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Phenotypic study for embryonic and larval development of common carp (*Cyprinus carpio* L., 1758)

A.M. Mojer

Marine Science Centre, University 0f Basrah, Basrah-Iraq
e-mail: ahmed.moajer@yahoo.com

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Abstract - The early development of common carp (*Cyprinus carpio* L., 1758) was studied from fertilizing until juvenile stage. The series developmental staging was done using morphological characteristics. Results identified eight main periods of embryogenesis: zygote, morula, blastula, gastrula, neurella, segmentation, pharyngula and hatching period. Results also identified and described nine larval development stages: hatching larva stage, rudimentary - pectoral fin and gill arch stage, melanoid-eye with gas bladder emergence stage, one chamber gas bladder with yolk absorption stage, two chamber gas bladder stage, pelvic fin bud with dorsal fin formation stage, anal-caudal and pelvic fin formation stage, squamation stage and juvenile stage. Hatching occurred at 38h after egg fertilization. The fertilized egg was spherical, yellowish, transparent and 2.5 mm in diameter. The transition from larva to juvenile occurred in 30 days.

Keywords: *Cyprinus carpio*, Phenotypic, Embryogenesis, Zygote, Larva.

Introduction

Fishes have a basal position in the vertebrate lineage, and their embryos share general chordate characters with other vertebrates. In addition, most fish embryos develop externally from transparent eggs. These characteristics made them a good model for the study of vertebrate embryogenesis (Langeland and Kimmel, 1997). The common carp (*Cyprinus carpio* L., 1758) is regarded as one of the oldest suitable food fish, and its cultivation dates to the fifth century B.C. in China (Balon, 2006). It reproduction by many methods such as the natural, semi-artificial and artificial. In modern times, most fingerlings are produced by artificial breeding through the use of induced hormones at suitable temperature and saturated oxygen (Drori *et al*., 1994).

The study of embryonic development by sequentially considered an instrument provides researchers with insight into evolutionary processes because embryos within the same group may grow at slightly different rates (Kimmel *et al*., 1995). For example, rapid development in fish embryos requires the availability of environmental conditions such as temperature, saturated oxygen and good biological characteristics of egg-bearing mothers (Kane and kishimoto, 2002; Nakagawa *et al*., 2002). In the case of temperature, a decrease in the temperature of incubator water caused a delay the development of embryos, may have effects on their survival ratio, and may lead to abnormalities of embryos (Arenzon *et al*., 2002; Ojanguren and Brana, 2003). On the other hand, elevated water temperature in incubators has gross effect of deteriorating the cellular symmetry and breakage of the oil granules which caused 100% mortality (Jennings and Pawson, 1991).

Embryonic and larval development in cyprinid fishes is well documented (Kemmel *et al.*, 1995; Sado and Kimura, 2002, 2006; Al-Hazzaa and Husein, 2007; Haniffa *et al.*, 2007; Mukhaysin and Jawad, 2012). The present study aimed to highlight critical stages in the embryonic and larval development of common carp by (i) reviewing and recording the most important development stages that include the primary divisions and appearance of germinated layers; (ii) reporting on organ formation which may allow field workers (e.g. fish farmers) to identify important indicators related to success of artificial propagation of this species; and (iii) identifying critical periods in embryonic development when temperature may affect the growth and development of the embryos.

Materials and Methods

Eight females (average weight 1.7 ± 0.2 kg) and four males (average weight 1.6 ± 0.1 kg) of common carp were selected from the broodstock pond in Marine Science Centre, and put in the breeding tank of the hatchery. The doses of pituitary gland hormone (PG) used were 4 mg/kg fish for female and 2 mg/kg for males. Artificial fertilization was based on methods published by FAO (1985) and United Standard (2006). Samples were taken before and after the fertilization at regular intervals for microscopic examination and photography by a digital camera (magnification about 32 X). Phenotypic traits were recorded from these images to follow development of the eggs until hatching, at age 30 days. Dissolved oxygen and pH were measured daily during the experiment using a Yassi digital meter; average pH was 8, and dissolved oxygen concentration was 8.5 mg/l during the experiment. Water temperature was regulated between 25 and 27 ^{0}C using an automatic heater.

Results

Artificial fertilization of common carp was successful. The incubation period lasted 38 hours and hatching ratio was 85%. The average size of mature egg was nearly 1.7 mm (Plate 1, A), and reached 2.5 mm after fertilization. Fertilized eggs were spherical, yellowish and transparent. The membrane of fertilized eggs was separate from the yolk, and formed the privetelline space. Zygotes increased in size gradually as a result of absorption of water during the washing process, and stopped increasing in size due to water absorption after neutralization of solutions in the privetelline space (Plate 1, B).

Embryonic development:

In general, embryonic development of common carp can be divided into two stages: the first is the embryonic stage, and the second is the post hatching stage (larval stage), which extended for thirty days after hatching. The embryonic stages were divided into eight main periods; each one was subdivided into many sub-stages (Table 1). The different stages of embryonic and larval development were described as follows:

Embryonic stages:

Zygote period: (First through sixth stages) began immediately after fertilization and continued for two hours. The first stage of this period is called cleavage stage, and the first cleavage is meroblastic that occurs in the animal pole of the germinal disc (Plate 1, C). The second cleavage led to form four cells, two of them (blastomers) were not completed (Plate 1, D). The third cleavage was indicated by vertically parallel lines, and led to formation eight cells (Plate 1, E). The fourth

cleavage was indicated by two horizontally paralleled lines and led to form 16 cells (Plate 1, F). The fifth cleavage was indicated by two vertical paralleled lines, and led to form 32 cells. These cells were large with regularly shape, and then became smaller with irregular shape (Plate 1, G).

Morula period: (seventh and eighth stages) occurred after (2.00-2.50 h) of fertilization, the cells continued to divide to form 64 cylinder cells, then to form 128 cells. These cells decreased in size, and the cytoplasm moved toward the animal pole (Plate 1, H-I).

Blastula period: (ninth to eleventh stages) occurred after (2.50- 3.50 h). The blastoderm appeared in the early blastula as circular shapes, and epiboly was begun. Whereas the shelling process continues and the embryonic disk became flat at late blastula, (Plate 1, L-N).

Gastrula period: (twelfth to fourteenth stages) occurred after (3.50- 6.30 h) and divided into three stages (early gastrula, mid gastrula and late gastrula). The gastrula disc began to protrude gradually toward inside and formed the germinal ring. The continuation of the shelling process led to form the germinal layer, while blastoderm was occupying most of the yolk (Plate 1, O-S).

Table 1. Different periods of the common carp embryonic stages at 25-27°C.

Stage No.	Main period	Stage	Time (h., min)
1	Zygote	1cell	00.15
2		2-cell	00:15- 00:45
3		4-cell	00:45- 1:05
4		8-cell	1:05- 1:20
5		16-cell	1:20- 140
6		32-cell	1:40- 2:00
7	Morula	Early morula	2:00- 2:30
8		Late morula	2:30- 2:50
9	Blastula	Early blastula	2:50- 3:10
10		Mid blastula	3:10- 3:30
11		Late blastula	3:30- 3:50
12	Gastrula	Early gastrula	3:50- 5:30
13		Mid gastrula	5:30- 6:00
14		Late gastrula	6:00- 6:30
	Neurella		6:30- 7:30
15	Segmentation	Blastopore closure	7:30- 8:00
16		Somatic appearance	8:00- 8:50
17		Optic primordium	8:50- 10:30
18		Optic vesicle	10:30- 12:00
19		Olfactory placodes	12:00- 13:00
20		tail bud	13:00- 13:40
21	Pharyngula	Otic capsule and tail vesicle	13:40- 14:30
22		Caudal fin and lens	14:30- 17:00
23		Muscular effect	17:00- 18:30
24		Heart rudiment	18:30- 24:00
	Hatching		24:00- 38:00

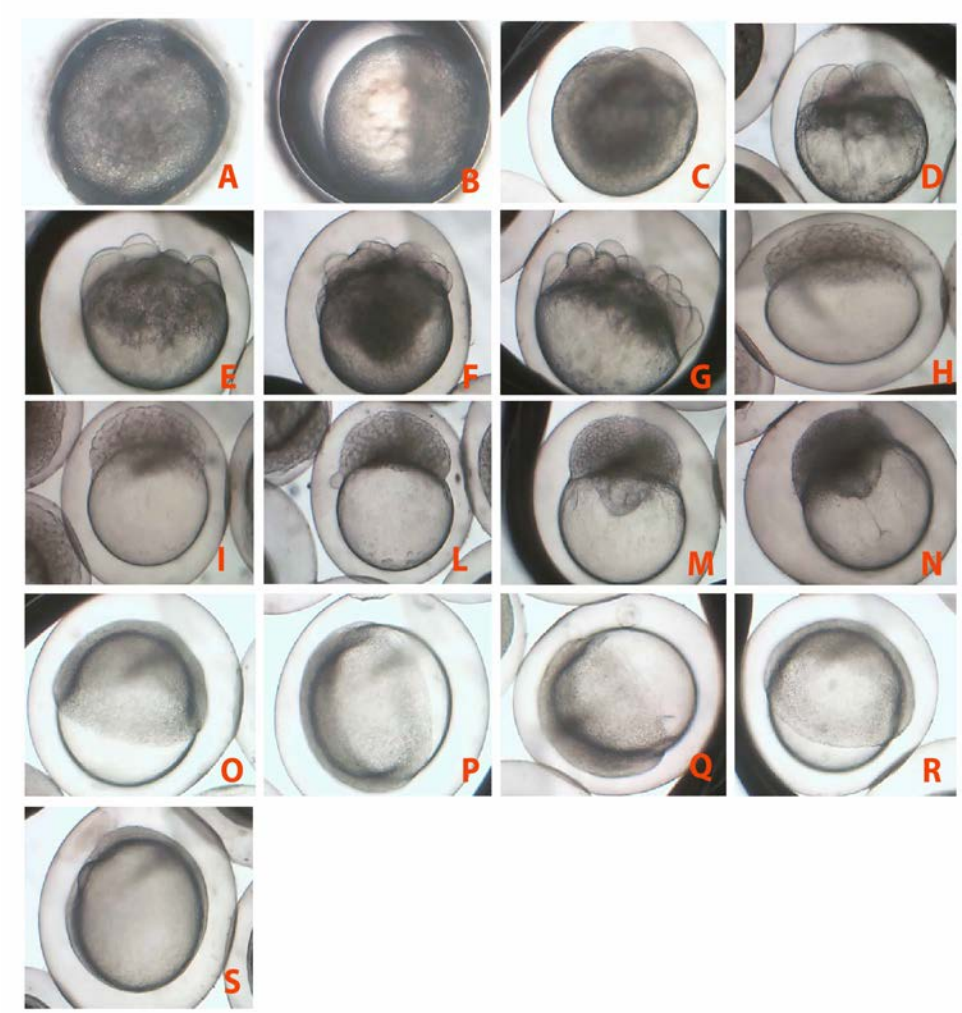

Plate 1. (A) mature egg, (B) formation of privetelline space, (C) 2cells, (D) 4cells, (E) 8 cells, (F) 16 cells, (G) 32 cells, (H-I) morula period, (L-M) blastula period, (O-S) gastrula period.

Neurella period: (6.30-7.30 h), the embryo had become a little elongated and the blastoderm was thick and rudimentary brain undifferentiated, while the blastopore was not closed (Plate 2, A).

Segmentation period: (16t^{h} to 21th stages) occurred after (7.30-13.40 h), the embryo became thick, especially at the frontal end with slight differentiation of the brain (Plate 2, B); a pair of somites appeared, and the embryo elongated a little, while the blastopore was incompletely closed (Plate 2, C-D) and optic primordium was formed. In this stage, the embryo became more elongated with tail end slightly extended; the brain became clearly differentiated, the primordial elongated and ovate with the appearance of notch at the lower edge and the embryo occupying one fifth of the yolk (Plate 2, E-G). The optic vesicle became little elongated, the embryo

enveloped most of the yolk, and the brain differentiating into three main regions (fore brain, mid brain and hind brain). The tail became more prominent with increasing of somites number up to more than 10 pairs. The head region approached with the tail (Plate 2, H), then olfactory placodes were appeared, but unclear at this stage, the embryo increased in the growth with an increase of somite number (Plate 2, I). The differentiation of embryo continues and the tail bud was appeared, as the embryo was elongated and clarity, the caudal bud became visible with an increase in the number of neural segments (Plate 2, L).

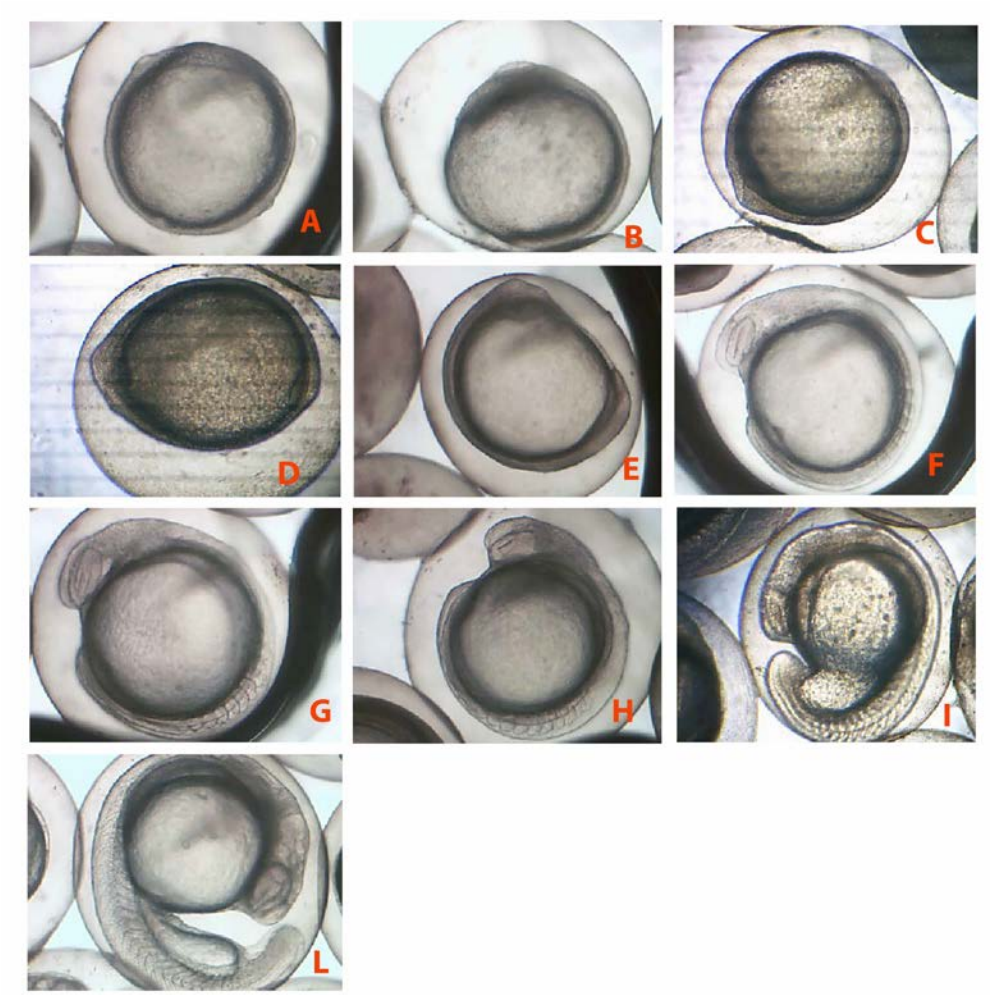

Plate 2. (A) Neurella period, (B-L) Segmentation period.

Pharyngula period: (22th to 26th stages) began after (13.40-24.00h). Otic vesicles differentiation synchronized significantly with brain development and was thick among the three divisions of the brain. The optic vesicles elongated and increased the number of somites, and small tail vesicles appeared with interiorly extension of the tail (Plate 3, A). The embryo continued to grow by increasing the distance

between the head and tail and lens formation (Plate 3, B-C). Next, muscle contraction commenced, and the embryo gradually moved with differentiation of most parts and a visible eye lens becoming more differentiated (Plate 3, D-E). The rudimentary heart was formed and started to pump slowly. As the pulse increased gradually, the number of somites as well as differentiation within the brain also increased (Plate 3, F-H).

Hatching period: (24.00-38.00 h), where the movement of embryo significantly increased, and differentiation within the eye and brain increased greatly; the distance between the head and the tail also increased. The embryo became elongated, and the thickness of the egg membrane was reduced. The yolk converted into kidney shape, the tail vesicle moved backward. Hatching occurred after 38h. The embryo cleaved the egg membrane and began to move freely (Plate 3, I-N).

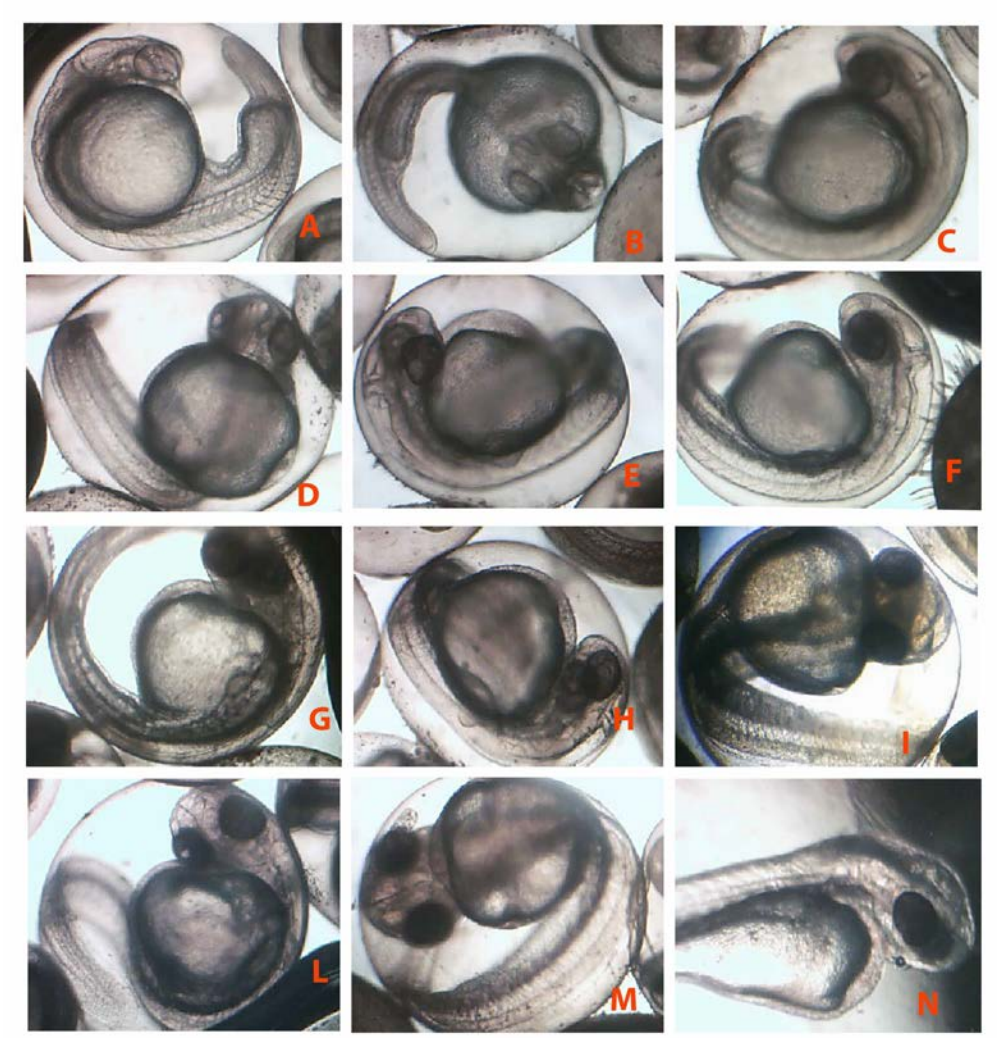

Plate 3. (A-H) Pharyngula period, (I-N) Hatching period.

Larval stages:

Nine developmental stages were distinguished in the common carp larvae, starting from the hatching larval stage to the juvenile stage (Table 2).

Table 2. Different stages of larval development.

No.	Stage	Time
1	Hatching larva stage	After hatching
2	Rudimentary-pectoral fine and Gill arch stage	12 hours
3	Melanoid-eye stage with gas bladder emergence stage	2 days
4	One chamber gas bladder with yolk absorption stage	3 days
5	Two chamber gas bladder stage	8 days
6	Pelvic fin bud with dorsal fin formation	11 days
7	Anal, caudal and pelvic fin formation	13-18 days
8	Squamation stage	22-28 days
9	Juvenile stage	30 days

Description of larval development stages:

Hatching larval stage: The larva was liberated from the egg membrane as a result of the strong movements of the tail. The larva was small (3.5-4.1 mm), transparent, cylindrical, and with an oval yolk sac. This larva moved slowly and attached to the walls of the zug jar's. At this stage the mouth was still closed, while the head was clear, and differentiated brain regions with a two black triangular spot down the front of the eye were visible (plate 4, A).

Rudimentary-pectoral fin and gill arch stage: In this stage, the larva (5.3 mm), develops dramatically with the emergence of vestigial pectoral fin and simple differentiation of four gill arches. The mouth was still closed and the larva fed by absorption of the yolk sac. A black patch was clearly visible in the anterior lower part of the eye (Plate 4, B-D).

Melanoid-eye with gas bladder emergence stage: The larva (7 mm) has black pigments in the top of the eye and extended around it. The yolk sac became slimmer with the appearance of many astral melanopores cells at the ventral edge. The gas bladder formed, the mouth became more differentiated, the intestine extended, and the gill arch length increased (Plate 4, E-H).

One chamber gas bladder with yolk absorption stage: The pigment cells in the head region of larva (7.4 mm) increased along the mouth. The yolk sac decreased and came to resemble a small yellow knot. The number of stellar pigment cells increased in the abdominal region and on both sides. A gas bladder with a single chamber appeared, and the number of pigment cells on the dorsal side of the head increased; with breadth at the end of caudal (Plate 5, A-C).

Two chamber gas bladder stage: The second chamber of the gas bladder of the larva (8.63 mm) became partially differentiated; the anterior chamber was transparent while the posterior appeared dark due to the presence of many pigment cells. The number of pigment cells increased to cover most of the larva body (Plate 5, D-E).

Pelvic fin bud with dorsal fin formation stage: The larva increased to 10 mm in size. Buds for pelvic and dorsal fins appeared (Plate 5, F).

Anal, caudal and pelvic fins formation stage: The larva increased to 12 to 18 mm in size. The anal fin has clearly emerged at the beginning of this stage as it continues to develop to its final shape. The pelvic fin was differentiated also. The radials of caudal fin seemed to form a two lobes at the end of this stage (Plate 5, G-I).

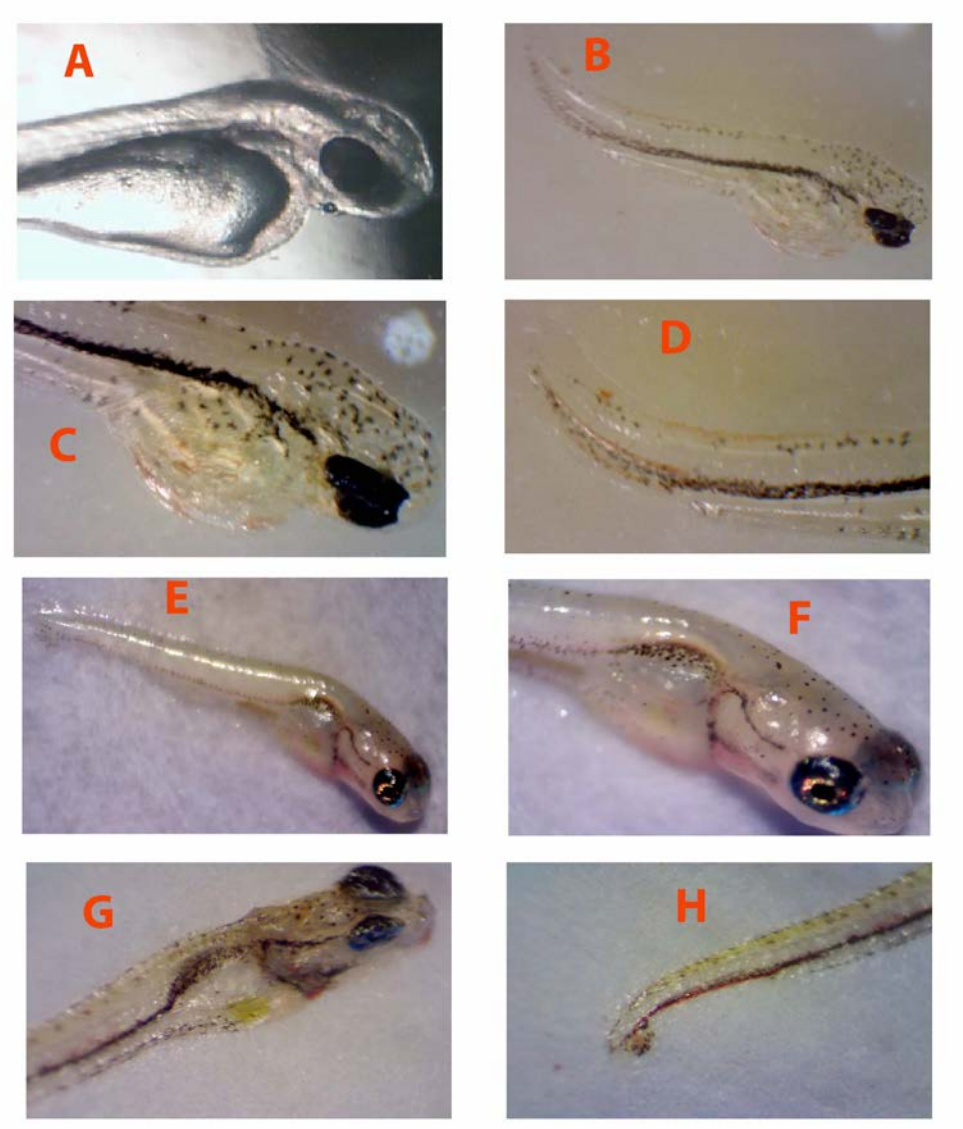

Plate 4. (A) Hatching larva stage, (B-D) Rudimentary-pectoral fin and gill arch stage, (E-H) Melanoid-eye with gas bladder emergence stage.

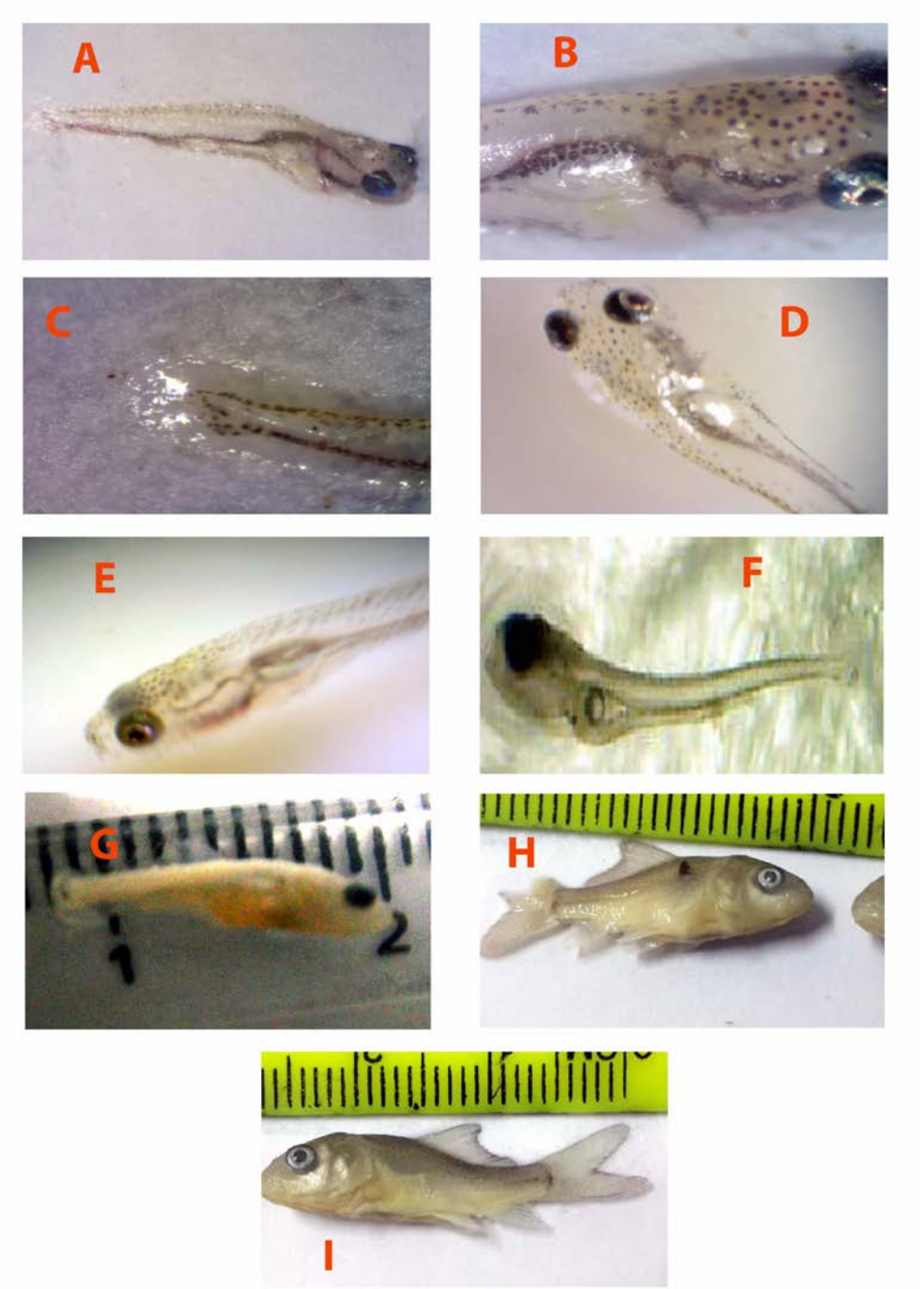

Plate 5. (A-C) One-chamber gas bladder with yolk absorption stage, (D-E) Two-chamber gas bladder stage, (F) pelvic fin bud with dorsal fin formation stage, (G-I) anal, caudal and pelvic fins formation stage.

Squamation stage: The larva has reached 25-29 mm in size. Scales appeared gradually, and most of them were complete in composition (Plate 6, A-C).

Juvenile stage: The size of juvenile was 35-40 mm. Scales were complete, and juvenile looked as adult fish (Plate 6, D-E).

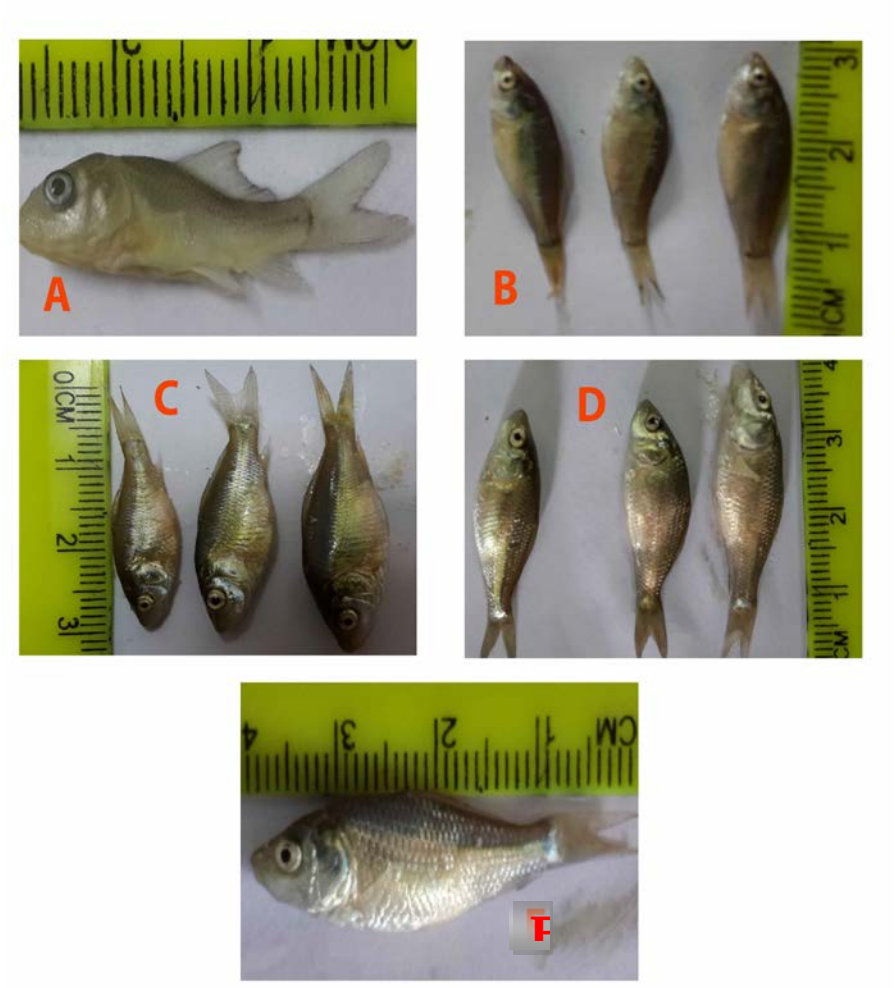

Plate 6. (A-C) Squamation stage, (D-E) Juvenile stage.

Discussion

Information is very rare about the embryology of fish in Iraq. Al-Nasih (1992) and Pyka *et al.* (2001) give brief accounts on the embryological development of the *Barbus sharpeyi* (= *Mesopotamichthys sharpeyi*). Saleh *et al.* (2011) studied the main stages of embryonic development of the common carp. All these studies lack detailed description of the morphology for larvae and juvenile and also lack illustration of development stages. Mukhaysin and Jawad (2012) reported the embryonic and larval development of the *M. sharpeyi* with more accuracy and detail. However, recording observations on the developmental stages is difficult (Kovac, 2000).

Changes in structure emphasize the thresholds between embryonic, larval and post-larval development from the onset of cleavage or epiboly, or at the time of organogenesis, respectively (Carlos *et al*., 2002). The current study is the first one present in detail embryonic and larval development for common carp in Iraq. Egg size is a key feature in the early history of fish. It may be expressed as egg diameter, wet weight, dry weight, energy content per egg, or content of a key substance such as carbon, nitrogen, or protein (Kamler, 2005). The size of eggs in common carp of the current study were a bit larger than other types of cyprinids, such as *M. sharpeyi* (Mukhaysin and Jawad, 2012), *Carassius gibelio* (Rahman, *et al*., 2011) and strain of common carp (Koi carp) (Haniffa, *et al*., 2007; Gosh, *et al*., 2012). Calta (1998) pointed out that egg size may vary amongst species of the same family as a function of different ecological niches, or differences in parental or ecological requirements of these species (Kamler, 1992).

The range of egg diameter and length of the newly-hatched larvae in current study were 1.7-2.5 and 3.5-4.1 mm respectively. Haniffa *et al.* (2007) reported 0.9-1.10 and 2.7-2.9 mm, respectively, while Gosh *et al.* (2012) reported 0.8-1.0 and 2.7-2.9 mm. It is important to note that variations in water temperature and salinity might have a direct effect on the diameters of eggs and size of newly-hatched larvae (Almatar *et al*., 2000).

The last few decades have seen many various problems about lack of standardization of nomenclature applied to early development in fishes (Urho, 2002). Embryonic development stages recorded in this study were completed more quickly than found by other authors, including: Saleh *et al.* (2011), at 48 hours; Haniffa *et al.* (2007), at 73 hours; and Gosh *et al.* (2012), at 75 hours. The reason may be due to differences in environmental factors, as the internal structure of the changes in response to the environment, and so is crucial to assess the development of species accurately (Balon, 1999). Temperature is one of the most important factors that stimulates embryonic development (Blaxter, 1992). Drozd *et al.* (2009) reported that the ratio of the incubation period to the total hatching duration was inversely proportional to the incubation temperature and ranged from 17.5 days at 9°C to 1.8 days at 24°C. As noted above, dissolved oxygen and pH were carefully maintained during this project. In the current study, dissolved oxygen of 8.5 mg/l corresponded with high hatching ratio (85%), which supports the observations of Mallya (2007) that a high percentage of saturated oxygen in the water has positive effects on the growth and development of embryos. Spence *et al.* (1996) reported that the effects of low levels of dissolved oxygen can include changes in embryo growth rate, time of hatching and ultimately the proportion of embryo births to death. Oyen *et al.* (1991) reported that low pH caused negative effects on embryonic growth of the common carp. A pH of 8 was recorded in the current study.

The high hatching rate and healthy larvae without deformation suggests that the environmental conditions maintained during the current study were optimal for common carp. The results included larger size and more rapid development of larval stages in comparison with other studies under similar environment on the common carp or other cyprinids (Haniffa *et al*., 2007; Gosh *et al*., 2012; Pyka *et al*., 2001; Mukhaysin and Jawad, 2012).

Another interesting results was the rapid and complete absorption of the yolk sac in current study which took only three days after hatching. This is faster than the duration of other cyprinids, such as *Barbus luteus* (=*Carassobarbus luteus*) (Al-Hazzaa and Hussein, 2007) and *M. sharpeyi* (Mukhaysin and Jawad, 2012).

These differences may be attributed to rapid development of common carp in current study or to the large size of their larvae compared with the others.

As for many fish larvae, access to air at the water surface is necessary for swim bladder inflation (Chapman *et al*., 1988). Larvae during this study showed no failure to activate their swim bladders which seem to be common among of many species (Battaglene and Talbot, 1990). These movements may serve a respiratory function, as suggested by Weihs (1981) for the newly hatched anchovy, *Engraulis mordax* larvae. This explanation seems plausible for carp larvae for two reasons: first, the cyprinid larvae after hatching respire almost exclusively through the body surface (El-Fiky *et al*., 1987); second, this pattern in the newly hatched larvae was found in other cyprinid species such as *C. luteus* (Al-Hazzaa and Husein, 2007) and *M. sharpeyi* (Mukhaysin and Jawad, 2012). The transition of common carp larvae to juvenile was fast, lasting less than one week. The reason may be attributed to various factors, such as environmental conditions, availability of suitable food and genetic heterogeneity (Ara *et al*., 2009).

The many changes in morphology that occur during the transition from larva to juvenile result in the stabilization of relative growth and the attainment of the adult morphology (Pinder and Gozla, 2004). There are many characters that can be used to classify cyprinid larvae, including relative pre-anal length, eye shape, pre-anal myomere numbers and ventral pigmentation (Fuiman *et al*., 1983). Thus, the results from this study should enable fish farmers to increase rates of successful breeding of carp, as well as survival of larvae.

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دراسة مظهرية للتطور الجنيني واليرقي لأسماك الكارب الشائع (*Cyprinus carpio* L. 1758)

أحمد محسن موجر
مركز علوم البحار، جامعة البصرة، البصرة - العراق

المستخلص - درست مراحل التطور الأولي لأسماك الكارب الشائع (*Cyprinus carpio* L. 1758) من مرحلة البيضة المخصبة Zygote وصولاً الى مرحلة اليافعة Juvenile. وتم متابعة سلسلة المراحل التطورية باستعمال الصفات المظهرية. أظهرت النتائج وجود ثمان مراحل رئيسية من التطور الجنيني: البيضة المخصبة Zygote، الطور التوتي Morula، الاريمة Blastula، المعيدة Gastrula، العصبونة Neurella، التعقيل Segmentation، تكون البلعوم Pharyngula ومرحلة الفقس Hatching فيما عرفت مراحل التطور اليرقي بتسع مراحل: اليرقة الفاقسة hatching larva، تكون بادئات الزعانف الكتفية وتكون القوس الغلصمي، تصبغ العين وتكون الكيس الغازي، تكون أحد فصي الكيس الغازي مع امتصاص المح، تكون فصي الكيس الغازي، نشوء براعم الزعانف الحوضية مع تكون الزعنفة الظهرية، تكون الزعانف المخرجية والذنبية والزعانف الحوضية، تكون الحراشف ومرحلة اليافعة. حدث فقس البيوض خلال 38 ساعة بعد الإخصاب وكانت البيضة المخصبة دائرية مصفرة وشفافة قطرها 2.5 ملم. كما حصل الانتقال من المرحلة اليرقية إلى اليافعة بعمر 30 يوماً بعد الفقس.