Observations on Some Techniques Employed for the Removal of Egg Adhesiveness of the Common Carp, *Cyprinus carpio*, During Induced Spawning

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Abstract

Existing techniques for the elimination of adhesiveness of eggs of some fish species used in artificial fish spawning programmes were adapted and improved for the European common carp *Cyprinus carpio*.

Introduction

One problem associated with the large-scale spawning of the common carp, *Cyprinus carpio*, is the removal of the egg adhesiveness which develops when the eggs come into contact with water. This problem can be overcome by using a mixture of 3 g urea and 4 g NaCl dissolved in one litre of water. in which the fertilized eggs are rinsed for a period of 30 to 45 min (Woynarovich, 1962; Rothbard, 1978). This problem of glutinosity in fish eggs can also be overcome by using enzymes such as hyaluronidase and bacterial alkaline proteases (Soin, 1977; Horvàth, 1979, 1980), or by coating the fertilized eggs with an inert powder such as talc, which prevents the eggs from aggregating, once they are released into water (Klotsch *et al.*, 1977; Soin, 1977). Several attempts were made to improve on the existing Woynarovich rinsing technique, as modified by Hulata and Rothbard (1979), by increasing the concentration of the urea used in the mixture. Alternative methods based on the experience of authors such as Soin, (1977) and Klotzsch *et al.*, (1977), by using fresh and powdered cow's milk, were also investigated during the present series of experiments conducted during the period 1979–1980.

Hatchery procedures

A number of 1 000 ℓ PVC plastic tanks, provided with running water and aeration were used to hold the male and female spawners in the hatchery after selection at the ponds on their ready release of sperm and gonad development, respectively. All spawners were treated for 1 – 3 h with a dosage of 25 ppm formalin (40%) and 0,05 mg/ℓ Malachite green (Leteux and Meyer, 1972). Female spawners were kept separately from the males in order to avoid natural spawning in the tanks. At the onset of the experiment the male spawners were all injected with carp pituitary gland extract (PGE), irrespective of body mass, for the purpose of inducing a semen thinning response, thus facilitating the release of the semen (Clemens and Sneed, 1962; Clemens and Grant, 1964, 1965; Rothbard, 1978).

The female spawners were all injected with PGE in two dosages, 9 h apart. The total dosage strength followed was one gland per female spawner applied at the ratio of 1/3 and 2/3 gland per dosage. The mass ratio of the donor fish and female spawner was 1:1 respectively. In the case of any deviations in the mass of the spawner from that of the donor, the dosage strength of the PGE was adjusted accordingly. In order to obtain the highest potency in gonadotrophic hormones, the carp pituitary glands used were collected from mature females at the onset of the spawning season (Yashouv *et al.*, 1968) and preserved in absolute ethyl alcohol, (Shehadeh, 1975). Each gland was homogenized in 1 mℓ of 0,9% sodium chloride solution. Injections were made intramuscularly, usually dorsally to the pectoral fin. Female spawners were ready to spawn 10 – 11 h after the last injection.

Collection and Preservation of Semen

Six hours after injection with PGE, the males were anaesthetized in 200 mg/ℓ MS 222 (Sandoz) for 2 – 3 min. They were then thoroughly towel-dried, in order to prevent water contaminating the sperm, and the semen obtained from each male by gentle stripping. A similar procedure was followed for the females at the time of spawning. Volumes of 1 – 9 mℓ of sperm was obtained from males weighing 1 – 2 kg. During spawning trials in 1979 some of the sperm collected was stored in a Wyonarovich solution in the ratio described by Hulata and Rothbard (1979). Use was also made of a 0.9% sodium chloride solution for sperm storage in the same ratio as that used for the Wyonarovich mixture. The semen collected in this way was kept in a thermos flask on ice at temperatures ranging from 0 – 3°C for 14 – 18 h before being used to fertilize the eggs. As a third alternative, fresh semen was directly stripped from male spawners onto the eggs at the time of spawning of the females.

Stripping of Females, Fertilization of Eggs and Removal of Egg Adhesiveness

The procedure followed in the artificial spawning of the females in the present series of experiments resulted in more than 90% of the cases to spawn successfully within 9 – 11 h after the last

fractional injection of PGE. The eggs were then stripped from the towel-dried anaesthetized females into dried, clean plastic bowls. Whereas in the 1979 trials, semen preserved with the Woynarovich solution and a 0,9% sodium chloride solution as well as fresh sperm, were used to fertilize different batches of eggs obtained from the same females, only fresh sperm was used in the 1980 trials. In all cases the eggs and semen were gently mixed with a soft rubber cake spatula for a period of approximately 3 min to enable fertilization to take place. After fertilization, the eggs were then rinsed with the appropriate egg-degumming solutions.

Fresh cow's milk, diluted 5 and 10 times, according to Soin (1977) and Klotsch *et al.*, (1977) as well as full cream powdered milk (11 g/ℓ of water) (Soin, 1977) and the Woynarovich mixture referred to above (Rothbard, 1978; Hulata and Rothbard, 1979) were used in the 1979 experiments to remove egg adhesiveness.

In the case of the egg rinsing procedure, the techniques as originally described by Woynarovich (1962) and later revised by Rothbard (1978), were closely followed. The correct volumes of the various milk solutions were made up at the onset of each spawning. After fertilization of the eggs an amount of 500 mℓ of milk solution was added and gently stirred for 30 s after which the entire volume of milk (8 — 10 ℓ of the diluted milk solution per 1 ℓ of eggs) was added and continuously stirred for a period of 35 — 40 min in each case. In all cases where milk was used in the rinsing process the eggs initially tended to stick together in small clumps but gradually separated towards the end of the rinsing period. All fertilized eggs, irrespective of the treatment, were then rinsed once or twice with tap water prior to the transfer of eggs to the breeding funnels. The eggs were then transferred to each of the glass hatching jars (Schoonbee *et al.*, 1978) or perspex funnel-type jars which received the surplus eggs. Waterflow through the funnels was adjusted to approximately 1 ℓ/s.

As a result of the unexpected clumping of some batches of eggs in the hatching jars, which occurred in each of the rinsing processes, special attention was paid during the 1980 trials to the uniformity of procedures followed for each specific rinsing process (Table 1).

Discussion of Results

Many methods have already been employed successfully elsewhere to induce the females of *C. carpio* to spawn by PGE and/or other gonadotrophic hormones such as PMS (Pregnant Mare Serum), (Tay, 1973; Woynarovich, 1975; Kausch, 1975; Kossman, 1975; Donaldson, 1975; Rothbard, 1978; Van der Merwe, 1981). The present authors found the prescribed procedure of one gland per female spawner of equivalent mass to that of the gland donor, applied in two fractional injections of 1/3 and 2/3 gland (9 — 10 h apart), a reliable method to induce spawning in the common carp and to obtain viable eggs under local conditions. However, experience showed that special attention should always be given to the gonad condition of the female spawners at the time of their selection at the ponds. Selection was based on an extended and soft belly as well as a swelling and reddening of the genital papilla, if complete success is to be obtained by the spawning method just described.

The various ways in which the semen was preserved and in which the eggs were subsequently treated in the removal of

TABLE 1
RESULTS ON THE DIFFERENT EGG DEGUMMING METHODS WITH THREE CONCENTRATIONS OF UREA (WOYNAROVICH TECHNIQUE) AND FULL CREAM POWDERED MILK WITH OBSERVATIONS ON EGG ADHESIVENESS AND THE PERCENTAGE SURVIVAL OF THE EMBRYOS UNTIL HATCHING[1]

Trial No. and Rinsing Method	Remarks on stickiness of eggs	Sampling periods and percentage survival recorded for embryos and larvae						
		6 h	12 h	24 h	36 h	48 h	60 h	72 h (hatching)
1. Normal Woynarovich (3 g Urea + 4 g NaCl/ℓ)	Eggs completely free	92	84	68	72	74	74	82
2. -do-	-do-	94	96	92	90	92	94	96
3. -do-	-do-	100	96	90	88	90	86	88
4. Woynarovich (4 g Urea + 4 g NaCl/ℓ)	-do-	96	100	90	86	84	88	86
5. -do-	-do-	100	92	84	96	84	86	94
6. -do-	-do-	98	96	92	94	92	94	94
7. Woynarovich (5 g Urea + 4 g NaCl/ℓ)	Some clotting in funnel.	96	92	92	88	84	90	94
8. Full cream powdered milk[2] (12 g + 1 g NaCl/ℓ)	Eggs completely free	84	88	56	74	62	62	54
9. -do- (18 g + 1 g NaCl/ℓ)	-do-	100	96	88	84	90	84	80
10. -do- (24 g + 1 g NaCl/ℓ)	Some clotting in funnel	98	96	98	96	96	94	92

[1]Fresh semen used during fertilization process

[2]Nespray (Nestlé) instant spray dried full cream milk powder

the adhesiveness from the eggs during the 1979 trials, did not allow for definite conclusions on the actual advantages that each of these procedures may have had on the eventual hatching success of the eggs. However, it was possible to make certain deductions from the 1979 experiments in order to adapt techniques and execute with success the removal of egg adhesiveness during the 1980 trials. These were:

- Refrigerated semen stored for 12 — 18 h in either the Woynarovich or 0,9% sodium chloride solutions, does not appear to be inferior to fresh semen used. These results confirm the findings of Hulata and Rothbard (1979).

- The results obtained furthermore suggest that fresh or full cream powdered milk was just as successful in the elimination of the adhesiveness of the carp eggs as the Woynarovich (Rothbard, 1978) rinsing process. Due to the varying quality of cream in fresh milk, depending on the breed of the cow and the time of season, etc., full cream powdered milk merited further investigation as a rinsing egg-degumming substance.

- The occasional and unexpected clumping of the eggs obtained during each of the different rinsing processes, pointed towards inconsistencies in the procedures followed. As a result of this, problems with *Saprolegnia* were encountered, which affected the eventual hatching success of such eggs.

The rinsing techniques for carp eggs followed during the 1980 experiments are summarized in Table 1. As can be seen from this table, an attempt was made to increase the concentration of substances responsible for either removing the egg adhesiveness (urea, rinsing methods 1—7), or isolating the eggs by means of fat globules present (full cream powdered milk, rinsing methods 8—10). With the exception of the eggs in trial 8 which had a 54% survival of larvae 72 h after fertilization, the results on the use of full cream powdered milk at concentrations of 18 and 24 g/ℓ were exceptionally good (80 — 94%). According to Huisman (1975) and Kossman (1975) the fertilization incidence considered as satisfactory for carp may range from 60 — 99%. According to the present results obtained, the fertilization success for all the Woynarovich type and powdered milk egg-rinsing solutions were therefore very satisfactory. The increased concentration of urea (4 — 5 g/ℓ) also did not appear to affect the fertilization incidence at all.

In those instances where clumping of the eggs did occur (trials 7 and 10), the problem was attributed to experimental error of a specific rinsing technique rather than to the technique itself. A close observation during the various rinsing trials in 1980 clearly pointed towards one ostensible deviation in these instances where clotting of the eggs did occur after rinsing. Here the final rinsing of the eggs with tap water prior to transfer to hatching jars was not repeated often enough to remove all the sticky substances released by the eggs. In these cases the rinsing water still had some traces of turbidity (milkiness) in it. Repeated washing of the eggs with tap water at the end of the stickiness removal process, clearly did not have any deliterious effects on the survival of the eggs. Even in the case of the milk rinsing processes, the necessity to remove all substances in solution from the eggs, was apparent. At the time of release of the eggs into the hatching jars there must be some flow of water in the breeding funnels in order to prevent compaction of the eggs, which may also initially contribute towards some kind of clumping of eggs in the jars. Experience showed that in both the Woynarovich as well as the milk rinsing processes, the more rapid stirring of the eggs during the initial 3—5 min facilitated the separation process of the eggs.

From the results it can be concluded that both the Woynarovich egg-degumming technique employed by Rothbard (1978) and modified by the present authors (4 — 5 g urea/ℓ) as well as the full cream powdered milk rinsing technique (Soin, 1977) as modified by the present authors, (18 — 24 g/ℓ), were both equally efficient in the separation of the eggs of the common carp. The full cream powdered milk technique is however, preferred, partly because of its general availability and the ease in which it can be prepared, and partly because this process does not interfere with the structure of the egg membrane which may be the case when the Woynarovich and/or enzymatic methods are employed. Huisman (1975) is of the opinion that substances used in the Woynarovich technique may be toxic to the eggs of some fish species. His findings are substantiated by Horvàth (1979, 1980) and Schoonbee *et al.*, (1980) in the cases of *Silurus glanis* and *Clarias gariepinus*, respectively.

Since the completion of the present experiments at the Marble Hall Fisheries Research Station, the full cream powdered milk rinsing technique (to isolate the carp eggs from each other for the purpose of the large scale laboratory spawning of fish) was not only repeated with success in 1981, but was also successfully applied to *C. gariepinus* (Schoonbee *et al.*, 1980) and the largemouth yellow fish, *Barbus kimberleyensis* at the Lydenburg Fisheries Institute, Transvaal.

At the time of the present series of experiments on techniques for the removal of egg adhesiveness in carp, Woynarovich and Woynarovich (1980) further adapted the technique (originally described by Woynarovich, 1962) by increasing the concentration of urea in the solution from 3 g to 20 g. By first rinsing the eggs in the original solution containing 3 g urea and 4 g NaCl/ℓ for 3 — 4 min and then transferring these eggs to the solution containing 20 g/ℓ urea and 4 g NaCl, it was found unnecessary to stir the eggs, but only to replace the solution once every 10 — 15 min in order to remove the egg adhesiveness. The latter technique has since also been employed with success in Israel by Rothbard (1981) and will be tested during the 1982 summer season under local conditions.

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