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Stickiness Elimination of Himri Barbel (*Barbus lutes*, Heckel) Eggs

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Abstract

Various treatments were applied on himri '*Barbus luteus,* Heckel' eggs to eliminate adhesiveness and attain good swelling by using inexpensive materials. Observations of treating by tannic acid; carbamid in saline; powdered milk dissolved in saline; and sodium sulfite then tannic acid on hatching have been recorded. Use of tannic acid after completing the fertilization showed better results in observed parameters namely time spend for desticking, swelling and treating cost of chemicals.

Key Words : Barbus luteus, adhesiveness, gamete management, Euphrates-Tigris.

Introduction

Progressive efforts to develop himri barbel (*Barbus luteus,* Heckel) culture and to sustain its abundance in natural fisheries of Mesopotamian Rivers could be improved by controlling and developing gametes management procedures.

Eliminating egg stickiness is one of the most critical treatments in artificial reproduction of himri which reduce adhesive effect of cortical envelope to avoid mass adhesion. These treatments enable the using of incubators, enhance embryonic development, rise hatching, and facilitate controlling and effecting of ambient conditions.

Mass accumulation occurs because of specific composition of the vitelline envelope (V.E.). In most cyprinids, the outermost layer made up of two parts identified by their cytochemical differences in carp ,*Cyprinus carpio,* the two outer-layers are rich in protein and contain acid-phosphatase activity (Kudo, 1982a,b). The composition of external layer is responsible for the great stickiness of carp eggs which occurs after eggs are immersed in fresh water but not in saline solutions (Billard *et al*., 1986). Better understandings to V.E. composition and biochemistry during fertilization and embedding in aqueous media can facilitate desticking techniques and consequently the propagation.

Vitelline envelope's proteins could be dissolved in biased pH media with or without saline solutions when acid anions calibrate proteins to sedimentation (Woynarovich 1962, 1964, 1975; Woynarovich and Woynarovich 1980), organic dissolvent in unheated conditions (Soin, 1976; Horváth, 1978), and by enzymes (Horváth, 1980; Linhart *et al*., 2003; 2000). Simultaneously, many procedures try to cover the VE by using inert materials instead of dissolving such as powder milk (Soin, 1976).

The aim of this study was to examine procedures used previously with some fish eggs to eliminate the egg stickiness of himri. Herewith, the faster; less expensive and most suitable procedure for desticking and water hardening could be determined.

Materials and Methods

Mature males and females were obtained from Euphrates River in May, transported to Mreaiya hatchery farm in Deir ez Zor (Figure 1), and propagated following the method of Al Hazzaa and Hussein (2003) in 20-22^{o}C and 8 mg.l^{-1} DO in the water.

Eggs were collected from 4 females and mixed with milt collected from 4 males in dry conditions, then separated into four batches (n=3000-3500) each fertilized and treated with specific treatment as mentioned below then incubated in Zuger jars. Micrometer eye-piece built on stereomicroscope used to measure egg's diameter. In course of estimating expenses of such procedures in mass-production; total costs of chemicals were calculated for each treatment treating one liter of eggs.

First treatment

Saline of (3 g NaCl.l^{-1}) was added to the first milted egg batch at ratio of 100 ml saline: 1 kg egg mass. After mixing for 2 min, eggs were rinsed four times in water for 10 min to start hardening, rinsed in tannic acid (1 g.l^{-1}) for 15 sec then washed with hatchery water and incubated.

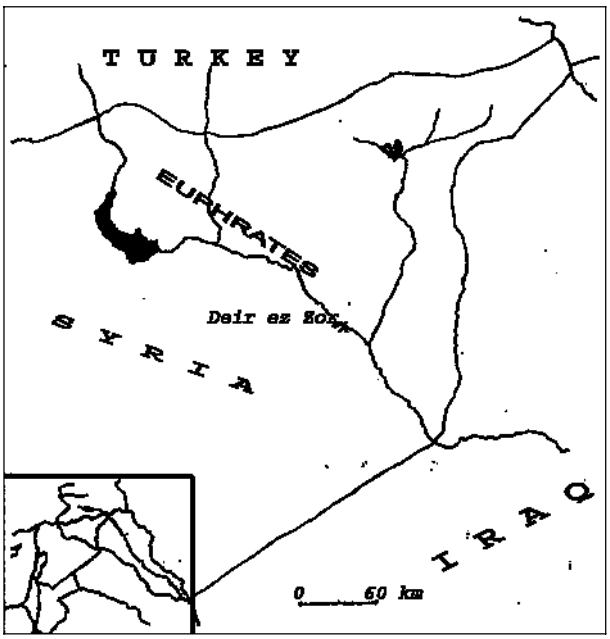

Figure 1. Experiments site at Mreaiya fish hatchery farm nearby Deir ez Zor.

Second treatment

After mixing of eggs with milt; they were rinsed with a solution of 4 g NaCl + 3 g carbamid per liter of water. This solution was added up to 10% of the eggs over it for two minutes with constant slight stirring for few minutes. Thereafter, changed many times with covering fresh quantities of the same solution and stirred continuously for 30 min, then let constant in new covering quantity of that solution for another 30 min changed many times. Hardening development was measured through treating.

Third treatment

Sperms mixed with eggs have been initiated by using saline (6 g $NaCl.l^{-1}$) for two minutes, then a composition of: (10 g full cream powder milk "Nido®, from Nestle" + 1.2 g NaCl) per liter of water added to cover fertilized eggs in the tray which were stirred softly and continuously for 40 minutes. Desticking solution has been changed whenever sufficient turbidity observed. Free egg mass washed with hatchery water and incubated in Zuger jars then.

Fourth treatment

After mixing eggs with milt; fertilization solution (4 g $NaCl.l^{-1}$) was added eggs rinsed in aqueous solution of sodium sulfite (15 $g.l^{-1-}$) for 5 min with constant stirring. This solution covered the egg mass slightly in the tray and changed two times trough that 10 min, then decanted and washed to add tannic acid (1 $g.l^{-1}$) twice for just 10 sec for each time with stirring. Eggs washed thoroughly in hatchery water and incubated in jars.

Statistical analysis

Experiment designed in randomized complete blocks (R.C.B.D.) considering each treatment as independent block with three replicates. Results of stickiness and swelling in each treatment analyzed using analysis of variance (ANOVA).

Results

High fertilization percentage could be attained but hatching didn't increased more than 58% as reported in Al Hazzaa and Hussein (2003) after about 62.5 degree-day of incubation. Clutches of the adjoined eggs in doubles and more recorded in Table 1 as median of percentages. Water swelling of fertilized eggs started after 5-10 minutes of applying the different treatments and completed about one hour later by reaching the maximal diameter as shown by each treatment in Table 1. ANOVA showed no significant differences between treatments in eliminating egg's stickiness. Cost of treating one liter of eggs was less for tannic acid than powder milk. Carbamid in saline was the highest.

Discussion

Hatching didn't affected by treatments which assure that the used chemicals in mentioned concentrations weren't toxic or hatching-hinder. Low survival rate of the incubated himri's eggs couldn't be explained, but the similar problem noticed in bunni *Barbus sharpeyi* and gattan *Barbus xantopterus* (Pyka

Table 1: Suitability of different treatments: (A) tannic acid; (B) carbamid in saline; (C) powdered milk dissolved in saline; and (D) sodium sulfite then tannic acid in stickiness elimination; water-swelling; time consumed to perform desticking and hatching. Treating cost in (A) considered 1.

Treatments	Fertilization (%)	Adjoining pairs (%)	Swelled egg diameter ($x\pm$ S.D.)	Time (min)	Cost	Hatching
A	98.6 - 97.8	8	2.19±0.18	2.25	1	57.3 ± 2.2
B		0	2.29±0.14	62	2.22	56.5 ± 1.4
C		4	2.28±0.19	42	6.65	56.8 ± 2.8
D		14	2.45±0.15	7.30	24.44	56.2 ± 2.0

et al., 2001) of the same genus and habitat. Such observations could approved that factors affecting egg quality are determined by intrinsic properties of the egg itself and the environment in which the egg fertilized and subsequently incubated and other physiological variables (Brooks *et al.*, 1997; Schreck *et al.*, 2001), or by the females producing these eggs (Dlaboga *et al.*, 1998; Papała *et al*., 1998; Marteinsdottir and Steinarsson, 1998; Pauly and Pullin, 1988).

In many fish species; fertilization envelope's outermost layer after being swollen in water for 0.5-1 h appears as a homogenous sticky layer (Vorobiyova *et al*., 1966) which will cause mass mortality in fertilized eggs due to inconvenient development conditions in hatchery incubators. Stickiness elimination of himri's eggs raised the predicted hatching percentage in all treatments to convenient rates which may be raised more by incubating in another types of incubators.

Because of the osmotic gradient, water flows in and subsequently the volume and weight of the egg increases until an equilibrium with the tension of the chorion is reached (Alderdice, 1988) which increased viability and protection of embryo. Eggs of high quality had significantly higher water uptake rate than did poor quality eggs (Lahnsteiner and Patzner, 2002). Himri eggs showed good water-hardening and increased in diameter from 1.10-1.18 mm (dry diameter) to 2.19-2.45 mm (wet diameter) comparing with 1-1.5 mm (dry diameter) to 2-2.5 mm (wet diameter) in common carp (Horváth and Lukowicz, 1982; Horváth *et al.*, 1985; Coche and Muir, 1998). Lahnsteiner *et al.* (2001) considered that the well water-hardening in many cultured cyprinid's eggs could be a good bio-marker for egg quality.

Since no significant differences could be found between the chemical treatments in eliminating the stickiness and affecting the swelling of the treated eggs in these treatments; the best treatment could be determined according to the time spend to perform the treatment and its cost when using in mass production. In our experiment, we noticed that using of tannic acid solution was more appropriate in required time, cost and desticking hirmi's eggs than the other treatments without significant differences in water-swelling.

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