ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(6): 474-482

www.fisheriesjournal.com
Received: 02-09-2016
Accepted: 03-10-2016

Hamad Haider Abbas
Astrakhan State Technical University, Institute of Fisheries, Biology and Environmental Sciences; from Ministry of Agriculture, Iraq

Bawazir Abdullah Salem
Hadhramout University, Faculty of Environmental Sciences and Marine Biology, Yemen

Ali Attaala M
Hadhramout University, Faculty of Environmental Sciences and Marine Biology, Yemen

Correspondence
Ali Attaala M
Hadhramout University, Faculty of Environmental Sciences and Marine Biology, Yemen

Embryonic and larval development of (Gattan) *Barbus xanthopterus* (Heckel) from Tigris River system, Iraq Title???

Hamad Haider Abbas, Bawazir Abdullah Salem and Ali Attaala M

Abstract
The study presents preliminary observations on the embryonic and larval development of *Barbus xanthopterus* within the joint laboratory and pond conditions of the middle reaches of the Tigris River in Iraq. Broodstocks were collected from Tigris-Tharthar canal in March and April months. The eggs were obtained through induction of spawning under 22–24 ºC, by using spawning protocols of carp hormones. Perivitelline space formed 35 minutes after fertilization reaching maximum 1.8 mm in diameter at about 70 minutes. The first cleavage occurs in 1 hour 10 minutes, blastula was observed in 7 hours. The formation of first somites was observed 18-20 hours after fertilization. The first hatching occurred 55 hours after fertilization and lasts up to 105 hours. The true larvae develop into the juvenile period within about 60 days after hatching. Here we do not adhere to certain numerous developmental schemes but describing the general course of early development of *Barbus xanthopterus*.

Keywords: Embryonic, larvae, development, *Barbus xanthopterus*, spawning, cleavage, Iraq

1. Introduction
The freshwater fish *Barbus xanthopterus* (Heckel) locally known as "Gattan" is a commercially important and distinctive wild riverine cyprinid in Tigris and Euphrates system and is an indigenous food fish in Iraq.
Inland waters provide people in Iraq with a large amount of animal protein as fish resources. These resources were affected by several reasons such as pollution, overexploitation and physical changes in tributaries of both Euphrates and Tigris rivers. Changes in migration ways, shortage of water supply in nursery grounds were cause in reducing new recruitment of Iraqi commercial fishes. These reasons in turn increase the stock of the noncommercial fish species and cause in acute competition on the expense of the stock of the local commercial species (e.g. Gattan). Damaging the migratory scheme, resulted in reduce or ban of mature fishes to reach the natural spawning grounds in mid and high rivers and its tributaries. Other ecological parameters such as water depth, current, become worse and in turn the spawning ground not suitable for reproduction and growth of larvae. Continues shortage of water and construction of dams in the basins of Euphrates and Tigris rivers make it difficult and not applicable to reconstruct and save spawning grounds [1, 2].
Due to the above reasons, the suitable way to save and compensate the stocks of Gattan and other barbell fish in the Euphrates and Tigris rivers and its tributaries in a certain balance is the artificial breeding. This controlled methods applied to reproduce larvae of a suitable sizes and physiological condition that can live safely when release in the natural habitat [3, 4, 5].
Based on the above scientific background, this paper aimed to study of early embryology and larval development of Gattan-*Barbus xanthopterus*. These studies were conceders as an important link in the Fisheries development programs. The results can be used to increase the efficiency and developed the quality of the results in the controlled artificial breeding of the commercial fish species. Moreover in aquaculture, the adaptation of new species is based on understanding the reproduction cycle, including larval stages [6].
The scarcity of scientific data about life cycle of these species in natural habitat will give specific importance of such subject [7, 8].
Thus the present study is the first time trial to rear in the control condition and to develop the larval developmental stages of the species.

2. Materials and Methods

In this study we took in attention that, in natural habitat, fertilized eggs of *B. xanthopterus* coated by mucus by which fish eggs can adhere temporarily to spawning ground. This ground is characterized as gravely with moderate to slow water currents. The fertilized eggs attached to the ground bed for a particular period during which development is initially takes place. For progress development these eggs reattached off the ground surface and complete evolution beneath and under stones of the spawning bed [7].

Development and growth of fertilized eggs and larval stages of Gattan (*Barbus xanthopterus*) were following up. Eggs and sperms were taken from mature fish specimens (collected from Tigris-Tharthar system) by hormonal stimulation in April. Eggs were artificially fertilized and incubated. Fertilization carries out by dry method. Incubation and development take place in 10-liter jars (Weiss apparatus) [9], water temperature was maintained, between 22-24 °C, as possible as in the natural environment.

B. xanthopterus produce 113.8 thousands eggs by artificial stripping (its average weight about 210 gm). These amount of eggs was produced by a female of total weight 4.2 kg and 64 cm SL from a group of females varied between 2.5 and 5.7 kg total weight (average wt. about 3.8 kg) and standard length 50-66 cm (average SL 61.5cm).

Development variation was directly followed up after eggs fertilization. The steps of embryo development that specified the genus *Barbus* were recorded. To facilitate these steps samples of 10-20 developed eggs or larvae were taken each 2-3 hours during incubation period. During larval development, 10-20 specimens were analyzed each 5 hours and each12-24hours during swimming post larva. Samples were analyzed by dissecting microscope (X10MBC) using different magnifications according to different stages. In all cases micrometer slides were used to measure sizes of embryos and larval stages and then modified to their actual size according to magnification used. Development variations in larval features were then drawing directly by hand throw microscope or using naked eye.

All phases and stages counted in relative to fertilizing moment tell complete organogenesis following Makeeva [10], Balone [11] and Cerny [12]. The age of stages is given here by hours and minutes, it abbreviated here as (after fertilization AF). Measurements expressed as egg diameter and as total length of extracted embryo, larva, and postlarva in millimeters, by micrometer slid (ocular micrometer). Early juveniles measured by Vernier slide. For expounding the identity of measurements and interspecific differences, measurement included most of the body parts, where it available [13]. Regarding feeding habits of early developmental larva and fries of Gattan, it was concentrated mainly on type of organisms in food mass, for evaluation of quality. Thus from each sample it had taken about 10 individual, dissected and investigated, all components identified as detailed as possible, following Duka and Synukova [14]. The main pattern of differentiation is the progress in tissue and organs development [15].

Morphometric measurements were made before preservation with an ocular micrometer accurate to 0, 1 mm. Total length (TL) is defined here as the distance from the tip of the snout to the posterior end of the caudal fin or fin-fold. Final drawings were made with the aid of a camera Lucida. Each drawing was based on a single specimen of the size indicated.

3. Results

3.1 Newly released eggs

Embryology and larval metamorphism in *B. xanthopterus* takes place through different stages. These stages characterized by anatomical variations and organs developments for each fish species.

Ripe eggs of *B. xanthopterus* normally have faint yellow and occasionally tend to orange color. After fertilization color slightly changed to light pink. For the purpose of artificial fertilization, fertilized eggs were cleaned of the sticky substance by washing with tab water for about 15-20 minutes. Average diameter of ripe eggs was 1.1-1.2 mm which increased gradually after fertilization to reach 1.6- 1.8 mm after 35 minutes. In that case privitilline space was formed. At age of 5-6 hours (hr) after fertilization, eggs diameter stabilized at an average value of 1.75mm. The post activation water intake by the eggs lasted approximately 15 minutes. The perivitelline space, formed as a result of this process, and took up some 40-50% of the egg volume.

Embryonic development consists of seven steps leading to hatching. These steps are the zygote period, cleavage period, blastula period, gastrula period, segmentation period, pharyngeal period, and finally hatching.

3.2 Initial development (zygote)

At 30-35mins after fertilization (AF or age), oval membrane was isolated and the blastomeres disc was formed and occupies central position in the lumen. Thin thread-like was noticed to prolonged start from the base of blastodisc vertically towards the yolk diameter. Yolk noticed to be turbid at the mid area. Blastodisc was gradually completed and its size and shape becomes constant at the age of about 50 minutes (Fig.1a). Blastodisc was elevated about 0.2-0.3 mm of the yolk base. No oil droplets in yolk seen at this stage. This stage was the primary period of embryo development which constitutes of formation of blastomeres.

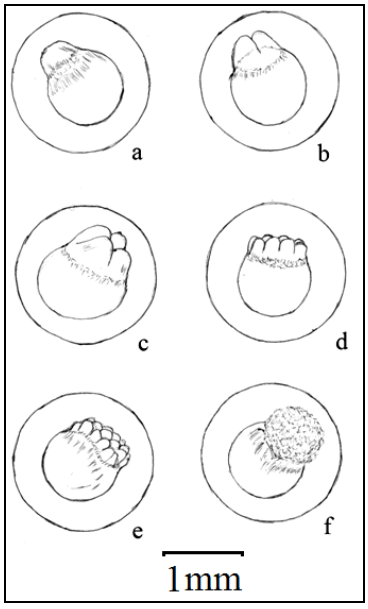

Fig 1: Cleavage period: **a**) Completed Blastodisc at the age of about 50 minutes AF; b) Blastodisc cleavage at the age of 1 hr &10 minutes AF to form two (blastomeres); c) 1hr &15 min, cleavage continued to form four blastomeres; d) 1hr & 30 mins-1hr & 45 min, eight blastomeres; e) 2-2.5 hrs AF, the number of blastomeres increased to 16; f) 4hrs AF, cleavage fastened, cells crowded, and becomes difficult to be counted.

3.3 Cleavage period

This is the cytoplasm plastomer cleavage stage to form the plastula which starts at one hour old. Blastodisc start cleavage at the age of 1 hr &10 minutes by means of transverse groove to form two cells (blastomeres) (Fig. 1b). At this period, longitudinal folds were formed in the cleavage line and the number of rod-like found in the diameter line of yolk was reduced. Granulated cytoplasm not yet formed. At 1hr &15 min, cleavage was continued to form four blastomeres cells (Fig. 1c). At the age of 1hr & 30 mins-1hr & 45 min, eight blastomeres cells were formed (Fig. 1d). At this stage, small granules were seen in cytoplasm and number of threads and folds was increased in the yolk. In this period, yolk was no longer transparence. At 2-2.5 hrs age, the number of cells increased to 16 whereas average diameter of egg was 1.8 mm and both yolk diameter and height of blastomeres was 0.95 mm and 4 mm respectively (Fig.1e). At 3hrs age 32 cells was seen but after that (at 4hrs AF), cleavage fastened and cells crowded and becomes difficult to be counted (Fig. 1f).

3.4 Blastula and gastrulating periods

Cellular growth was continued and at about 7 hrs age blastula situated in the first quarter platform of the yolk sphere (animal pole). Protoplasmic granules were seen in the opposite pole of the yolk (Fig. 2 a). This is the beginning of gastrulating where dermal cells discarded around yolk mass, surrounding and covering the upper pole portion of the yolk. Dermal cells continue discarded to cover about 80% of the surface area of the yolk sphere as thin layer of cellular tissue (Flat marula) at the age of 12-13 hrs AF (Fig.2 b). At 16 hrs age, tissue covers more than 90% of marula surface which is transparent and its internal margins more thick. In this period the embryo body start formed (late gastrula). Gastrula was completely formed at the age of 16 hrs. This progression in the development of an embryo allows for the primary determination of the anterior–posterior and dorsal–ventral axis. After 18hrs of the fertilization, all steps and dermal cleavage processes was completed and embryo plate was formed. Two lateral lines were formed in thicker end of the embryo plate in mid layer of the tissue. These lines were conceder as the boundaries of notochord; moreover it is the first step of organogenesis.

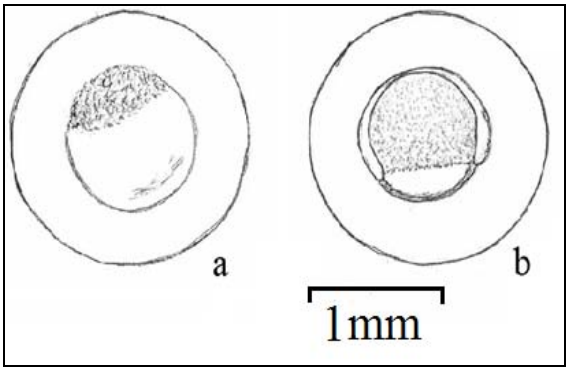

Fig 2: a) 7 h AF beginning of gastrulating; b) Flat marula at 12-13 hrs AF

3.5 Segmentation period

This is the organ initiation stage (organogenesis) which started at about 18 hrs after fertilization. This stage characterized by cleavage of the internal layer of the embryo disc forming equal sized somites in the middle of the embryo body disc. Six segments were formed at the first two hrs – 18-20 hrs AF (Fig. 3a). The somite number was increased and extended to both ends of embryo disc, dabbled in number at the age of 22hrs & 15mins (Fig. 3 b). At this age, few small oil droplets was seen in the middle of yolk which may aid in movement of yolk and embryo and in turn helps in development of gas exchange of embryo and floating of eggs. The optic vesicle primordium begins to show (Fig. 3b). Throughout the next three hours, segmentation in mid layer was accelerated to form 25 somites at 25 hrs AF. Optic funnel becomes more visible and embryo much thicker, completely surrounded the egg yolk (Fig. 3c). At 27 hrs AF, majority of eggs yolk sac was elongated and becomes oval shape where the front portion (spherical) was easily differs from the hind part (elongated) of yolk. In the end of this step, embryo start moving, somites reach the end of yolk sac and eye crystals becomes more visible in the middle of optic vesicle.

At the age of 29-30hrs, neural vesicle evolution was progressed and caudal fin fold elongated. These were initiated accompanied with the initiation of embryo movements in the eggshell developing gas exchange pattern between embryo and outside habitat. Neural vesicle developing was complete at the age of 35 hrs. The movement of yolk fluid was seen makes the embryo more active and the mesoderm layer was completely segmented. At the age of 38 hrs, neural cord elongated starting from neural vesicle backward. The tail curled continuously makes the embryo turned to left and right sides. At this age, the average length of the embryo was about 3.8 mm. At this point the otic (acoustic) vesicle were observed in the fore portion of the embryo; brain folds characterized as three brain vesicles and somite segments was completed (about 46), in the auditory capsule the otoliths are formed, whereas oval diameter not changed (Fig. 3d). Impulse of the elongated, tube-shaped heart (underneath optic vesicle) along with movements of nutriment fluids (from yolk sac) was noted at the age of 40-42 hrs. A backward movement of yolk fluid was first noted beneath notochord and around optic vesicle at the age of 45 hrs. At the same time the head of the embryo was slightly elevated above the front of yolk sac. The embryo becomes more active at the age of 48-49 hrs and yolk fluid movement was clearly visible in the majority of body tissues and parts, in the head in front of eyes. Eye lens was clearly visible at this age as well as peduncle of tail area was distinguished from the rest of body area.

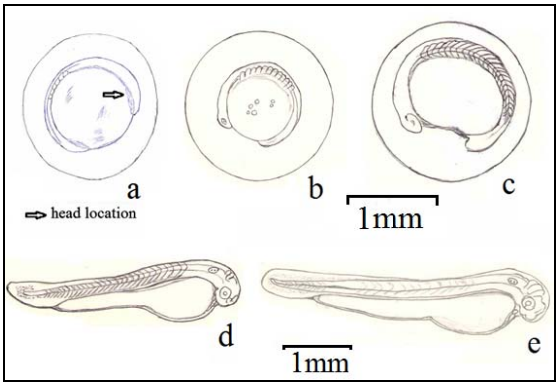

Fig 3: a) six segments formed at the age of 18-20 hrs AF; b) twelve segments at 22hrs AF age with few small oil droplets; c) 25 somites at 25 hrs AF, optic funnel becomes visible, embryo completely surrounded the egg yolk; d) extracted embryo at age of 38 hrs AF; e) Newly hatched larva – 52-55hrs AF (L 3.1 mm body length).

3.6 Hatching

At the age of 51 hrs, early hatching takes place where some free embryos (Prolarva) were released. Hatching of these embryos was accelerated in the next few hours. These embryos was fast swimming and jumping and has characterized by the following morph metrics: 52-55hrs old (Fig.3e), the average total length was 4.4mm, the maximum body height was 0.6 mm, height of fore portion of yolk sac was 0.45mm, height of the elongation of yolk sac was 0.3mm, total length of yolk sac was 0.3 mm, length of tail was 0.9mm, eye diameter was 0.15mm, the number of body segments was 45-47 and no sign of pigment cells. In the next hrs, hatching was continues and no changes noticed in the shape of embryos.

At the age of 59-60 hrs, embryo tends to be more quite than at the period just before hatching. At this age, yolk sac was quickly absorbed and the base of pectoral fin was clearly seen at the end of fourth somatic segment. A penetration of blood fluid between segments was first noted at the age of 61 hrs and mostly evident between the 10^{th} and 11^{th} segments. At the age of 66 hrs, nutriment fluid in blood vessels becomes light purple in color in the position between yolk and notochord and at the base of tail. At this age formation of the base of pectoral fin was noted as a pectoral bud, even in unhatched embryos (Fig. 4a).

At the age of 70 hrs, no particular change in the embryo body whereas hatching continues. Free embryos swim actively forwards and upwards for seconds and finally collected in incubation machine by means of water current. More than 30% of incubated eggs were hatched at 72 hrs old.

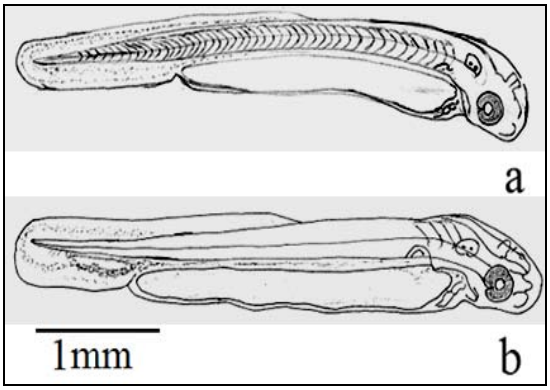

Fig 4: free larva: a) formation of the pectoral fin base, the age of 66 hrs AF; b) larva of 5 mm total length - at 74 hrs old.

3.7 Free embryo

At 74 hrs old, the majority of *B. xanthopterus* eggs were hatched but unhatched ones bearing completely developed embryo. At this age, larvae has the following characters: The acoustic vesicle appeared at the first somite segment, total length - 5 mm, total height of the body - 0.5 mm, yolk sac becomes thin and elongated, head pointed forward, the average of somatic segments – 44; sign of mandible base at the margin of yolk sac and pericardium and gas bladder not yet formed (Fig. 4b).

3.8 Pharyngula fold

At 81 hrs old, eye coloration was initiated and gradually becomes dark, inferior lip clearly formed and gill slit with pharyngeal folds develops into first gill arch well seen from bottom view. Also blood motion was clearly noticed and the base of pectoral fin occupied the second somite segment. It is important to note that the majority of embryos hatched between 70-80 hrs time period after fertilization.

At the age of 90-91 hrs, margins of inferior lip clearly distinguished but no movement. Nostril opening was also primitively formed and seen as very thin slits as well as hyaline gill arches. At this time, eyes become darker and numbers of folds in brain increased. The first melanophor cells created at dorsa- midline position of the body in faint color pigments (Fig.5).

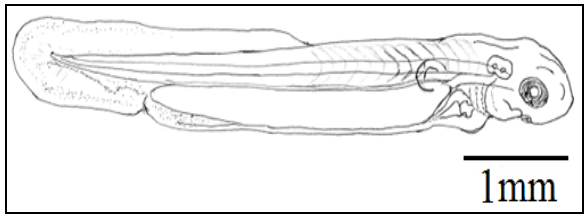

Fig 5: larva at age of 90-91 hrs AF with the average 5.4 mm total length

Figure 5 shows also a free embryo after hatching (3-4 days AF) with the considerable following measurements: Total length - 5.4 mm, head length (to the base of pectoral fin) 1.1mm, length of yolk sac - 3mm, eye diameter (horizontal) - 0.28mm, tail length - 1.35mm, length of dorsa-caudal fin fold (which started at the 14^{th} somite segment) - 2.9mm, body height (At the base of dorsal fin layer and at the 14^{th} somite segment) - 0.86mm, height of yolk sac -0.28mm and height tail (at the end of notochord) - 0.7mm.

3.9 Larval period

At the age 100-102 hrs, pectoral fine increased in length and the inferior lip moves from time to time. At this time the first branched melanophor cell was initiated dorsally on the acoustic vesicle area. At this time of life, the free embryos of *B. xanthopterus* was started its larval stage which initiated by hibernation. At this phenomenon the embryo becomes less active and moves passively by means of water currents (beginning of resting stage). During this period, embryos were grows considerably.

At 116 hrs old, the number of branched pigment cells (melanophor) was accelerated in low dense. It is visible up the yolk sac underneath notochord and above the acoustic vesicle and both on dorsal and base of the pectoral fin. At this time, the buccal parts frontally prolonged, mouth becomes semi-inferior.

At 128 hrs old the reduction of yolk material in the yolk sac clearly noticed, it become more elongate. Tube-like shape was visible in the remaining of yolk sac which represents the developing digestive tube. Faint red spot, was remarked upward the front portion of tubular yolk sac. This spot may represents developing liver or kidney. At this age, hyaline branches of gill filaments were formed on gill arches. Arch membrane (operculum) concaved to cover the majority of gill lumen (approximately third gill arch) and showing continuous movements. Pigment lines were seen along lateral line area particularly in hind part of the body where the base of caudal fin clearly noted. Larval bodies have hyaline coloration. The hibernation period of larvae was completed at the age of 150 hrs. Thereafter, the first larvae started to become active again and swim actively by pectoral and caudal fins. Larvae were continuously leaving hibernation and become active till the age of 165 hrs.

Before completion of hibernation, organogenesis was

continually takes place where the red spot becomes clearer. It is underneath the fourth somatic segment and upward and left to the intestinal tube. Underneath of this spot, other yellow spot was formed (which is the base of gall bladder) in the right side of the tube. At the age of 143 hrs, the first larva was noticed bearing primordial of gas bladder as gas bubble underneath the 5th and 6th body segments. Later on gases was continuously absorbed in the air sac. By 1-2 hrs the air sac prolonged to occupy the area of 2-6 somatic segments. The larva at this time of life (143 hrs (about 10days)) characterized as:

Total length - 7.8 mm, head length - 1.5mm, horizontal eye diameter - 0.43mm, length of acoustic vesicle was 0.43mm, lengthy of yolk sac - 4.1mm, length of abdominal fin fold was 3.3mm, length of dorsal fin fold - 4.6mm, length of tail - 2.1mm, length of rostrum was 0.3mm, maximum height of head - 0.97mm, height of body at the end of the head was 0.9mm, height of body in front of dorsal fin fold - 0.9mm, height of peduncle (at the end of notochord) - 0.86mm, and width of forehead was 0.3 mm.

At the age of 158 hrs, still there are some hibernated embryos with the remaining of yolk sac and the size of air sac was increased, fills with air to occupy more than seven somatic segments. The fourth gill arch still not covered by operculum. The tissue accumulation of caudal fin was increased. Same phenomenon was noted at the position of dorsal and anal fins at the age of 165 hrs AF. At this age eye colors becomes darker and pigment cells distributed on the upper area of air sac in sparse manner.

This age seems to be a transition period for larva from feeding by yolk to external feeding where operculum covers the fourth gill arch at the age of 173-175 hrs (Fig.6a). In such case, the yolk sac has only a trace of yolk material by some larvae and completely absorbed by the others at the age of 185 hrs. Some rotifer cells were observed in the alimentary tract that occupies the remaining of yolk sac which elongated to anus. The alimentary tract was clearly visible through hyaline clear body; the air sac is located under an 8-9 somite segments. The brain noted in its complicated form and its deferent parts was clearly distinguished. Lips become clear and thick.

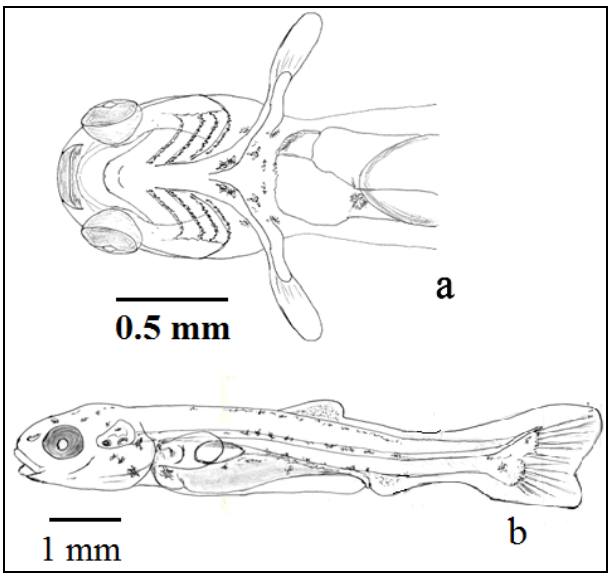

Fig 6: a) operculum covers the fourth gill arch, the age of 173 hrs AF; b) 8.9 mm standard length at 360 hrs AF old

At the age of 207 hrs (8 days and 5 hrs), the forming of larval stages was completed by totally absorption of yolk and all larva externally or exogenously feeding. Pelagic larva known by forming of the base of dorsal fin above the 15th somite, folds of caudal fin was deepened and density of visceral tissues in caudal area was increased. It is easy to see some short neuromast copula on both sides of head, operculum and on the lateral line. The number of the sensory neuromasts on the lateral line was 37. The average body length at this age (8 days) was 7.9 mm and total weight was 3.3 mg.

In the next hundred hours, development of *B. xanthopterus* was restricted only on the increment of morphometric characters but no organogenesis developments. During this period, the larva was fed actively which is pronounced by fullness of alimentary tract with rotifers and diatoms. Also visceral tissue of dorsal and anal fins is well seen at this time age.

At 280-300 hrs old, the average length of the pelagic larva was 8.6 mm. For part of the air sac starts to be distinguished for the majority of larvae.

At 360 hrs old, coloration of the pelagic larvae was still hyaline helps to see the alimentary tract that full with food due to intensity of effective external feeding. Fins still not yet isolated except slit separation in both dorsal and anal fins (Fig.6b). Both lops of caudal fin was slightly separated and bend upward with the end part of notochord. The number of vertebrae was 44, 14 of them were caudal vertebrae. The pelagic larvae at this age were very active with total length of 8.9 mm and total weight equal to 6.2 mg. The melanophor cells were faint in color and slightly distributed through the body. It is denser on the rostrum and along the midline of the body.

Approaching to the age of 406 hrs AF, melanophor cells was increased in number and size. The rostrum was elongated, head was relatively, and mouth becomes totally terminal. The melanophor cells become denser on the operculum and on top surface of the alimentary tract and air sac. Calcification of operculum (opercle) was identified; initiation of the base of pelvic fin within the longitudinal pelvic fin fold was also noted. Dorsal fin was well formed with seven fin rays. Anal fins, with clear rays as well as caudal fin were well identified. Upper mouth lip was terminated and becomes longer than the lower one (Fig. 7a). Larvae at this age were mainly fed on phytoplankton (diatoms). Measurements of larva at this age (17 days AF) was: Total length- 12.1 mm, standard length – 10.5 mm, head length – 2.5 mm, horizontal eye diameter – 0.68 mm, length of rostrum – 0.55 mm, vertical eye diameter – 0.6 mm, forehead width – 0.8 mm, distance between rostrum and dorsal fin origin – 5.26 mm, height of head – 1.8 mm, height of body in front of dorsal fin – 1.3 mm, length of tail and peduncle – 3.66 mm, height of peduncle – 0.55 mm, and height of tail – 1.6 mm.

The larvae were mostly totally developed at the twenty-first day of age (20 days and 16 hrs AF or about 18 days AH) (Fig. 7a). Dorsal fin was of semi cercal shape with eight branched fin rays anlagen. Anal fin also formed but still connected with dorsa-ventral fin fold. Pelvic fins were completely isolated of the abdominal fin fold which is still wide and long. Center of the operculum bone was completely calcified with clear and active outer leather tissue. Nostril was also isolated and air sac becomes bi-chamber as seen in the adult fish. Vertebrae and rips connection was completely developed. The head was relatively big about 23.1% of the body length.

By the age of 660 hrs (27.5 day), larvae developed to the

feature of *B. xanthopterus* fingerling but with some larval characters. Although dorsal, anal and pelvic fins are well developed, parts of dorsal fin folds still connected with folds of caudal, anal and pelvic fins.

The body is still transparent when the gills and otic vesicles with the advent of two tiny otoliths can be seen through the operculum. The melanophor cells were thoroughly aggregated in the top of head and trunk area in patches at the outer lines of the somite segments (Fig.7b). At this age, at the end of the snout and in the corners of the mouth (upper lips) being laid tubercles of the future barbels. It can be well diagnosis by prolongation of lips and when mouth opened. The average total length and weight of the larvae at this age (27.5 day) is 14 mm and 33 mg respectively.

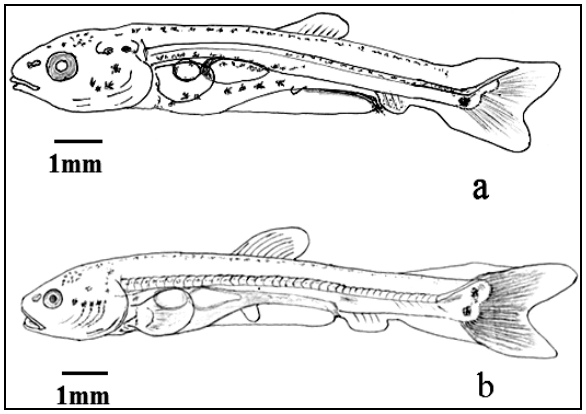

Fig 7: a) Total length- 12.1 mm, standard length – 10.5 mm, age - 406 hrs AF; b) age (27.5 day) is 14 mm and 33 mg respectively**.**

Growth in size of these pelagic postlarva was continued with normal external feature of adult fish. The organogenesis was continued gradually in the next 16 days. In this period, study of samples was restricted only on measurements of growth in size and weight and analyses of food and feeding development. The only distinct feature noted that branched pigment cells were thoroughly distributed on the upper half of the body. Pigmentation was more concentrated on top surface of the head.

At the age of 44th day, squamation was started which initiated in the front lateral part of the abdominal half of the trunk. It is also takes place on the beginning of the lateral line, towards the upper side of the pectoral fin. In the position of the lateral line, one line of branched (relatively big) pigment cells was observed which is less condensed and small on the back. Pigment cell was also aggregated beside the base of anal fin and at the end of the body forming a black dot in the end of caudal column which is disappeared with age progress. At this age, spine rays were start forming (three spines) behind the third serrated spinous ray of dorsal fin.

The average measuring characters of specimens at this age (44th day) were: Total length – 25.1 mm, standard length – 19.8 mm, forked length – 22.3 mm, head length – 6.1 mm, distance between the beginning of rostrum to the dorsal fin origin – 10.5 mm, distance from the rostrum to the base of dorsal fin – 11.2 mm, length of peduncle – 4.6 mm, distance from the end of base of dorsal fin to the end of trunk – 7 mm, distance between pelvic and anal fin – 4.2 mm, length of pectoral fin – 3.7 mm, length of rostrum – 1.4 mm, eye diameter – 1.86 mm, distance behind eye – 2.55 mm, height of head at position mid of eye area – 3.5 mm, height of head at its hind margin – 4.4 mm, height of body in front of dorsal fin – 3.95 mm, height of peduncle – 2.1 mm, length of anal fin – 1.4 mm, height of anal fin 2.8 mm, length of pelvic fin – 2.8 mm, length of upper barbell – 0.46 mm, and length of lower barbell – 0.93 mm.

Regarding physiological and anatomic development of organs at this age (44th day) was as following:

Digestive system: alimentary tract of bended form consist of three lops. Gas bladder situated above of the alimentary tract and connected by tubular tissue on the pharyngeal area.

At this age (44 days) development of larvae was completed and beginning of early squamated juvenile stage. Juvenile stage is characters by active feeding and fast growth.

During the next five days (49 days old), the number of scales on the lateral line were 47, pharyngeal was completely formed and gill rakers was well identified. The standard measurement at this age are (Fig. 8): Total length – 36 mm, standard length – 29 mm, Total body weight – 384 mg, length of head – 8.5 mm, eye diameter – 2.3 mm, length of rostrum – 2.5 mm, distance behind eye to the end of operculum – 3.4 mm, length of upper lip – 1.6 mm, height of head at mid of eye – 4.9 mm, height of the head at the end of operculum – 6.8 mm, distance between rostrum and dorsal fin – 15.1 mm, distance from rostrum to pelvic fin – 15.5 mm, length of peduncle – 6.5 mm, distance between rostrum and anal fin – 22.2 mm, height of body at the beginning of dorsal fin – 6.8 mm, height of peduncle – 2.97 mm, length of pectoral fin – 4 mm, length of the base of dorsal fin – 4.1 mm, height of dorsal fin – 6.2 mm, length of pelvic fin – 4.4 mm, length of base of anal fin – 2.1 mm, height of anal fin – 4.6 mm, length of barbell – 1.1 mm, length of alimentary tract – 35.6 mm, number of scales on the lateral line – 47, the number and formulae of pharyngeal teeth – 4,2,1,-1,2,4, number of vertebrae (including atlas vertebrae) 42, the number of gill rakers in the first gill arch were 5 as well as other 15 in bud form.

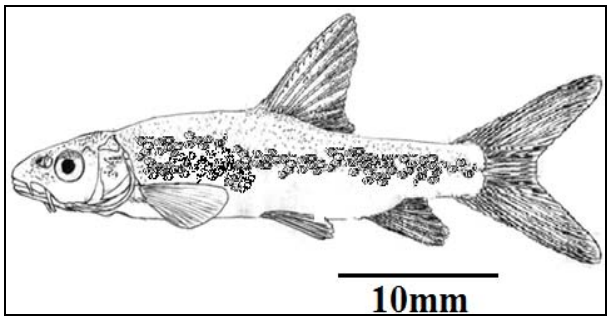

Fig 8: age (44 days) development of larvae was completed, beginning of early squamated juvenile

Development of squamated juvenile was continued towards organogenesis of the adult fish to get the characteristic of *B. xanthopterus* species.

At 0the age of 60-66 days (Fig. 9), general characteristic of the species (male and females) was completely identified. Coloration was limited only in the upper half of the body (above lateral line). Pigment cells were relatively big and branched at the end of lateral line at the base of caudal fin. Maxillary barbells were relatively long, the posterior on to reach mid of eye orbit. All fin rays was completely developed at this age. Also 7-9 posteriorly recurved spikes at the serrated third hard ray of dorsal fin were completely formed; by this age metamorphosis was completed, total length-at-age (L_T) here is best described by the exponential equation: $L_T = 5.103\ e^{0.0016t}$ ($r^2 = 0.938$) (Fig.10).

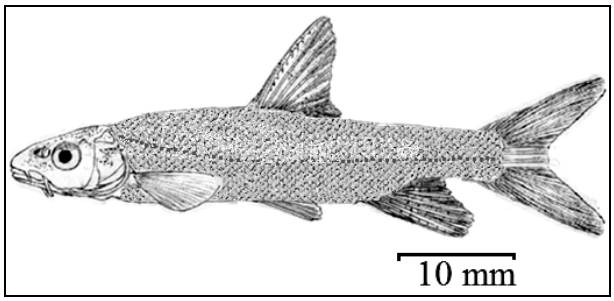

Fig 9: 63 day after hatching squamated juvenile representing of the adult characteristic of *B. xanthopterus* species – 43 mm standard length

The average total mass was 1000 mg, total length – 52 mm, standard length – 43 mm, number of scales in the lateral line – 54-56, number of scale lines above the lateral line – 9, under lateral line theirs 6 lines. There were three spiny rays in dorsal fin and one primitive ray (which disappear later on) as well as 8 cartilaginous branched soft rays. The anal fin has 3 hard rays and other 6 branched ones as well as 19 branched rays in both lop of caudal fin. The number of fin rays and spines were standard taxonomic feature of the species *B. Xanthopterus* adults [7].

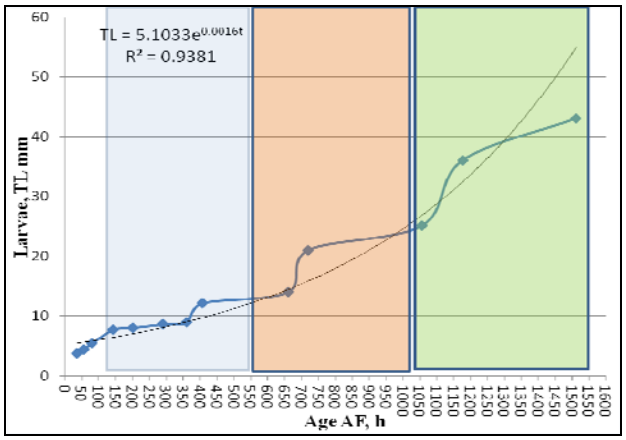

Fig 10: The length-at-age, exponential regression for *B. xanthopterus* larvae, ages 1 – 63 days AF

4. Discussion

Due to the fact that we are in passed sections thoroughly and for the first time described the initial development of the Gattan *B. Xanthopterus*, in this section we will concentrate on some important issues.

The individual operating fecundity of Gattan was relatively low, where not more than 42-45% of absolute fecundity during the activities of artificial reproduction and natural stimulation in places of natural reproduction [17, 18].

One of the key questions in studying early stages of fish is identification problem. Allocate the cyprinid larvae, in many cases, particularly complicated, because of their similarities, so there is a definite need for morphological description of their larvae [19, 20].

We believe that within the sub-period or period one can allocate steps. Theory of qualitative 'etaps' (ontogenetic intervals) of fish was developed by Russian scientists academician school of Severtsov [21], Kryzhanovskii [22], and Vasnetsov, [23]. At each step, the body is characterized by a specific adaptation to the environment, i.e. definite structural features, breathing, nutrition, and growth. During the phase (steps) the body is growing, but significant changes in its structure and relationship with the environment does not occur. In this case the properties are developed that ensure the transition to the next step. Stage may refer to any current moment of development. How would it can be formulated, we find no strong common agreement about phasing of the embryonic and larval development [24-29], so we combining the terminology come up with a detailed description of the initial stages of the development of this species, particularly that this description is implemented for the first time. Nevertheless we comprehensively defined the nature of *B. Xanthopterus* development for more than 2 month AF.

So we are here, in this presentation does not follow particular scale of development of embryos and larvae of fish, as in our direct and continuous observation we have not noticed an milestone indicating developmental changes in the morphological, or anatomical, deserve to be considered as a stage or can be isolated as a step change in the form or anatomical structures of larvae, except changes related to ecobiological, behavioral consequences and genetic factors. Maybe all this is because of constant water temperature in our hatcheries [29].

Anyway, the study revealed that there was a very important fact that the crude carp pituitary extract used in this study could commonly employed in induced propagation procedures for all wild barbell species.

Herein as we prefer to call the main developmental periods:

- Embryo starts from the moment of fertilization within the egg-shell and continues until the egg has hatched. While hatching has profound ecological and behavioral consequences, it nevertheless occurs over a wide range of times [e.g., 48–72 hours post-fertilization (hpf)] and developmental morphologies in zebrafish and many other organisms.
- Free embryo or "yolk sac larvae" between hatching and the first food reception.
- True Larvae, the period from the start of the external feeding until the formation of a constant natatory behaviors (fins configure and their movements effectiveness)
- The transition from a larval phase into juvenile and adult lifestyle often includes a reconstruction of the larval basic body plan, leading to significant adult morphological changes.

Many studies demonstrated that the optimal temperatures, which were in the range of 22–25 °C, ensured proper development and resulted in hatched individuals having the highest values of condition factors (> 94%) [30, 31].

In general, eggs of *B. xanthopterus* have shiny pink color which quit differ of the color of *B. Sharpeyi* eggs that yellowish dusky [16].

Segmentation period here is covers the development from embryo cleavage of internal layer embryonic disc, forming somites of the same size in the middle of the embryo disk, till the moment of hatching.

A mixed feeding period can be defined as a time when yolk reserves are reduced and the larval fish must commence external feeding. Mixed feeding usually occurs between the initiation of external feeding and the complete resorption of the yolk sac. In our results *B. Xanthopterus* had noticeable transition rate of the larvae to exogenous feeding after absorption of the yolk (~165 – ~185 hrs AF (4-5 days after hatching)) that can be explained by a deficit or even the absence of the oil globules which is considered to be one of the most important sources of energy during this larval period [32, 33].

Latter significantly less than that of many cyprinids species larvae - *Leuciscus cephalus*, *Barbus luteus* and in which expiration of yolk sac observed by about the 8th day after hatching [34, 35], but almost equally to *Barbus sharpeyi* - 5 days [16] and more than *Barbus grypus* and *Barbodes gonionotus* - 3 days [36, 37]; most likely it depends on the temperature at which larvae appeared in the natural environment.

The pigmentation throughout the embryonic and larval organogenesis was characterized by weak pattern along almost the entire body.

Many of the few researchers studying the formation of scale cover in juvenile fish assert that the site of the first scale appearance can be on several parts of the body in bony fishes (either on the caudal peduncle, lateral line, belly or pectoral peduncle or even around the anus). In our results the squamation begin by several scaly flakes appears at the site under the sensory line forepart, at the upper edge of the pectoral fin. Two days later, a series of scales appears at the mid of the caudal peduncle. Scale formation on this site proceeded anteriorly along a midlateral line, and met with the scales from the first locus on the middle of the trunk. While this event happened here only nearly 41th day AH at 25mm total length larva, it happened at 33th day about at the same site in 31mm total length of *Barbus sharpeyi* from Iraqi waters [16]. However, in some teleost species the squamation pattern is initiated in different foci at the same time [38, 35]. Usually scales can indeed start to form either in the anterior region in some cyprinids or the posterior locations as in some cyprinids and cichlids (McCrimmon and Swee, 1967; Fujita, 1971; Armstrong, 1973). [39, 40, 41]

As shown here, *B. Xanthopterus* can be easily maintained, bred, stripped and raised in large numbers under laboratory conditions.

It must be noted that in pattern of Gattan larvae growth one can observed conspicuous after hutching growth acceleration at nearly equal 3 intervals (Fig. 10).

In the present study, another species of the Barbus genus had a satisfactory response to artificial reproduction and presented relatively high fecundity [42, 36, 16].

5. References

1. Anonymous. The Techno- Economical Report of Iraqi water resources utilization. Chapter 3, Fishery Problems. "Gydrorybproject". Ministry of Fisheries wealth, USSR. 1982, 335-347.
2. AM Ali, NK Tomas. Some Ecological Aspects of Bizz *Barbus Esocinus* Heckel, 1843 (Actinopterygii, Cyprinidae) From Tigris and Euphrates Rivers – Iraq. Tropical Freshwater Biology, 2009; 18(1):27-50.
3. Taylor AA, Britton JR, Cowx IG. Does the stock density of still water catch and release fisheries affect the growth performance of introduced cultured Barbel. Journal of Fish Biology. 2004; 65:308-313.
4. Bolland JD, Cowx IG, Lucas MC. Movements and habitat use of wild and stocked juvenile chub, *Leuciscus cephalus* (L.), in a small lowland river. Fisheries Management and Ecology. 2008; 15:401-407.
5. Pegg J, Britton JR. Effects of inter-and intra-specific competition on the growth rates of juvenile European barbel *Barbus barbus* used in the stock enhancement of UK fisheries. Fisheries Research. 2011**;** 112(1-2):8-12.
6. May RC. Larval mortality in marine fishes and the critical period concept. In J.H.S. Blaxter (ed) The early life history of fish. Berlin: Springer-Verlag. 1974; 3-19.
7. Ali, AM. Study of morphological and biological characteristics of Gattan *Barbus xanthopterus* from Tigris River and Al-Tharthar reservoir. The Arab Guff, 1979; 11(1):181-197. (In Arabic): 3-14.
8. Ali, AM, Tertat, M, Tomas, NK. Morphological and biological characteristics of the fish shaboot Barbus grypus in Al-Tharthar Reservoir and Tigris River. Journal of the Arab Gulf. 1981; 13(3):117-128.
9. Privezentsev YA. Practicum on pond fish culture. High school publishing house. Moscow. 1982, 208.
10. Makeeva, AP. The development of the Aral Barbel *Barbus brachycephalus* Kessler. Problems of Ichthyology. 1958; 11:86 -101.
11. Balon EK. Reproductive guilds of fishes: a proposal and definition. J. Fish. Res. Board Can. 1975; 32(6):821-864.
12. Cerny K. The early development of chub—*Leuciscus cephalus* (L., 1758), rudd—*Scardinius erythrophtalmus* (L., 1758) and roach—*Rutilus rutilus* (L., 1758). Acta Univ Carol Biol. 1977; 1:149.
13. Lange IO, Dmitrieva EN. Methods of ecological and morphological investigation of the development of fish juvenile. The methodical handbook: Research and development of fish reproduction. Nauka press. 1981; 68-88.
14. Duka LA, Sinyukova. Guide for Study of Feeding of Larvae and Fry of Marine Fish in Nature and under Experimental Conditions. Naukova Dumka Kiev, [in Russian], 1976.
15. Vasnetsov VV, Ye F Yeremeyeva, IO Lang, Dmitrieva EN, Braginskaya Y. Etaps of development in semi-anadromous commercial fishes of Don and Volga rivers-bream, carp, roach and pike-perch. Trudy Inst. Morph. Zhiv. Severtsova. 1957; 16:7-76 (In Russian).
16. Ali Attaala Mukhaysin, Jawad LA. Larval development of the Cyprinid Fish *Barbus sharpeyi* (Gunther, 1874). Journal of Fisheries and Aquatic Science. 2012; 7(5):307-319. DOI: 10.3923/jfas.2012.307.319
17. Szypula J, Ali AM. Age and growth rate of *Barbus xanthopterus*, *B. grypus*, *B. luteus* and *Aspius vorax*, in lakes of middle Iraq. IV congress of European ichthyologists. Hamburg, West Germany. 1982; 9:20-24.
18. Uryn BA, Ali AM. Artificial spawning and breeding of hatchlings of *Barbus grypus*, and *B. xanthopteru*. IV congress of European ichthyologists, Hamburg, W.G. 1982, 20-24.
19. Fuiman, LA, Conner JV, Lathrop BF, Buynak GL, Snyder DE, Loos JJ. State of the art of identification for cyprinid fish larvae from eastern North America. Transaction of the American Fisheries Society. 1983; 112:319-332.
20. Pinder AC. Keys to larval and juvenile stages of coarse fishes from fresh waters in the British Isles. Scientific Publication, Ambleside: Freshwater Biological Association. 2001, 60.
21. Severtsov AN. Collection of papers. Akad. Nauk Press, Moscow-Leningrad. 1949, 4. (In Russian).
22. Kryzhanovsky SG. Theoretical foundations of embryology. Uspekhi Sovremennoi Biologii. 1950; 30:382–413. (In Russian).
23. Vasnetsov VV. 'Etaps' in the development of bony fishes. *In:* E.N. Pavlovsky (ed.) Othcherky po Obshtchim Voprosam Ikhtiologii, Acad. Nauk SSSR Press, Moscow-Leningrad. 1953, 207–217. (In Russian).
24. Kimmel CB, Warga RM, Schilling TF. Origin and

organization of the zebrafish fate map. Development. 1990; 108:581-594.
25. Fuiman LA, Poling KR, Higgs DM. Quantifying developmental progress for comparative studies of larval fishes. Copeia. 1998; 3:602-611. Doi: 10.2307/1447790
26. Leis JM, Carson-Ewart BM. The larvae of Indo-Pacific coastal fishes: a guide to identification (Fauna Malesiana Handbook 2) Brill. Leiden. 2000, 850.
27. Penaz M. A general framework of fish ontogeny: a review of the ongoing debate. Folia Zoologica. 2001; 50:241-256.
28. Trevarrow B, Robison B. Genetic backgrounds, standard lines, and husbandry of zebrafish. Methods Cell Biol. 2004; 77:599-616.
29. Parichy DM, Elizondo MR, Mills MG, Gordon TN, Engeszer RE. Normal table of postembryonic zebrafish development: Staging by externally visible anatomy of the living fish. Dev. Dyn. 2009; 238:2975-3015. doi:10.1002/dvdy.22113
30. Rezničenko PN, Gulidov MV, Kotlarevskaâ NV. Vyzivanie ikry lina *Tinca tinca* (L.) pri postoânnyh temperaturah ikubaci [Survival of tench roe (*Tinca tinca* (L.)) under constant temperatures of incubation]. Vopr. Ichtiol. 1968; 8(3):492-499 [in Russian].
31. Geldhauser F. Some aspects of embryonic and larval development of tench (*Tinca tinca* (L.)). Pol. Arch. Hydrobiol. 1995; 42(1–2):87-95.
32. Kamler E. Early Life History of Fish: An Energetic Approach. Chapman and Hall. Fish and Fisheries, London. 1992; 4:287.
33. Fuiman LA. Special considerations of fish eggs and larvae in Fuiman LA, Werner RG. (eds.). Fishery Science, the unique contributions of early life stages. Chapter 1. Blackwell Science Ltd. 2002.
34. El-Finky N, Wieser W. Larval styles of development gills and muscles in larval cyprynids (Cyprynidae: Teleostei). J Fish Biol. 1988; 33:135-145.
35. Calta M. Morphological development and growth of chub, Leuciscus cephalus (L.), larvae. J. Appl. Ichthyol. 2000; 16:83-85.
36. Sahinoz E, Dogu Z, Aral F. Embryonic and Pre-Larval Development of Shabbout (*Barbus grypus* H.). The Israeli Journal of Aquaculture–Bamidgeh. 2007; 59(4):235-238.
37. Siddhwartha Kumar Basak, Biplop Basak, Nipa Gupta, SM Mustafizur Rahman, Mohammad Mahfujul Haque. Embryonic and Larval Development of Silver Barb (Barbodes gonionotus) in a Mobile Hatchery under Laboratory Condition. IOSR Journal of Agriculture and Veterinary Science. 2014; 7(4):81-90.
38. Sire JY, Arnulf I. Squamation chronology and scale development in cichlids. *In* Proceedings of the 4th Workshop on Cyclid Biology and Conservation. Ann. Mus. Roy. Afr. Centr. 1989; 257:97-100.
39. Mc Crimmon HR, Swee UB. Scale formation as related to growth and development of young carp, *Cyprinus carpio* L. J. Fish. Res. Bd. Canada. 1967; 24(1):47-51.
40. Fujita K. Early development of the squamation in *Tilapia sparrmanii* Japan. J. Ichthyol. 1971; 18(2):90-93.
41. Armstrong JG. Squamation chronology of the zebrafish (Cyprinidae), *Brachydanio rerio*. Copeia. 1973; (4):823-824.
42. Al Hazzaa R, Hussein A. Larval development of himri, *Barbus luteus* (Cyprinidae: Cypriniformes) reared in the laboratory. Turkish J. Zool. 2006; 30:1-7.