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**محاولات تفريخ اصطناعي في البني الحمري**  
**(*Barbus luteus*, Heckel)**

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ملخص

استخدم الحقن بمستخلص نخامة الكارب العادي CPE في البني الحمري *Barbus luteus*, Heckel) قبل شهر و نصف من صرفه الطبيعي. أعطت الإناث المعاملة بحقنيتين متتاليتين خصوبة نسبية أعلى ( $7464.96 \pm 481$ ) بيضة من تلك المحقونة مرة واحدة ( $5286.62 \pm 392.6$ ) بيضة. لم تلاحظ فروق معنوية بين الذكور للمعاملة ( $22.5 \pm 0.4$ ) ملل سائل منوي و تلك غير المعاملة ( $23.2 \pm 1.6$ ) ملل. لم ترفع نسبة الإخصاب العالية (98.6%) من نسبة الفقس التي لم تتجاوز (57%). لوحظت تبدلات لونية و إطلاق أصوات خلال فترة الاستعداد للصرف. كان وزن البيضة الواحدة  $1.57 (\pm 0.40) \times 10^{-3}$  غرام بقطر 1.18-1.10 ملم. زادت حركة النطاف معنوياً (145%) في محلول ناقص التوتر 150 مللي أوزمول  $\times$  كغ<sup>-1</sup>. كان التطور الجنيني مماثلاً لتطور الشبوطيات الأخرى بالتوقيص الزمني، إلا أن المرحلة المميزة بتلون العين لم تلاحظ في الحمري إلى ما بعد الفقس الذي حصل بعد 74 ساعة.

# Propagation attempts in himri barbel (*Barbus luteus*, Heckel)

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## Abstract

Carp pituitary extract (CPE) injections applied to himri (*Barbus luteus*, Heckel) month and a half before its natural spawning. Females treated with two consecutive injections gave higher relative fecundity ( $7464.96 \pm 481$  eggs) than those treated with one injection ( $5286.62 \pm 392.5$  eggs). No significant differences noticed between treated ( $22.5 \pm 0.4$  ml) and non-treated males ( $23.2 \pm 1.6$  ml). High fertilization (98.6-97.8 %) could not raise hatching more than (57 %). Colors modifications and acoustic acts have been observed during ripping. Spawned egg has  $1.57(\pm 0.40) \times 10^3$  g weight and 1.10-1.18 mm diameter. Spermatozoon motility increased significantly (145 %) in hypotonic solution ( $150 \text{ mOsm.kg}^{-1}$ ). Embryonic development was similar to other cyprinids in durations, but the important stage of eye pigmentation has not been noticed even after hatching which occurred after about 74 h.

**Key words:** *Barbus luteus*, propagation, genital products, embryo, Mesopotamia.

## Introduction

Himri (*Barbus luteus*, Heckel) is endemic cyprinid in Mesopotamian Rivers system. Beckman (1962) reported its presence in Orontes River, but that hasn't proved again.

Some signs and observations indicate to promising possibility of being used for aquaculture in polyculture ponds, since it is herbivorous and detritus feeder (Epler *et al.*, 2001a). Adaptation to earthen ponds with common carp *Cyprinus carpio* and other cyprinids has been noticed when it entered accidentally with water flux to ponds nearby the Euphrates (Al - Daham *et al.*, 1991) which could consider it as a new species for the aquaculture practices in spite of clear decrease in body growth after the second year of life (Szupla *et al.*, 2001) Their tasty scented flesh raises popular demand on this fish.

Few investigations have been performed to investigate himri's reproductive biology. It matures within 1 - 2 years of age at 14 cm average total length (Epler *et al.*, 2001b). Spawning in middle and lower Euphrates occurs between June and July among waterweeds and roots of the floating plants. It has high fecundity and extended ovulation period for many days, so Al-Daham and Bhatti (1979) describe it as partial-spawner. Histological gonad analysis of both sexes showed annual changes leading to extended spawning season (Epler *et al.*, 2001b; Al-Daham and Bhatti, 1979; Bhatti and Al-Daham, 1978) whereof increasing the total fecundity of himri.

The aim of this study is to examine the response of himri to exogenous hormonal induction by common carp pituitary extract (CPE) for simple propagation practices to be used in fish hatcheries, some specifications of gametes, and embryonic development. All these studies performed with non-complicated techniques can be used in field.

### **Materials and Methods**

Experiments were performed in Mreaiya fish farm hatchery on the left bank of Euphrates River, Deir ez Zor, Syria (Figure 1) with river water heated by solar energy from 4 to 11 May.

#### **Animals**

Sexually mature females (n=10) [355-395 g body weight (W), 23.2-27.5 cm standard length (SL)] and males (n=9) [110-413 g (W), 21.8-26.5 (SL)] were caught by gill net (4x4) cm from Euphrates River and transported to the hatchery farm. Acclimation with hatchery water was adjusted to (20-22°C) temperature in (6.5-7.5) pH.

#### **Induced spawning**

Sex and weights of broods were determined, tagged and divided into two groups. Broods in group I (G. I) injected with CPE at 3 mg.kg<sup>-1</sup> Body weight (BW) in 1 ml saline as preparatory hormone treatment. After 10.5 h of the injection of G. I broods, genital papillae of all females sutured and the decisive injection at 8 mg.kg<sup>-1</sup> BW in 1 ml saline was applied just for females in G. I. Females of G. II were given 8 mg.kg<sup>-1</sup> BW in single injection. Males of G. II were treated just with saline. All injections were intraperitoneal under the base of pectoral fins. Broods were stocked together at 20-22°C and 2.5 l.min<sup>-1</sup>.kg<sup>-1</sup> BW. After 520-540 degree-hours (dh) since first injection; fish were stripped by slight abdominal pressure. Estimations of fecundity were accomplished and eggs fertilized in dry conditions by adding 25 ml milt to 1kg eggs.

Fertilized eggs swelled, desticked (Al Hazzaa and Hussein, submitted), then put into Zouger jars of 8 l. Water flow adjusted to 0.5-1 l.min<sup>-1</sup>, temperature and dissolved oxygen (DO) measured at hourly basis by using the oxygen-meter W. T. W. type OXJ-92. Turbidity and salinity of

water estimated by Yellow Springs 6600 multi parameter at the beginning of the experiment. Malachite green used as fungicide.

### **Embryonic development**

Developmental stages of fertilized eggs were observed and examined under stereomicroscope (4-7×) and microscope (10, 20, 40×). Observations were documented in hourly basis and categorized according to distinguished development intervals.

### **Gametes**

Egg (n=40) diameter was measured by micrometer eyepiece attached to stereomicroscope and weight by quantitative analysis on electronic balance (Denver). Milt volume was measured for each male. Sperm viability was estimated in distilled water and saline (3g NaCl.l<sup>-1</sup> dist. water). This saline raised osmotic pressure of water to 150 mOsm.kg<sup>-1</sup>.

Duration of motility and stages of viability examined under microscope on 60× and 100× after 10 seconds of motility initiation. Viability was divided into three stages of mobility: fast forward (FF), rotational waves (RW), and then the last stage starting by tail immobility and vanished swinging in head till quite immobility (VD).

## **Results**

### **Induced spawning**

By watching the spawning behavior, ripping irritations of broods were noticed gradually about 10 h after second injection (the single injection in G. II) with some chafes among males and females. Body color of males turned to reddish brown in anterior body part and greenish around caudal peduncle. Females were less pigmented than males. Acoustic behavioral changes were observed in males during ripping. Ripping males emitted short and sharp pulse trains accompanied with springs over pond's water. These sounds were not aggressive behavior within or between sexes. Fertilization rate rose over 98.6 %, but hatching still less than 57.3 %. Statistical analysis showed significant effect of two injections on female fecundity ( $P < 0.001$ ) since female's mean fecundity estimations were 11.72 g.kg<sup>-1</sup> BW in G. I and 8.3 g.kg<sup>-1</sup> BW in G. II in spite of the high quantities of unspent ova seen after post-spawning dissections of ovaries. No differences in milt production were observed between induced and non-induced males ( $P > 0.1$ ) as shown in (Table 1). Additional milt could be collected for second and third time after 6-10 h but decreased in volumes gradually.

### **Embryonic development**

Embryonic development is defined in (Table 2); where embryos developed to final formation outward after 64 h of fertilization. Hatching

started after 74 h of fertilization in 20-22°C; 6-11 mg.l<sup>-1</sup> DO; and neutral pH, but embryo's eyes still without pigmentation (Figure 2).

#### Gametes

Unfertilized eggs were demersal, spherical, adhesive and dark yellow in color with mean weight of  $1.57(\pm 0.40) \times 10^{-3}$  g and size range of 1.10-1.18 mm.

Milt look homogenous milky in color with mean pH value of 8.5. Viability of sperm rose up to 145 % in hypotonic solution (150 mOsm.kg<sup>-1</sup>) comparing with distilled water (Table.3).

#### Discussion

Himri showed response to exogenous stimulation by pituitary extract which appeared by accelerating gametogenesis and ability to spawn one and a half month before its natural spawning season. Emission of vocalizations by males near the incoming spawning time referring to acoustic mating behaviour in himri, but it has failed to make females release eggs due to suturing genital papilla. Such behaviour has been noticed in other species during spawning interactions in sand gobies *Pomatoschistus marmoratus*, *P. canestrini*, and *Knipowitschia panizzae* (Lugli and Torcceli, 1999), mating in *Hypoplectrus unicolor* (Lobel, 1992), before and during oviposition (Bisazza *et al.*, 1989). This act may be positively affecting gamete production of both brood genders. It is recommended to observe the natural spawning of himri in natural habitats or mimicking conditions to discover the details of spawning acoustic behavior and its relation with female fecundity and spawned eggs. These acts could be manipulated to enhance total gamete production in propagation practices. However, Lugli *et al.* (1996) found that not all prespawning sounds may be a functional component of the spawning behaviour such as the male Arno goby *Padogobius nigricans*.

Significant differences in fecundity between females received two doses of CPE of the priming dose lesser than one third of the last one and females received single dose indicates to use the CPE in two doses to attain good results in oocyte maturation, ovulation, and fecundity. The priming dose induces a significant GtH increase one hour after the injection and then GtH levels continue to increase for 3 hours and remain high during the next 12 hours (Weil *et al.*, 1980). Main exogenous stimulation to induce the spawning in some cyprinids could be the second injection, in other cyprinids it could be unique and massive (some Chinese carps). We found that application of consecutive CPE doses as injected stimulator in himri raised female fecundity more than single dose. Similar results have been reported in some other cyprinids such as Indian major carps *Catla catla*, *Labeo rohita*, *L. calbasu* *Cirrhinus mrigala* (Jhingran, 1986); Chinese carps *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, and *Aristichthys nobilis*, (Shigang, 1989); tench

*Tenca tenca*: (von Lukowicz et al., 1986); nase, *Chondrostoma nasus* (Szabó et al, 2002)

Himri eggs are slightly different in size and weight which could be interspecific differences observable between individuals according to their age and weight. Differences in egg size in many fishes have been related to spawning season (Bagenal, 1971; Papala *et al.*, 1998), fish individual size (Bartel *et al.*, 1999; Bonisławska *et al.*, 2000; Marteinsdottir and Steinartsson, 1998), or brood protection (Filney and Hecht, 1993).

Sperm production in males was not effected by CPE injection. However, males have produced in a single stripping more milt than Horváth *et al.* (1985) described for the common carp.

Collection of additional milt quantities and the high quantities of unspent ova indicates that application of CPE in himri propagation were not highly satisfactory. Thus, all future attempts to propagate wild himri broods should consider that it is a partial-spawning species like the bunni barbel *Barbus sharpeyi* from the same habitat of himri (Al-Daham and Jasim, 2000). Balon (1995) described that the wild common carp was portional spawner in Danube, Volga and Amur Rivers. Adoption of himri with ecology of fish-culture's ponds could modify its sexual behavior as modified in domesticated common carp.

Embryonic development outlines in himri look identical to other cyprinids reported by Peňáz (2001) from first cleavage to gastrula and organs formation, but appearance of optical vesicle without pigmentation till several days after hatching seems to be specific. Embryonic development calendar of himri was almost same as bunni barbel described by Pyka *et al.* (2001) at the same temperature but absence of pigmentations in optical vesicle were specific for himri.

Fertilized eggs of himri hatched after about 64 degree-days (dd) in well oxygenated water (7-9 mg.l<sup>-1</sup>). This is close to incubation period of bunni: 60 dd (Al-Nasih, 1992) to 70 dd (Pyka *et al.*, 2001), common carp: 60-70 dd (Coch *et al.*, 1998); and tench 60-70 (von Lukwicz *et al.*, 1986).

Low hatching rate of himri's eggs couldn't be explained, but the similar problem noticed in bunni and gattan *Barbus xantopterus* (Pyka *et al.*, 2001) from same genus and habitat. Using other incubation techniques with investigating microbial loading of eggs and other ambient incubating conditions could raise hatching rates. Variations in egg dimensions could affect developmental strategy and hatching percentage in himri.

Himri's spermatogenesis is generally similar to other teleost species (Bhatti and Al-Daham, 1978). Spermatozoa of many fish species including himri are imotile in the testes and the genital tracts. They are activated only after release into external medium and have a short period of motility (Scott and Baynes, 1980). It was shown that a simple hypotonic shock of 60-100 mOsm.kg<sup>-1</sup> amplitude could activate common carp's sperm (Billard *et*

al., 1995). But dissimilarities of osmotic pressures in aqueous media according to variant concentrations of dissolved particles originate different responses of motility and viability of fish sperms. Billard (1978) also ascribed these variants to the original structure of the spermatozoon. Intermix of teleost spermatozoa with ionic factors in aqueous media could depolarize sperm membrane which initiate motility, but it appears that freshwater is not the best media for the survival of the spermatozoa of freshwater fish (Billard, 1978). So, there is no use of this practice in artificial insemination. Osmotic pressure seems to be the major controlling factor in cyprinids sperms motility (Linhart et al., 1991; Morisawa et al. 1983). Osmotic pressure of seminal plasma of many cyprinids is about 300 mOsm.kg<sup>-1</sup> in pH values of 7.6-8.6 as measured by Zhukinsky and Bilko (1984). So, when himri's sperm activated by hypotonic Na<sup>+</sup> solution (150 mOsm.kg<sup>-1</sup>); we accomplished longer viability comparing with freshwater, which can raise fertilizing rates.

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Figure 1. Experiments site on Euphrates River bank.

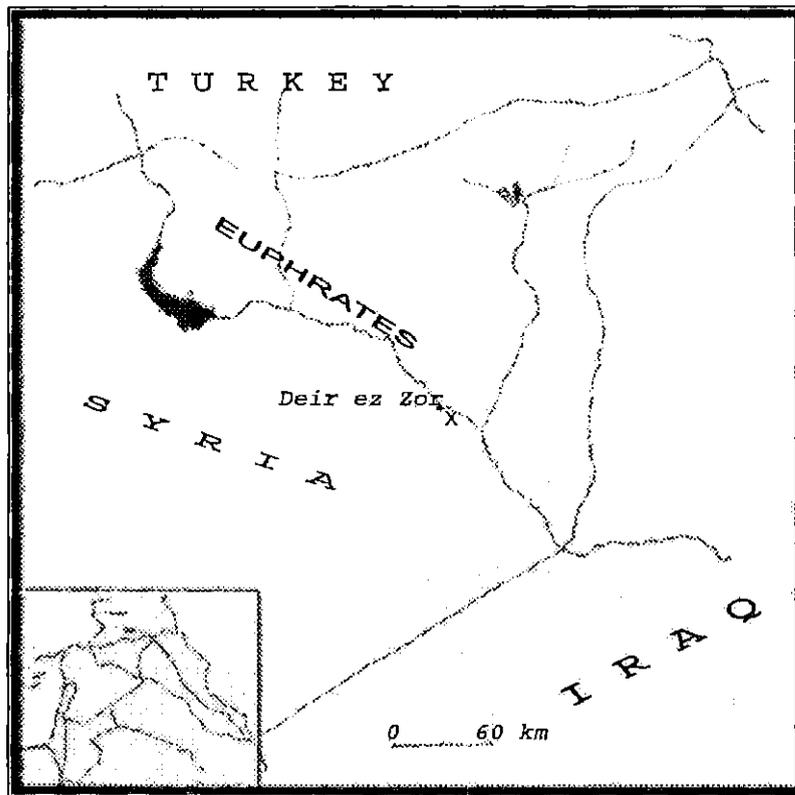


Figure (2): Newly hatched *Barbus luteus* larva, total length 5 mm. No pigmented optical vesicle could be noticed.



Table 1. Effect of hormonal stimulation by common carp pituitary extract on fecundity, fertility, and hatching of himri *Barbus luteus* at 20-22° C.

Group	1 <sup>st</sup> dose mg.kg <sup>-1</sup> (BW)	2 <sup>nd</sup> dose mg.kg <sup>-1</sup> (BW)	Relative fecundity Mean ± S.D.	% Fertilization	Degree-day Mean ± S.D.	% Hatching
1	Females (n=5)	3	8	7464.96 ± 481†	98.6 ± 1.2	57.3 ± 1.2
	Males (n=5)	3	-	22.5 ± 0.4‡		62.5 ± 2
2	Females (n=5)	-	8	5286.62 ± 392.5†	97.8 ± 0.7	56.8 ± 0.8
	Males (n=4)	-	-	23.2 ± 1.6‡		

†: Eggs.kg<sup>-1</sup> BW, ‡: ml.milt.kg<sup>-1</sup> BW.

Table 2. Embryonic development of himri *Barbus luteus* at 20-22°C of water.

\*: approximately

<i>Time*</i>	<i>Description</i>
1 to 2.5 h	Segmentation, first cleavage followed within 30 min by second cleavage up to 8-cell stage
3.5 h	16-cell Morula, cells are medium in dimensions
6 h	Morula, small and many cells
10 h	Blastula
13 h	Gastrula, epiboly increased. Cells ring closing yolk sac.
26 h	Completion of yolk invasion, blastopore, and embryonic rudiments
29 h	3, 6 then 12 myotomes. Yolk sac elongation. Formation of intestine, notochord, and caudal bud.
31 h	Head and tail elongated, tail separating from yolk sac. Head is clearly developed and bent toward yolk. Colorless, slowly-circulating blood in rudiments. Sudden movements turning embryo around.
63 h	Development of Myotomes. Well developed eye lens present but unpigmented.
64 h	Final outward, tail moving freely. Continuous movement. 32 myotomes. Regular heart contractions and red blood circulation in vessels.
74 h	Hatching continued 6 h in our experiment. Final formation of hatching larva is straight, head is slightly bent towards yolk. Single chamber swim bladder. Total length 5 mm (Fig. 1)

Table 3. Motility of himri's spermatozoa in different solutions (sec.). FF: fast forward, RW: rotational waves, VD: vanished till death.

<i>Treatment</i>		<i>FF</i>	<i>RW</i>	<i>VD</i>	<i>Viability (min.)</i>
Hatchery water	Min.	22	27	70	1.983
	Max.	22.5	30	75	2.125
3g NaCl.l <sup>-1</sup> water	Min.	28	47	98	2.883
	Max.	32	54	100	3.1
Total increment <sup>0.0</sup>		127-142	174-180	133-140	145