Techniques and hatchery procedures in induced spawning of the European common carp, *Cyprinus carpio* and the Chinese carps *Ctenopharyngodon idella, Hypophthalmichthys molitrix* and *Aristichthys nobilis* in Transkei

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Abstract

Techniques followed in the induced spawning of the European common carp as well as the Chinese grass, silver and bighead carps under conditions in Transkei are described. Experience showed that procedures developed to spawn these fish elsewhere in southern Africa required further adaptations to suit local environmental requirements.

Introduction

Since the establishment of a hatchery at the Umtata Dam in 1980, the Chinese grass and silver carps as well as the Aischgrund and Dor 70 varieties of the common carp have all been successfully reproduced by means of induced spawning. In November 1982, the bighead carp was artificially spawned for the first time in southern Africa at the Umtata Dam Fish Research Station [Prinsloo and Schoonbee, 1983].

This paper concentrates on procedures involved during the artificial spawning of the three Chinese carps in Transkei. Reference is also made to the techniques employed in the induced spawning of the common carp, *C. carpio*. As a result of differences in climatic conditions at Marble Hall, where the Chinese grass and silver carps were first spawned [Schoonbee *et al.*, 1978; Brandt and Schoonbee, 1980] and Umtata, Transkei, the hormones used, as well as the application thereof had to be adapted to obtain successfull spawns.

Pond Rearing of Brood Stock

Spawners of all the fish species under consideration were kept in special brood stock ponds and in polyculture at densities varying from 1 700 to 2 000 fish per ha. Although this figure is high compared to the densities suggested by Woynarovich and Horváth [1980], limited pond space necessitated this procedure. In order to avoid pond spawning of fish, especially by the common carp, males and females of all species were separated in August [late winter] prior to the onset of the breeding season. Specimens of the Dor 70 and Aischgrund varieties of the common carp were both specially branded in order to avoid possible confusion of broodstock during the spawning programmes. The spawners were kept under optimal conditions with pelleted fish feed provided *ad lib* by means of a demand feeder. The water from the higher-lying experimental ponds, which had already developed phyto- and zooplankton, was regularly released into the brood stock ponds. Freshly cut kikuyu grass was added daily as supplementary feed for the grass carp. No problems were experienced with oxygen deficiencies in the ponds. The condition of the fish of all species concerned was excellent throughout. A good example of the condition of the ponds was reflected by growths obtained for the bighead carp, where some spawners reached a biomass of 6,6 kg in only 3 years, while common carp grew to 3-7 kg in size and the grass and silver carps reached biomasses of 2-7 kg over the same period.

Water Quality of the Broodstock Ponds

Water samples for chemical analysis were taken from the different ponds during the early morning hours, usually not later than 09h00. As a result of the application of lime and the manuring of ponds, the electrical conductivity of the water was found to increase from 50 μS/cm at the point where the raw water entered the pond system, to values fluctuating between 220-280 μS/cm. Values for pH were found to fluctuate widely from 6,5 to 9,6. These extreme values in pH could be ascribed partly to algal activity in the ponds. With the exception of ammonia, organic loads as reflected by nitrate and phosphate were not abnormally high, considering the constant addition of cattle manure to the pond water. Even though ammonia values were on isolated occasions found to be abnormally high [maximum 7,194 mg/ℓ] no fish kills were experienced. The fact that a fish polyculture system existed in the broodstock ponds where benthic, algal, zooplankton, detritus and macrophyte feeders were present, might have played an important role in maintaining the balance of pond conditions. Values obtained for alkalinity [40-80 mg/ℓ], calcium and total hardness [32-64 mg/ℓ] showed the water in the brood-stock ponds to be well buffered compared to the raw water which entered the system.

Selection of Spawners

In conformity with established procedures of research in South Africa and overseas, female spawners were selected on the basis of a swelling of the gonads as well as a reddening of the genital opening. [Schoonbee *et al.*, 1978; Sinha, 1980; Woynarovich and Horváth, 1980; Rothbard, 1981]. In South Africa, these criteria are valid for the common carp and for females of the Chinese carps, which yield the best results during spawning when in this condition. In the case of the Chinese carps, well-hydrated gonads in females are usually a sign of over-ripeness of the eggs.

Once selected, spawners were immediately transferred to the hatchery and put into 1 000 ℓ tanks provided with lids or nets which prevented the fish from jumping out. The spawners were then treated for 3-6 h in well aerated water containing 40 mg/ℓ formalin and 0,05 mg/ℓ malachite green. After this treatment, each female was weighed and then transferred to special holding tanks supplied with a throughflow of water and aeration. Although the ideal situation is to keep one female spawner per 1 000 ℓ tank, 4-5 females were usually kept together without any deleterious effects. The fish holding tanks in the hatchery were then covered with net material which was tightly secured around the perimeter to prevent the fish from jumping out. Furthermore, the surface of each tank was darkened to prevent the fish from being disturbed by movements in the hatchery.

Injection Programme

In contrast to the injection programme followed by Schoonbee *et al.* [1978] and Brandt and Schoonbee [1980] for the grass and silver carps, where use was made of a combination of human chorionic gonadotropin [HCG] and carp pituitary gland extract [PGE] administered in two dosages, carp PGE only was found to be just as successful in the induction of ovulation of both the European common carps and the grass and silver carps in Transkei. The technique which proved successful here for the Chinese carp species, viz. two fractional injections of carp PGE applied eight hours apart, corresponds to some extent to that followed at the Gan Shmuel Fish Culture Station in Israel [Rothbard, 1981] and at the State Research Institute of Lake and River Fisheries, Leningrad, USSR [Konradt, 1968].

Alcohol or acetone preserved pituitary glands removed from mature male and female common carp at the onset of the spawning season when they had not yet spawned, were first dried for 15 min on filter paper after which they were homogenized in a small volume of 0,9% NaCl solution in a specially designed glass homogenizer. Freeze-dried carp pituitary gland was also used. In order to minimize stress, care was taken to keep the volume of the solution as small as possible and not to exceed 3 mℓ in volume of dosage per fish. Each dosage was applied with a clean sterilized syringe. Injections were usually made intramuscularly, and in the case of the Chinese carps, at the base of the dorsal fin where no scales occur. Injections were made 8 h apart.

The relative dosage strengths of each of the two injections were the following: First injection: 0,2 gland per kg recipient female. Second injection: 1 gland per kg recipient female. In both cases the concentration of the gland was based on the mass equivalent of the PGE donor and recipient female.

Usually males were injected once, simultaneously with the application of the second dosage of the females and with a concentration of 1 gland from a 1 kg donor per male, irrespective of the biomass of the recipient male. This was done to obtain a thinning response and easier release of the semen by the males when required for the fertilization of the eggs.

Ovulation and Fertilization of the Eggs

Although the approximate interval between the final injection and the anticipated time of ovulation of the females of the various carp species could be pre-determined, the females were kept under constant observation for specific signs indicating that they were ready to be stripped of their eggs. Females ready for spawning commenced swimming actively around in the tanks and usually approached the surface of the water in the vicinity of the water inlet. At the time of spawning, such females remained near the surface and splashed with their tails. It is important that the stripping of the eggs takes place within half an hour of ovulation in order to prevent overripeness of some of the eggs, which are then not fertilizable [Prinsloo and Schoonbee, 1983].

In the case of the Chinese carps, ovulating females were removed as soon as the first few eggs were released spontaneously or upon slight pressure of the abdomen. A certain sign that a female is ready for spawning is the onset of a slight trembling of the body which coincides with powerful contractions of the abdominal muscles and which may force the eggs out in a stream. Anaesthetics may be used to subdue large female and male spawners and facilitate handling during the stripping of the eggs and milt [Schoonbee *et al.*, 1978].

Female spawners were caught head first, in a tube-shaped scoop net and then laid tail upwards. In this way, the eggs, which at this stage had separated within the body cavity of the fish, were prevented from leaving through the genital opening. During this time a suitable male was selected for the stripping of the milt over the eggs immediately after they were discharged into a dry clean plastic bucket. Mixing of the eggs and milt was done with a soft rubber tipped cake spoon. In order to increase sperm activity and motility during the process of fertilization, a fertilizing solution, consisting of 2,4 kg urea/ℓ water, was mixed in with eggs and milt. The Woynarovich mixture, normally employed to remove egg adhesiveness in the common carp [Woynarovich and Horváth, 1980], may also be used to stimulate sperm activity during fertilization. The egg and milt mixture was subsequently stirred for approximately 30-60 s during which time fertilization of the eggs took place.

Rinsing of Eggs

In the case of the common carp the eggs become adhesive once they are released into the water. There are ways of removing or neutralising this adhesiveness [Woynarovich, 1962; Klotsch *et al.*, 1979; Soin, 1977; Woynarovich and Horváth, 1980; Woynarovich and Woynarovich, 1980; Rothbard, 1981; Schoonbee and Brandt, 1982]. The most recent methods tried for the common carp is that described by Woynarovich and Woynarovich [1980] and Rothbard, [1981]. In South Africa, Schoonbee and Brandt [1982] improved the method originally employed by Klotsch *et al.*, [1979] in which full-cream powdered milk at concentrations of 12-24 g/ℓ, is used to rinse the eggs. In this case small fat globules are formed around each egg which prevent the eggs from clumping. The recent improvement of the original Woynarovich method [Woynarovich and Woynarovich, 1980] where the concentration of the urea is increased to 20 gm/ℓ appears to be much more effective in the successful removal of the egg adhesiveness in the common carp. Both the milk rinsing technique and the Woynarovich and Woynarovich [1980] methods are at present being employed.

After removal of the egg adhesiveness, the eggs were first treated for 5-10 s in a 0,5 g/ℓ tannic acid solution. This was followed by the repeated rinsing of the eggs in tap water until the water used in the ringsing process became clear. This treatment not only toughened the eggs, but also assisted in preventing possible mould infections. Finally the eggs of the common carp were transferred to the breeding funnels in which they then hatched. The fertilized eggs of both the Chinese and European common carps were finally rinsed 3-4 times in tap water, preferably in water with a temperature close to that in the breeding funnels.

Hatching of Eggs in Funnels

After rinsing, the eggs were transferred to perspex-type breeding funnels similar in design to funnels normally used in Israel and in certain European countries. The funnels were connected to a water recirculation system, with water being released into the funnel from below. Considerable care was taken not to create too strong a flow of water as this damaged the delicate membrane which surrounded the embryo in the case of Chinese carps. Since the eggs of the Chinese carps are also pelagic, any strong flow of water through the funnels will result in the overflow and escape of eggs from the funnels.

In the present series of experiments where water temperatures ranged between 24 and 25 °C, the larvae of all three Chinese carp species hatched within 18 h. In the case of the common carp, larvae hatched only after 48 h at temperatures fluctuating between 22 and 23 °C.

After hatching, the larvae were allowed to migrate actively out of the funnels with the outflowing water through a connecting pipe into the larval holding tanks. These tanks were provided with fine mesh filters which prevented larvae from escaping but which still allowed recirculation of the water through the filter system. Inflow water was oxygenated by means of a shower-head spraying water into the tanks. No direct aeration was provided as this was found to disturb the larvae with the current it created and also caused problems during the feeding period of the larvae. Small air bubbles were mistaken by them for food, and such air accumulated in the stomachs of the larvae so that they floated on the surface of the tanks where they eventually died.

Concluding Remarks

Experiments with the holding of broodstock in special ponds show that the different species used for the spawning experiments can be kept in relatively large numbers without affecting the condition of the fishes concerned. However, it is felt that the density of fish kept as spawners should be reduced to the level recommended by Woynarovich and Horváth [1980], that is not exceeding 1 300 fish per ha.

The quality of the water used in the hatchery was improved by maturing piped water in an earthern pond before releasing it into the recirculation system of the hatchery. The best spawning season for the Chinese carps in Transkei appears to be the end of October – beginning of November [late spring – early summer]. [Schoonbee *et al.*, 1978; Brandt and Schoonbee, 1980; Prinsloo and Schoonbee, 1983]. Fish spawned earlier usually did not respond to hormone injections. On the other hand, when fish were spawned towards the second half of November, the eggs of a fairly large number of the Chinese carp spawners were already in an overripe stage. In cases where eggs were overripe, and the females injected with the necessary hormones, the eggs were released on slight pressure of the abdomen and contained a high percentage of ovarian fluid. A fairly large percentage of such eggs, which usually started embryonic development after fertilization, died off within 12 h of fertilization.

Although the injection programme for the common carp in Transkei is the same as that for the Chinese carps, the wide time margin within which ovulation takes place after the final injection of hormones shows this procedure to be less than satisfactory. The induced spawning method for common carp as employed by Schoonbee and Brandt [1982] shows a more consistent interval before ovulation, and is in consequence more satisfactory.

Rothbard [1981] showed that carp pituitary glands used in induced spawning in Israel can be removed at any time during the year. Our experience so far indicates that the carp pituitary gland, intended for spawning programmes, should be removed preferably during the early part of the natural spawning season. This substantiates the findings of Yashouv *et al.*, [1981]. Pituitary glands removed from mature fish can be preserved in either alcohol or acetone, or can be freeze-dried in order to minimize the deterioration in the gonadotrophic potency of the glands. All glands preserved in the ways mentioned, should be kept refrigerated, preferably at temperatures below zero in order to minimize loss of gonadotrophic potency (Yaron, 1983).

Where pond space and thus sufficient numbers of common carp donors are available, the use of PGE instead of HCG permits a considerable saving in costs. When an early season spawn is planned, the use of a combination of HCG and PGE as followed by research workers such as Schoonbee *et al.*, [1978] and Brandt and Schoonbee [1980] may be considered. It is not advisable, however, to use HCG during the latter half of the spawning season as this hormone tends to accelerate the maturation process and the consequent risk of overripening of the eggs.

Acknowledgements

The authors wish to thank the University of Transkei, the Rand Afrikaans University and the Department of Agriculture and Forestry of Transkei for equipment and pond space used during the growth of the various fish species. Our sincerest thanks are also due to the Departments of Co-operation and Development and Foreign Affairs of South Africa for financial support which made this study possible.

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