm³ eggs obtained. Development of eggs in breeding jars stopped after 18 h.
	3 500	63,0	43,0	0,68	45,3	0,72						500 I.U. SRGN + 750 I.U. HCG	250 I.U. SRGN + 2 000 I.U. HCG			No spawn.
C. idella	5 000	70,4					43,3	0,62	44,0	0,63	600 I.U. PMS + 5mg stilboestrol			250 I.U. SRGN + 2 500 I.U. HCG	3,0 mg gland	No spawn.
	4 500	66,0	41,5	0,63	42,0	0,64						500 I.U. SRGN + 1 000 I.U. HCG	250 I.U. SRGN + 2 500 I.U. HCG			No spawn.
TANK 2																
H. molitrix	4 750	66,5					46,1	0,69	48,0	0,77	600 I.U. PMS + 5mg stilboestrol			750 I.U. SRGN + 500 HCG	0,2 mg gland	Blood clots in gonads. Partial spawn at 01h10 on day 3.
	4 000	62,0	45,0	0,73	46,8	0,75						500 SRGN + 1 000 HCG	4,5mg gland			Partial spawn. Blood clots in gonads.
C. idella	6 500	74,4					47,5	0,64	48,0	0,65	300 I.U. PMS + 250 I.U. HCG			500 I.U. SRGN + 500 I.U. HCG	3,0mg gland	Spawn 01h10 on day 3 1 200 cm³ eggs produced
	4 500	67,3	43,5	0,65	44,2	0,66						500 SRGN + 1 500 HCG	4,5mg gland			Spawn 17h15 on day 3 1 100 cm³ eggs produced partial spawn in tank.
TANK 3																
H. molitrix	4 000	61,5					45,1	0,73	46,5	0,76	600 I.U. PMS + 5 mg stilboestrol			500 I.U. SRGN + 500 I.U. HCG	2,0mg gland	Partial spawn in tank. Spawn 00h20 on day 3 300 cm³ eggs.
	4 500	64,2	46,2	0,72	47,5	0,74						1,5mg gland	4,5mg gland			No spawn.
C. idella	4 250	66,3					40,7	0,61	41,8	0,63	500 I.U. HCG			500 I.U. SRGN + 500 I.U. HCG	3,0mg gland	Complete spawn 23h40 day 2. 2 200 cm³ eggs obtained.
	4 250	66,0	40,9	0,62	41,5	0,63						3mg gland	6mg gland			No spawn.

* Fork length BG = Body girth BL = Body length (in this case fork length) SRGN = Seragon HCG = Pregnyl

at certain hatcheries in Taiwan. Observations in these cases showed that when the gonads of female spawners were already partly hydrated and soft, injections with gonadotrophic hormones and/or pituitary gland extract easily led to an excessive hydration of the spawners, which then usually coincided with the loosening of gonadal tissue together with the eggs. This in turn resulted in some cases in the partial or total blockage of the genital opening of female spawners or in a release of eggs containing large volumes of body fluid. Fertilized eggs obtained from such fish usually only developed for 2—6 h before they died. A much lower dosage of hormones and PGE in such spawners might have yielded better spawning results and a higher survival rate of the fertilized eggs.

An overall better spawning performance in the present study was obtained for the grass carp when the combined injection of SRGN and HCG was made, followed by PGE, than was the case for the separate injections of HCG and PGE in the spawning trials of 1977 (Schoonbee, *et al*, 1978). The release of eggs by the grass carp (tanks 2 and 3, Table 1) was also in all cases completely free of blood clots. This was not always the case for this species in the 1977 induced spawning experiments.

Although no spawns were obtained where attempts were made to spawn the fish without the use of PGE, both the silver and grass carps achieved gonadal hydration, with a particularly

marked increase in body girth in the silver carp (tank 1). From the limited results so far obtained, it appears as if the use of PMS together with SRGN and HCG, and possibly by making greater use of mammalian prolactin LTH (Blüm, 1972) or even steroids (Jalabert, 1976) in promoting gonadal hydration, may possibly induce the Chinese carp species to ovulate during early summer without the use of PGE.

The present results further indicate that in the case of the grass carp, at least one successful spawn was obtained without the use of a starter dosage. This implies less handling of the spawner and a reduction in handling stress. This step is of considerable importance for the survival of spawners. This was found previously (Schoonbee, *et al,* 1978) and it was also indicated during the present spawning programme, that some of the female spawners did not survive after completion of the spawning trials.

In both the grass and silver carp specimens no spawn was obtained when only PGE was used (tank 3). In countries like Russia, Taiwan, Hungary and Israel it was, however, found that ovulation of the Chinese carp species could be effected through the use of PGE only, especially during the late summer season (Lin, 1965; Vinogradov, 1968; Antalfi & Tölg, 1971; Konradt, 1968; Rothbard, *et al,* 1978). Although the experiments using only PGE were unsuccessful, it may nevertheless be possible to achieve success with PGE, if a late summer spawn could be induced during December–January each year.

Conclusion

The present series of experiments in which the Chinese grass and silver carps were spawned following injection with mammalian FSH and LH hormones as well as pituitary gland extract of the common carp, provided favourable spawning results with the grass carp. An excessive hydration of the gonads occurred, however, in most of the silver carp female spawners, resulting either in partial spawns (due to blockages of female genital openings, by material derived from the gonads, mixed with the loosened eggs) or in a release of considerable amounts of body fluid together with the eggs. Such eggs, when fertilized, usually developed only for a few hours before dying. It is recommended that in the case of already partially hydrated female spawners and especially the silver carp, the dosages of hormones and pituitary gland extract be reduced to minimize this problem.

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