

Morphological and histological studies of brain development in embryos and larvae of the common carp *Cyprinus carpio* (L. 1758)

A.M. Mojer*, Z.A.K. Mezyad and J.H. Saleh
Marine Sciences Center, University of Basrah, Basrah-Iraq
*e-mail: ahmed.moajer@yahoo.com

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Abstract – In the present study, the morph-histological characterization of the brain development in common carp was studied in Basrah, Southern Iraq, by examining the several samples of eggs and larvae of Common carp *Cyprinus carpio*. The temperature of the water in incubators was 25 -27 °C and the incubation of eggs were carried out for 38 hour. Morphologically, neural stage was emerged after eight hours of incubation with continuation of epiboly processes Brain was progressively differentiated into three main parts namely forebrain, midbrain and hindbrain. Forebrain was differentiated into telencephalon and diencephalon. Optic primordial was appeared after 12 hours of fertilization in a form of evagination from both sides of the forebrain and slightly oval elongated and then characterized optic vesicle, then the lens was appeared. Hindbrain was clearly differentiated and neuromerose was marked after 22 hours of fertilization. Histologically, the brain at the age of 24 hours was composed of a mass of undifferentiated neuroectoderm, whilst at the age of 28 hours appeared more differentiated with clarity a cerebral hemisphere on both sides of the forebrain and it was a mass of undifferentiated neuron cells. Gradually, the brain parts were completed with a differentiation of several layers until the age of 20 days after hatching.

Keywords: *Cyprinus carpio*, Embryo, Larva, Brain, Telencephalon, Hindbrain and Histology.

Introduction

The common carp (*Cyprinus carpio*) belongs to the family Cyprinidae and has extending from shallow lakes and rivers slow flow with soft bottom covered with an aquatic plants to lakes and deep rivers fast and a bottom of sand and gravel, and from fresh water to brackish water (Mohammed *et al.*, 2006).

Fishes have the base site in the vertebrates and their embryos shares general chordate characters with other vertebrates, in addition most embryos developed externally of transparent eggs. This makes them a special model for the study of vertebrate embryogenesis (Langeland and Kimmel, 1997). The study of an embryonic development was sequentially considered as an instrument and it provides researchers with accuracy in development studies because the embryos grow in slightly different growth rates within the single batch (Kimmel *et al.*, 1995).

The brain integrator emits both internal and external signals and corrected the functions based on the normal brain development. Understand the evolution of the brain and unnatural manifestations or changes rearing environments will provides the frame work for evaluation and attaining solutions associated with major stages in fish farming (The Research Council of Norway, 2009). Vertebrates have a nervous system more complex than other animals (Castro and Huber, 1992; Jurd, 1997). The nervous system in fish extends along the body (Lagler *et al.*, 1962) which consists of a central nervous system including the brain and spinal cord, a peripheral nervous system which consists of cranial nerves, spinal nerves and the ganglia (Hyden, 1967; Hibiya, 1982). The embryonic development of the brain in vertebrates includes few stages begin with a neural plate of the dorsal side of the embryo and excellence along the front axle-rear dorsal-ventral and be the neural tube of the plate and then the brain ventricles develops to contribute the typical form of the brain (Schier *et al.*, 1996). During and after the neurolation stage, the inner part of the neural tube was formed by the plate nerve which suffers a series of bends formations and stretch-to divided the brain secondary divisions to form the forebrain (prosencephalon), midbrain (mesencephalon) and the hindbrain (rhombencephalon) and called all these areas in primary embryonic development the brain vesicles (Gray and Clemente, 1985). The brain vesicles were developed to be the central nervous system (CNS), the forebrain develops to the telencephalon and diencephalon, whereas midbrain develops to tectum and tectum, while the hindbrain develops to the cerebellum and medulla oblongata (Searimaki, 2012). Due to lack of information about the embryonic development of the cyprinid fish in Iraq (Al-Nasih, 1992; Pyka *et al.*, 2001), the present study was aimed to explain the stages of embryonic development of the brain in common carp.

Materials and Methods

Samples of embryos and larvae of common carp were obtained from the process of artificial fertilization carried out in the marine science center fish hatchery. Embryos were collected after eight hours of incubation at a rate of 6-8 samples per hour, then the period was increased to every two hours until hatching. While samples of the larvae were taken at the first, second and third days respectively to the tenth day and at the same rate. Then, the interval time was increased gradually.

All samples were fixed using formalin (10 %) for a period ranging from 8-48 hrs. Then prepared, stained and cutting slides histological section of eggs and larva according to the method of Bancroft and Stevens (1982). Samples were cut by a rotary microtome with thickness of 5-7 μ and mounted with D.P.X. The sections were stained with Cole's Hematoxlin-Eosin stain and examined using Olympus optical compound microscope. Eggs samples and tissue sections were observed by optical imaging microscope type Kruss (German) with a digital camera type HDCE-50B. Dissolved oxygen and pH were measured daily during the experiment using Yassi digital tool. Water temperature was regulated using automatic heater at 25-27 °C.

Results

Ecological and biological data of artificial fertilization in common carp was analyzed and the results were furnished in Table (1). Artificial fertilization of common carp has been successfully done in this experiment. Average weight of fish eggs were one kg and average value of pH 8, dissolved oxygen 8.5 mg/l and the

incubation period was lasted for 38 hrs by hatching of eggs 85 % (Table. 1). The results included the morphology and histology of the brain development in embryos and larvae of the common carp. The first sign of emergence of the neural stage occurred after eight hours of fertilization, blastoderm covered most of the yolk and the epiboly constituted more than 40 % with an increase in the thickness of the embryo, yolk plug remained uncovered (Plate 1. A). After that, the somites appeared with the differentiation of brain rudiment (it was thick at the front of embryo) was increased gradually but still their variations were indistinguishable. This was accompanied with an increasing embryo growth which became clearly differentiated without completely closed blastopore (Plate 1. B-E) but difficult to discriminate its regions.

Table 1. Some ecological and biological data of artificial fertilization in common carp.

Fish Type	No. of Females	No. of Males	Average eggs weight (Kg)	Hatching Rate %	Temp. (°C)	pH	O ₂ (mg/l)
<i>C. carpio</i>	10	6	1	85	25- 27	8	8

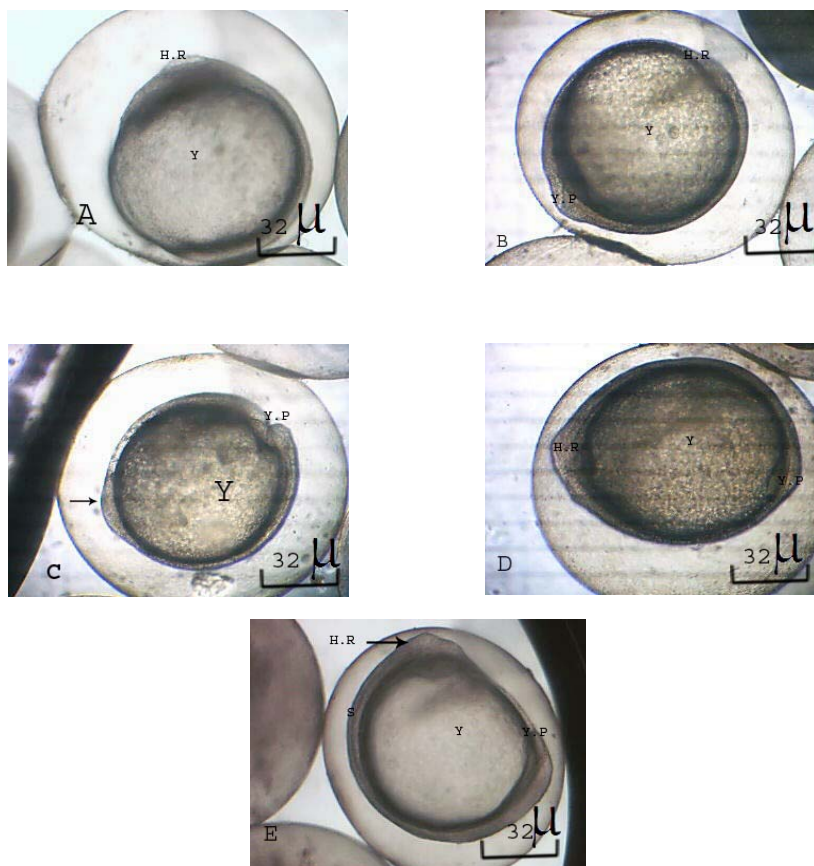


Plate 1. (A) Neurella stage after 8 hrs of fertilization, (B-E) Head rudiment formation stage after 8:30 hrs of fertilization, shows the head primordium (H.R), yolk (Y), the yolk plug (Y.P), somite (S).

The Plate (2. A) shows the gradual increase in Number of somites. The head appeared more pronounced with increased embryo growth. The brain began to differentiate into three parts (forebrain, midbrain and hind brain) and the optic vesicle was appeared with the lack of distance between the head submitted and the tail, as a result of embryo deflection (Plate 2. B-D). The eye lens was appeared initially after 18 hours of fertilization. Brain regions are well differentiated and the forebrain was formed as two parts namely telencephalon and diencephalon with clear notochord (Plate 2. E).

The hind brain was clear with distinction of neuromere which appeared after 22 hours of fertilization and differentiated most areas of the brain immediately after hatching (Plate 3. A-D).

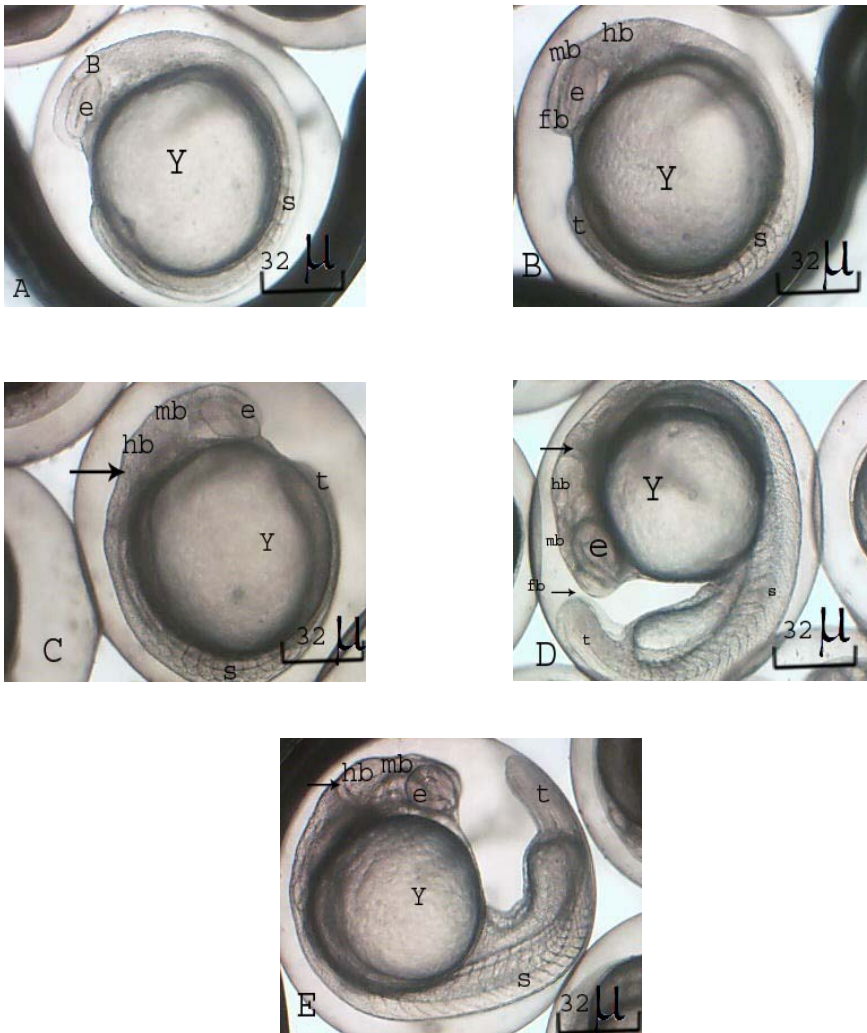


Plate 2. (A) Optic primordium stage, (B-E) Optic vesicles stage occur after 13-18 hrs of fertilization shows the brain (B), eye (e), forebrain(fb), midbrain (mb), the hindbrain (hb), arrows are neuromeres, yolk (Y), tail (t), somite (S).

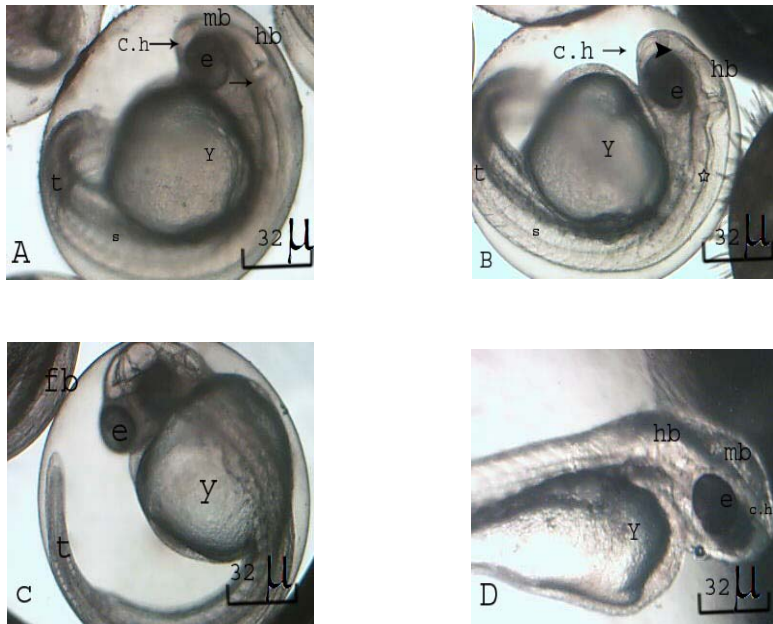


Plate 3. (A) lens formation stage, (E-D) hatching stage and completeness of the emergence of the members, we note cerebral hemispheres (ch), midbrain (mb), hindbrain (hb), yolk (Y), asterisk (neuromers), eye (e), somite (s).

Histologically, the brain consists at age of 24 hours and form a mass of undifferentiated neuroectoderm (Plate 4. A-B). Diencephalon was composed of mass of compacted cells that appeared with the emergence of hypothalamus (Plate 4. C-D); whilst at the age of 28 hour it was more differentiated with a clear a cerebral hemisphere on both sides of the forebrain and it was a mass of undifferentiated neuron cells (Plate 4. E). After 1-3 days of hatching, the forebrain have a spherical or oval cells which confine them a nervous with a little of myelinated fibers.

Brain became larger and more differentiated and olfactory lobes were emergence and cerebral hemispheres differentiated. The pineal body appeared consists of a single row of spherical neurons. The dorsal cord was surrounded by single rows of the cells (Plate 5. A-D).

After 4 days of hatching, the rhombencephalon which consists of two parts, myelencephalon and metencephalon as well as the telencephalon and the olfactory lobe were developed. Myelencephalon which consists of a thicker wall that represents the bottom and extends to the ventricular which consisting of neuroectoderm followed by several layers of modern neuronal differentiation cells which confine a nerve fibers and some myelinated; while metencephalon was large and consisted of two layers. Tectum, which composed of several layers represents the optic tectum and the tengmentum (Plate 6. A-D).

After 5 days of hatching, forebrain was appeared, differentiated and composed of two parts telencephalon and diencephalon. Neurons were spherical with many nerve fibers and differentiated into the hypothalamus which was a mass of compact cells (Plate 7. A-C), whilst the myelencephalon composed from two layers, granular layer and molecular layer (Plate 7. D).

Tectum composed from several secondary layers, these layers are distinguished by their relative content of unmyelinated and myelinated axons and presence or absence of neurons (Plate 8. A-B). T cerebrum consists of fields of interconnected neurons supported by an extensive neuropil; while the cerebellum was the major component of the dorsal metencephalon (Plate 8. C-D). Myelencephalon appeared composed of two layers, granular layer and molecular layer. The granular layer contains more small and rounded granular cells, while the molecular layer contains few cells with more myelinated fibers (Plate 8. E-F).

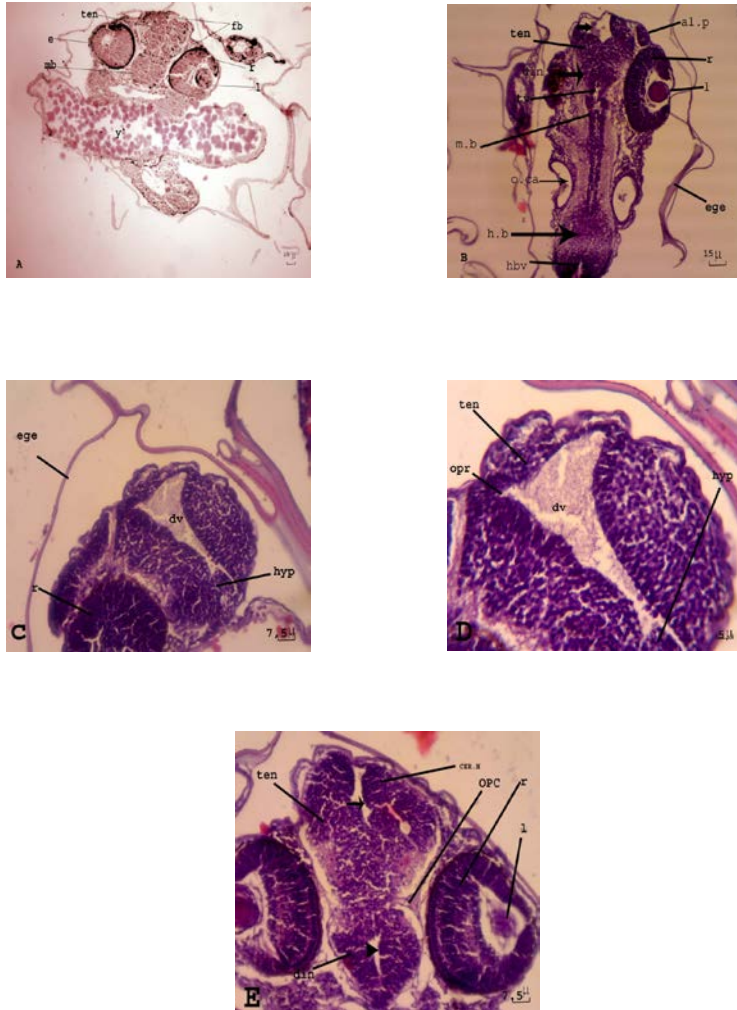


Plate 4. Longitudinal section of common carp embryo (A-B) embryo at age 24 hrs of incubation, (C-D) embryo age 26 hrs of incubation, (E) the age of 28 hrs of incubation, showing forebrain (fb), tectum (ten), midbrain (mb), eye (e), yolk (y), lens (l), retina (r), olfactory placodes (ol.p), diencephalon (din), otic capsule (o.ca), egg envelope (ege), hindbrain (hb), hindbrain ventricle (hbv), optic recess (opr), diencephalon ventricle (dv), hypothalamus (hyp), optic chiasm (opc), cerebral hemispheres (cer.h).

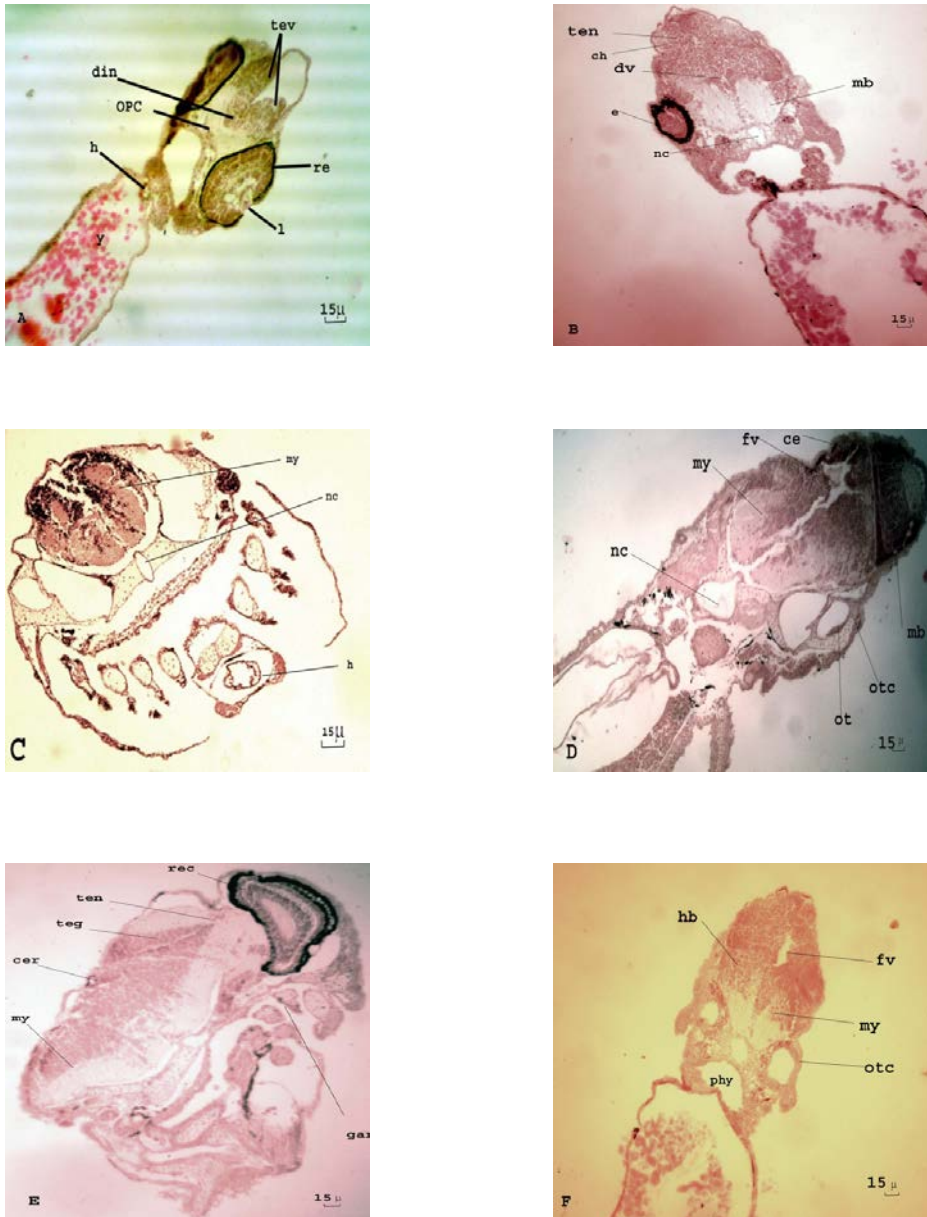


Plate 5. (A- B) Longitudinal section of larva common carp at age one day after hatching, (C) cross-section of the age 2 days after hatching, (D) Sagittal section of larva at age 2 days after hatching, (E-F) longitudinal section of larva, age 3 days after hatching, showing telencephalon vesicle (tev), midbrain (mb), eye (e), yolk (y), lens (l), optic chasima (opc), otic capsule (o.ca), diencephalon ventricle (dv), myelencephalon (my), (gar) gills arch (fv), Tectum (tec), fourth ventricle (fv), heart (h), neural canal (nc), otolith (ot), tengetum (teg), retina cup (rec), cerebellum (ce).

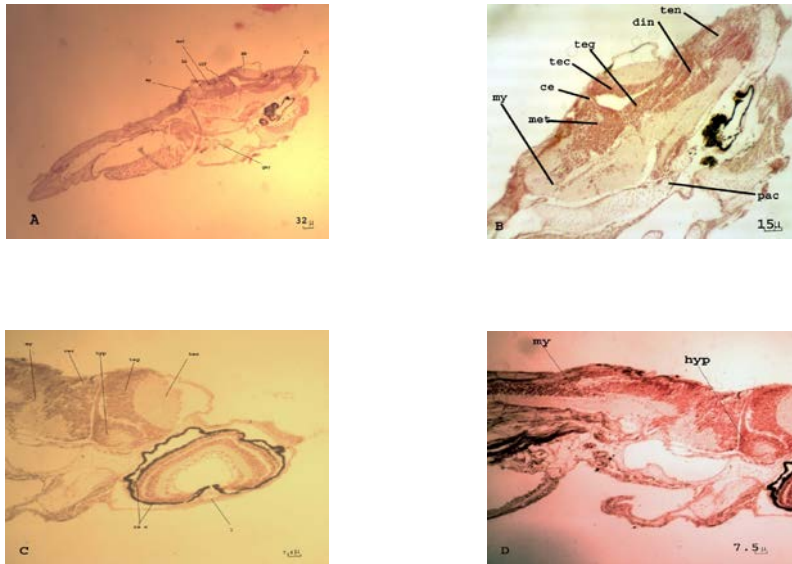


Plate 6. (A-D) Sagittal section of the larva 4 days after hatching, showing forebrain (fb), midbrain (mb), cerebellum (ce), hindbrain (hb), hypothalamus (hyp), metencephalon (met), myelencephalon (my), gills arch (gar), Tectum (tec), tetnetum (teg), retina cup (rec), lens (l).

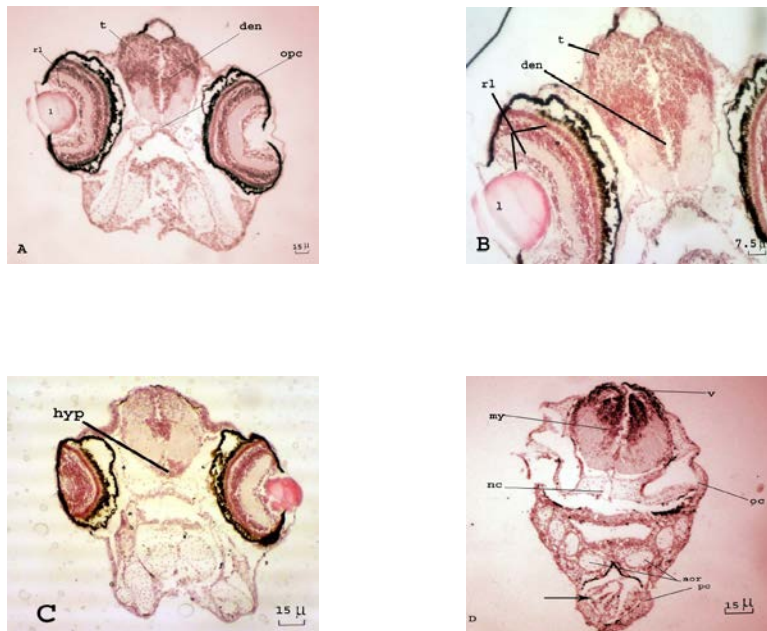


Plate 7. (A-D) Cross-section passes the brain of an larva aged 5 days after hatching showing telencephalon (t), diencephalon (den), retina layer (rl), lens (l), hypothalamus (hyp), optic chasima (opc), otic capsule (oc), ventricle (v), aorta (aor), neural canal (nc), pericardial cavity (pc), (→) ventricle.

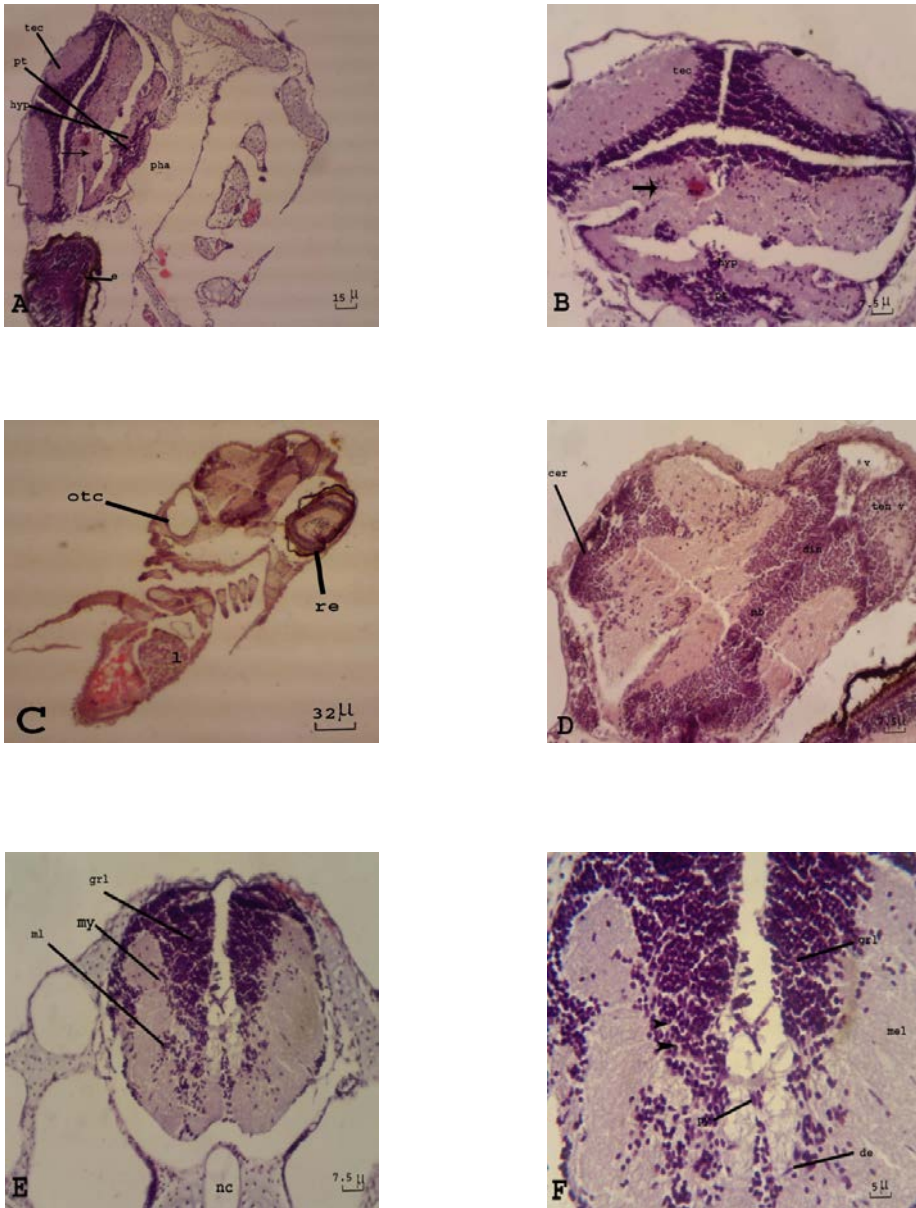


Plate 8. (A-B) Cross-section passes brain of larva at age 7 days after hatching, (C-D) Sagittal section of the larva aged 7 days after hatching, (E-F) Cross-section passes the hind brain of larva aged 8 Day after hatching, view pituitary gland (pt), pharynx (pha), hypothalamus (hyp), eye (e), Tectum (tec), retina (re), otic capsule (otc), tengmentum (teg), liver (l), diencephalon (din), ventricle (v), midbrain (mb), telencephalon vesicle (ten v), cerebellum (cer), granular layer (grl), myelencephalon (my), molecular layer (ml), neural canal (nc), purkinje cells (py), dendrites (de), (head arrow) small and rounded granular cell.

At the age of 8-14 days after hatching, the nerve layers of the brain was continued to develop (Plate 9. A-C), while at age of 20 days after hatching, telencephalon was consisted of a two large mass which represents the cerebral hemisphere (Plate 9. D-F).

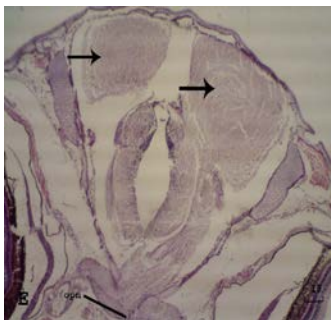
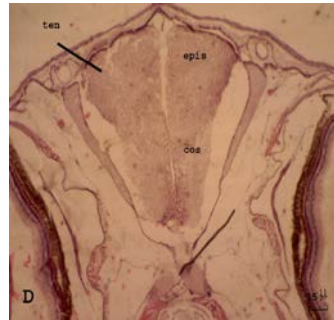
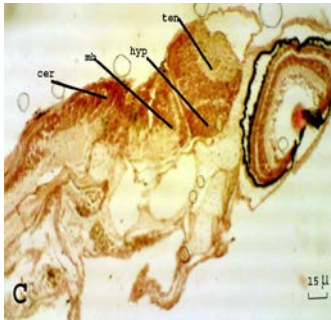
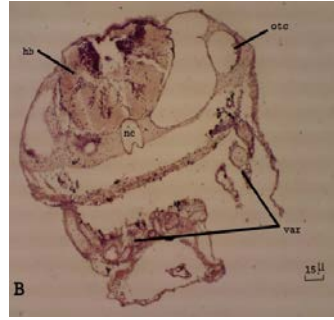
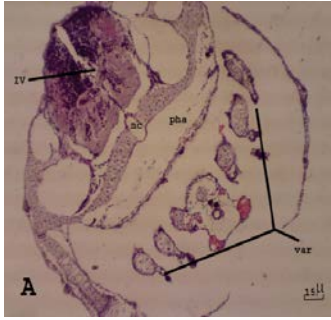


Plate 9. (A) Cross-section of the hind brain of larva 9 days after hatching, (B) Cross-section of the hind brain aged 11 days after hatching, (C) Sagittal section of the larva of age 14 days after hatching, (CF) Cross-section passes the front of the brain larva age of 20 days after hatching, showing fourth ventricle (iv), telencephalon (ten), cerebellum (cer), neural canal (nc), visceral arches (var), hypothalamus (hyp), epistriatum (epis), corpus striatum (cos), cerebral hemisphere (ceb), optic nerve (opt).

Discussion

Artificial reproduction is of great importance in the supply of fish farms with small fish (Kovac, 2000). There are several changes occurring at the beginning of fertilized fish eggs perception between the Prelarvae and the Post larvae stages (Carlos *et al.*, 2002). The embryonic development was affected by various environmental factors namely temperature, oxygen concentration, salinity and pH (Jones, 2002; Kamler, 2008). Temperature is the most important of these factors as it is the dominant factor in the development of embryos (Blaxter, 1992). Kucharczyk *et al.* (1997) found that the effect of temperature on the development of embryos includes growth and survival rates, while Blaxter (1992) mentioned that the hatching operations to fourteen species of freshwater and saltwater fish were shorter at high temperatures. The present study indicated that the fertilized eggs took 38 hour to hatch at incubation temperature of 25-27 °C, while the study of Saleh (2011) found that the incubation period for the same fish took 48 hour at 23°C. Mukhaysin and Jawad (2012) mentioned that the bunny *Mesopotamichthys sharpeyi* eggs need a period of 70-79 hour for hatching at ambient temperature 22-24°C, while Jordan and Kling (2003) explained that the incubation temperature accelerates the stages of embryonic development.

The results of the current study were in accordance with the timing of the follow-up stages of brain embryonic development with that presented by Kimel *et al.* (1995). In his study he clearly explained the various stages of embryonic development in Zebra fish. In both studies the emergence of brain rudiment was occurred after nine hours of incubation at temperature of 28 °C with an increased thickening of the brain and optic vesicle. Notochord primers were appeared after 11.40-18 hour when it was began to become an optical lenses and the emergence of the hind brain after 19-20 hour of incubation and may be attributed to the differences in the types of fishes.

The results of the present study was differed from some other related studies of Haniffa *et al.* (2007) who showed that the characterization of the brain was occurred during the plug stage and locked the hole blastopore between 6-13 hour of incubation in the common carp at 26-28 °C, while the notocord appeared and optical lenses formed within 14 hour and got hatching after 32-34 hour.

Mukhaysin and Jawad (2012) showed that the stages of embryonic development of *M. sharpeyi* were at a temperature of 22-24°C, that the emergence of brain rudiment and increase the somites into 12 pieces and form the forebrain and extension neural cord happened between 11-26 hours. In Nile tilapia *Oreochromis niloticus*, the embryonic axis was showed and formed the brain rudiment as well as notochord and the eyes during 30-31 hours of incubation at 27-30°C, while differentiated forebrain to diencephalon and telencephalon and also shows the midbrain and cerebellum at 41 hours (Morrison *et al.*, 2001).

In general, different results were obtained in this study in comparison with their similar investigations made in other geographical locations and this may be attributed to the different species responding to variable incubation temperature. Laurila *et al.* (1987) noted in their study on the evolution and growth of larvae and juvenile of *Carassius carassius* and revealed that the length of the embryonic stages of the fish depends largely on incubation temperature and showed that it takes four days when incubated at 20°C and two days at 27 °C.

The present study revealed that the brain consists of four particular ventricle cavities. The first and second ventricles showed the cerebral hemisphere, the third

one appeared in the diencephalon; while the fourth in the cerebellum, and the mesencephalon was consisted of several layers. This was in accordance with the findings of Al-Nuclear (2005) who stated that the optic Tectum consists of six layers which varies in thickness and cell diversity the brain in the common carp. Vanegas *et al.* (1974) pointed that the midbrain has six layers in Salomon fish brain which differed according to kinds and thickness of the layers. Northcutt (1983) noted that the layers of mesencephalon was varied in the number according to the type of fishes.

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دراسة مظهرية ونسجية لتطور الدماغ في أجنة ويرقات أسماك

الكارب الشائع (*Cyprinus carpio* (L.1758))

أحمد محسن موجر و زياد عبد الكاظم مزيد و جاسم حميد صالح

مركز علوم البحار - جامعة البصرة، البصرة - العراق

المستخلص - درس تطور الدماغ نسيجياً ومظهرياً في أسماك الكارب الشائع *Cyprinus carpio* في البصرة / جنوب العراق، وذلك باستخدام عدة عينات

من البيض واليرقات خلال التلقيح الاصطناعي في مركز علوم البحار، كانت درجة حرارة المياه في الحاضنات 25-27°م، وكانت فترة حضانة البيض 38 ساعة. مظهرياً ظهرت مرحلة العصبونة بعد ثماني ساعات من الحضانة مع استمرار عمليات التغليف. قسم المخ تدريجياً إلى ثلاثة أجزاء رئيسية (الدماغ الأمامي، الدماغ المتوسط والدماغ المؤخر). كما تميز الدماغ الأمامي إلى الدماغ الانتهائي والدماغ البيئي، وظهرت البادئات البصرية بعد مرور 12 ساعة من الإخصاب وعلى شكل اندلاق من جانبي الدماغ الأمامي ثم تميزت الحويصلة البصرية، ثم تكونت العدسة والتي كانت كروية الشكل، فيما بدا الدماغ الخلفي واضحاً، مع تميز القطع العصبية neuromerse بعد مرور 22 ساعة من الإخصاب. نسيجياً، ظهر لدماغ في عمر 24 ساعة متكوناً من كتلة من الأديم الظاهر العصبي غير المتميزة، بينما في عمر 28 ساعة ظهر أكثر تمايزاً مع وضوح نصفي كرة المخ على جانبي الدماغ الأمامي وهو عبارة عن كتلة من الخلايا العصبية غير متميزة، وبعدها تكونت تدريجياً أجزاء الدماغ مع تميز مختلف طبقاته حتى عمر 20 يوماً بعد الفقس.