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Morphometric and meristic variations in bream (*Abramis brama orientalis*, Berg, 1949) during larval development

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ABSTRACT
This study was conducted to examine morphometric and meristic characteristics alongside pigmentation patterns of bream larvae, *Abramis brama orientalis*, in four stages of larval development. Morphological characters including total length (TL), standard length (SL), notochord length (NL), head length (HL), head depth (HD), eye diameter (ED) and mouth width (MW) were examined from hatching time through 30 days after hatching (DAH). The results showed morphometric variations in the different larval stages. Growth and development of fins occurred mainly at the Post Flexion stage with the completion of caudal fin at 9 mm TL and pelvic fin at 13.33 mm TL. At all larval stages, the highest concentration of pigments was seen on dorsal, ventral and somewhat on the lateral sides of body in descending order. The highest density of melanophores at larvae yolk sac stage was observed on the yolk sac and the back of head zone, while at preflexion and flexion stages melanophores were dominant on the back of head and on the skin folds which resulted from yolk absorption. At post flexion stage, high density of melanophores was found on the back of the head, bases of fins and caudal fin, while less concentrations of these cells were evident on the lateral sides of fish' body. In conclusion, a clear change in the growth and main morphological characters were observed in postflexion stage. This may be due to the main development of fins, which would suggest enhanced swimming capabilities and also prey capture efficiencies.

Keywords: Larvae, Meristic, Morphometric, Pigmentation pattern, *Abramis brama*.

INTRODUCTION
Fish larvae undergo major morphological changes during the first months of their life. Such changes play an important role at the time of transformation from a post-hatching organism with simple organs to a juvenile with the definitive phenotype (Orton, 1953). Analysis of meristic and morphometric characteristics has been used for different purposes in the ecological studies of fish species. Usage of combinations of morphometric, meristic and pigmentation characters enabled us to identify fish populations (Ihssen *et al*., 1981; Melvin *et al*., 1992). They are also powerful tools for comparing the different developmental stages between and within species and also make the correct identification of the larvae possible. Interspecific and intraspecific variations in morphology is known to be related to genetic and environmental factors (Fowler, 1970; Lindsey, 1988; Turan, 2000; Leavy & Bonner, 2009). Imre *et al*. (2002) and Dunn (2011), for instance, demonstrated adaptive phenotypic plasticity of Galaxias fishes and brook charr by changing their body shapes and fin sizes in response to varied hydrodynamic conditions. Sfakianakis *et al*. (2011) has also studied the effect of rearing temperature on body shape and meristic characters in juvenile zebrafish, *Danio rerio*. Many studies have shown larval development and morphological characteristics in different Cyprinid species (Laurila & Holopainen, 1990; Sakai, 1990; Al hazzaa & Hussein, 2007; Jafari *et al*., 2009; Carrapato & Ribeiro, 2012; Mukhaysin &

Jawad, 2012). Previous larval development studies of fishes in Iran are mainly focused on sturgeons and kutum (Shafizadeh, 1993; Parandavar, 2004; Jafari *et al.,* 2009; Yousefian *et al.,* 2010; Falahatkar & Imani, 2011; Norouzi & Ghorbani, 2013) in which the morphological characters were studied over embryonic development. Bream, *Abramis brama orientalis*, is a migratory semi-anadromous fish which spawns on aquatic plants between March and June. This fish is one of the species of Cyprinidae family and is an important commercial fish in the Caspian Sea. During recent decades, the stock size of bream has considerably been decreased due to pollution, overfishing and spawning grounds destruction (Khara *et al.,* 2009). An extensive restocking program is currently underway for bream in the south Caspian Sea in which a considerable number of fingerlings are being released annually into the rivers. Morphological development of bream larvae has not been previously described despite the importance of such study for management and conservation of this species. It is essential to recognize fish larva in the recruitment studies and such recognition has, therefore, implication in fisheries ecology of a particular species. The present study aimed to describe meristic and morphological developments of bream. Pigmentation pattern and the degree of morphological variations among the three stages (preflxion, flexion and post flexion) were investigated in bream under captive conditions.

MATERIALS AND METHODS

Fertilized eggs were obtained from bream broodstock at the Shahid Ansari Fish Culture and Propagation Center in Rasht through semi-natural reproduction method. Broodstock were injected with Ovaprim (0.2 ml.kg^{-1}for females and 0.1 ml.kgr^{-1} for males) in May 2011 and transferred into an earthen pond for spawning with the sex ratios of two females to one male. The spawned eggs of bream, adhered to the established stems and branches in pond, were collected from different locations of pond and transferred to an aquarium (101×35×50 cm), in conjunction with the vegetation, for incubation under a natural light regime (12D:12L).The water conditions were daily monitored including temperature = 25^{o}C and pH = 8. The eggs were inspected daily to monitor the developmental progress of the embryos. Hatching occurred 9 days after fertilization. The bream larvae began their first exogenous feeding on the second day after hatching (DAH).

The larvae were fed *ad libitum* with egg yolk and soya been latex 8 times a day for the first 15 days, followed by commercial feed (SF1) for another 15 days. The number of feeding was reduced to 4 times a day due to larval growth close to the end of the experiment (Helfman *et al.,* 2009). During the study, 30 healthy larvae were randomly sampled every 2 days and then twice a week until 30 DAH. Specimens were fixed in 4% buffered formalin and measured to the nearest 0.1 mm under a binocular microscope connected to an ocular micrometer (M6c-10). The larval developments were also illustrated by a stereo microscope connected to a camera (SZX12).

Seven morphometric variables including total length (TL), standard length (SL), notochord length (NL), head length (HL), head depth (HD), eye diameter (ED) and mouth width (MW) and meristic characters were measured or counted in four larval stages including yolk sac, preflexion, flexion and post flexion (Ahlstrom & Ball, 1954). All measurements and counts were made on the left side of the fish (Helfman *et al.,* 2009). One-way analysis of variance (ANOVA) was applied to compare morphometric characteristics of fish in different larval stages. The Tukey post hoc test was used to identify significant differences among the various means while the error terms of ANOVA analyses were tested for homogeneity of variance and normality.

Values are presented as means ± standard errors. Statistical analysis was conducted using SPSS (Version 18, Inc., Chicago IL, USA). Differences of $P < 0.05$ were considered statistically significant.

RESULTS

The main morphological and meristic characters appeared during the larval periodwere classified into four stages. Duringthese stages fish underwent major changes as follow:

Table. 1: Morphometric characteristics of bream, *Abramis brama orientalis*, larvae in different larval stages. Values are expressed as mean ± SE. Values in rows with different superscripts noted are significantly different ($P < 0.05$).

Morphometric characteristics	Larval stage			
	Yolk sac(mm)	Preflexion (mm)	Flexion (mm)	Post Flexion (mm)
TL†	6.12 ± 0.45^{b}	7.28 ± 0.05^{b}	7.87 ± 0.09^{b}	12.28 ± 0.62^{a}
SL§		6.94 ± 0.05^{b}	7.50 ± 0.11^{b}	10.54 ± 0.41^{a}
HL‡	1.04 ± 0.14^{b}	1.32 ± 0.02^{b}	1.46 ± 0.03^{b}	2.81 ± 0.21^{a}
MW ˣ	0.24 ± 0.01^{c}	0.25 ± 0.01^{bc}	0.27 ± 0.01^{b}	0.34 ± 0.01^{a}
HD*	0.61 ± 0.04^{b}	0.72 ± 0.01^{b}	0.78 ± 0.01^{b}	1.80 ± 0.15^{a}
ED•	0.35 ± 0.03^{c}	0.40 ± 0.01^{bc}	0.51 ± 0.02^{b}	0.84 ± 0.03^{a}

Yolk sac stage

Hatching occurred at 9 DAH. The newly hatched larvae were transparent (Fig. 1a). In yolk sac stage, the notochord length was between 4.4 and 6.4 mm.

The larvae were characterized by a two-part shaped yolk sac extending from the end of head to the mid-part of the body towards the tail; the posterior part was also more elongated and slender than the anterior part. Yolk sac contents were hexagonal (polygon with six edges) under the microscope. The head was in a straight line of the body. The eyes were large from the beginning of this stage and well pigmented, whereas the mouth was not yet developed and the identification of the sub-terminal mouth occurred in the late period at 6.4 mm NL. The rudiments of the branchial arches did not appear in the yolk-sac larvae and larvae seemed to breathe through skin. The highest density of pigments was evident in dorsal and ventral parts including dorsum of head, forehead, snout, yolk sac while the lowest melanophores distribution appeared on the lateral sides of the body. Two rows of the stellate melanophores were also appeared along the dorsal surface to caudal area. These pigments were larger and clearer than those that appeared on the head region. By the development of larvae, the yolk sac continued to be elongated and changed to a narrow strip. The posterior part of the yolk sac changed into skin wrinkles having the highest pigment density on fish' body. In the remaining steps, the anterior and posterior parts of the yolk sac transformed into viscera and anal fin, respectively.

Preflexion stage

In this stage, the larvae have a standard length varying from 6.8 to 7.1 mm. At the beginning of the preflexion period, the rudiments of the branchial arches appeared (Fig. 1b). The body was more elongated in comparison whit the previous stage. The yolk sac hexagonal contents were not completely exhausted and changed into a part of the internal organs at the end of this period. The pattern of pigmentation was similar to the yolk-sac larvae and the only difference was that the highest concentration of melanophores on yolk sac and dorsum of head.

Flexion stage

The standard length of larvae at flexion stage varied from 7.25 to 7.9 mm. In this stage, the compress form and increased body depth, which is one of the most important characteristics of the adult fish, had not yet been observed (Fig. 1c). Pigment distribution was similar to the previous stages and the melanophores were limited to the lateral line of the trunk.

Post flexion stage

The standard length of larva was between 8.1 and 12.65 mm. In this stage, following the completion of fins, the highest concentration of melanophores was observed on fin-base regions, caudal fin and also on the posterior portion of head (Fig. 1d). Unlike the previous

stages, there were three rows of stellate melanophores on the dorsal surface of body which continues to caudal peduncle. Caudal fin was the most developed fin and made up a significant part of the larval body. The final stage of post flexion (Fig. 1e) was associated with the start of scale development. Fish size reached to 16 mm TL in this stage, in which larva was morphologically more similar to adults, hence is considered as the first size of prejuvenile.

Morphological variables

Morphological characteristics changed considerably during the larval development. The ratio of the head length (HL), head depth (HD) and eye diameter (ED) to total length (TL) reached the maximum value at postflexion stage. HL increased from 16.7% TL at yolk sac stage to 18.6% TL at flexion stage and then to 22.4% TL at postflexion stage. HD also increased with growth from around 10% TL at yolk sac stage to 14.26% TL at post flexion phase. ED increased slowly from 5.7% TL at yolk sac stage to 6.3% at flexion and then to 6.9% TL at postflexion stage. All morphometric variables in post flexion stage were considerably (P<0.05) larger than flexion or preflexion stages (Table 1). However, there was no significant (P>0.05) change in morphometric parameters between flexion and preflexion stages.

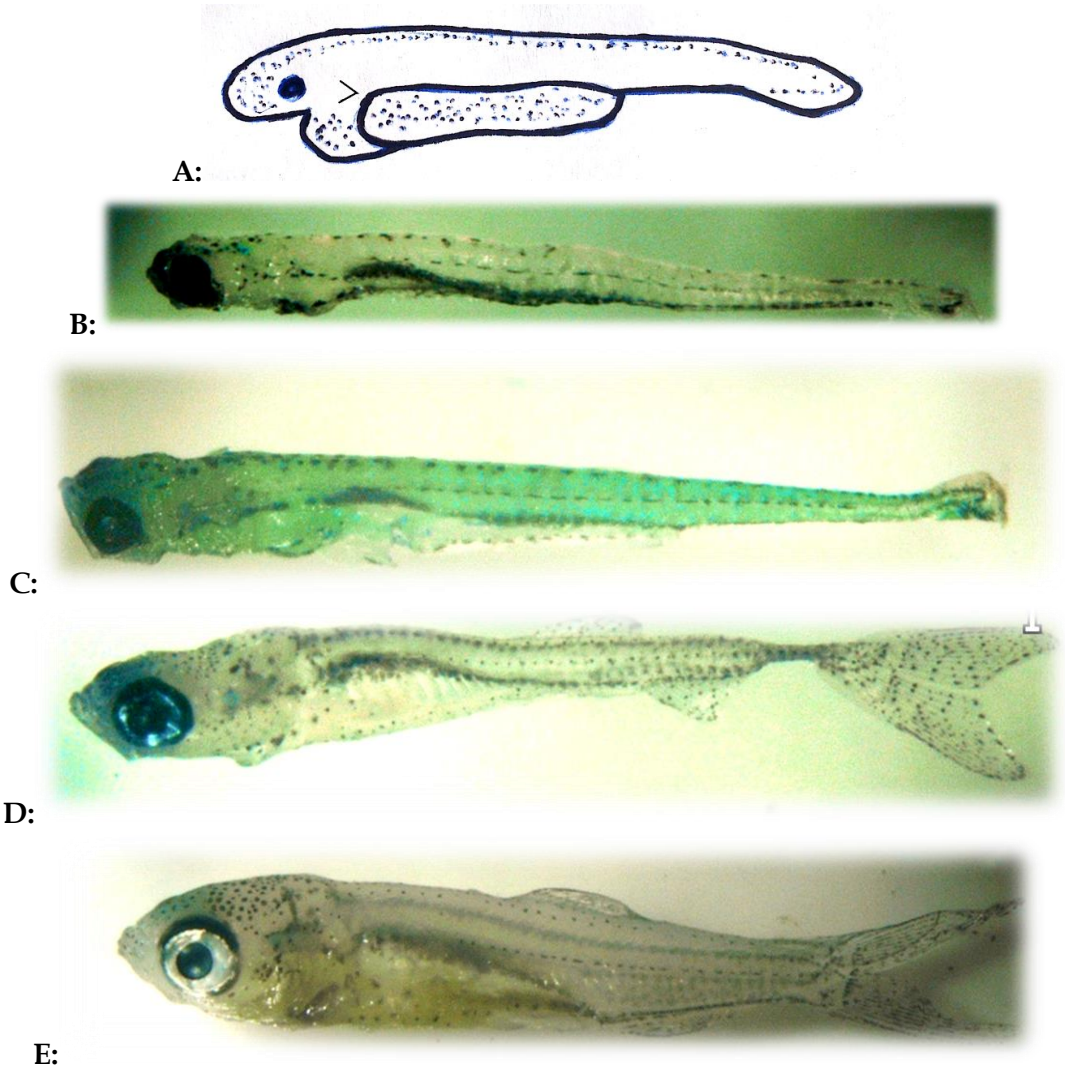

Fig. 1: Developmental stages of bream, *Abramis brama orientalis*, larvae (scale: 800 µm). A: yolk sac larvae, B: preflexion larvae, C: flexion larvae, D: early post flexion larvae and E: post flexion larvae.

Meristic variables of fin rays

Although the most observable fin bud of the newly hatched larvae was the caudal fin bud, the beginning of the pectoral fin bud was also evident in these larvae. In preflexion stage, anal fin bud began to develop at 7.28 mm TL. Dorsal fin bud development started at the beginning of flexion stage, at 7.5 mm TL, while there was no evidence of formation of pelvic fin bud until the end of this period. Formation of caudal fin rays appeared at the end of the preflextion stage, at 7.45 mm TL, continuing to develop at flexion larvae stage and attaining its full form at post flexion stage at 9 mm TL. The length of caudal fin was 2.57mm in postflexion larvae stage.

Both dorsal and anal fin rays began to develop in postflexion phase in which the number of fin rays was 8 and 28, respectively. Pectoral fin rays also developed in this phase. Pelvic fin bud started to appear at 11.9 mm TL and the fin rays continued to develop at 13.33 mm TL. Pre-dorsal and anal fin lengths were also measured at 5.3 mm and 6.65 mm on the average, respectively (Fig. 1).

DISCUSSION

Early life history of fishes is a complex phenomenon of growth and differentiation. In this study we investigated some morphometric and meristic characters as well as pigment cells patterns of bream larvae ranging from 4.9 to 15.66 mm TL at four stages. The succession of these stages involved not only morphological development but also behavioral and functional changes. These changes occur in nearly all systems and organs, when the larva metamorphoses to a juvenile (Tanaka, 1975).

In cyprinids, egg incubation period is short. Therefore many small eggs will be produced if the species live in warm water. The egg diameter obtained for *Abramis brama* (Penaz & Gajdusek, 1979) is smaller than *Cyprinus carpio* (Hoda& Tsukahara, 1971). Such differences can be based on the differences in parent size and ecology (Kamler, 2002). The size range of the newly hatched larvae, moreover, is a function of water temperature variation (Almatar *et al.*, 2000). The range of size of the newly hatched larvae recorded in this study was 4.5-5.2 mm TL, incubated at temperatures between 24 and 25 ºC. Temperature has an important effect on timing of ontogenetic events (Fuiman *et al.*, 1998) and growth of newly hatched larvae in cyprinid species may be applied to improve bream larvae rearing at hatchery systems. A decreased rearing temperature resulted in longer larval durations, reduce growth rates and slow swimming development in larvae . The complete absorption of the yolk sac in bream larvae took place 4 days after hatching at about 7.15 mm TL. This is faster than the duration taken by other cyprinid species; e.g. *Leuciscus cehalus* 8 DAH (Calta, 2000) and *Barbus sharpeyi* 5 DAH (Mukhaysin & Jawad, 2012). Depletion of the yolk sack and transformation period to exogenous feeding is considered to be a critical period in fish development influenced mainly by the environmental conditions such as food availability, temperature, predation (Blaxter, 1988) and may coincide with some significant improvements in the respiratory system and swimming activity.Cyprinid species are closely similar to each other at larval stages and are difficult to identify.

Fuiman *et al.*, (1983) stated a number of characteristics which can be used to separate cyprinid larvae. The flexion and post flexion stages in many fishes are accompanied by rapid development of fin rays, change in body shape, in locomotive ability, and in feeding techniques. The number of fin rays can be considered as an important indicator for larval identification. In this study, post flexion bream larvae showed 28 rays in anal fin and 8 rays in dorsal fin, while in crusian carp, *Carassius carassius*, they are 6 and 16-19, respectively (Trnski *et al.*, 2005).

Description of pigmentation patterns is also a constant feature for the identification of early larvae (Simon & Vondruska, 1991; Meijide & Guerrero, 2000). The pigmentation pattern is typical for cyprinid fishes with dark melanophores forming lines. The bream larvae

have a specific pigmentation pattern with three rows of stellate melanophores along dorsal surface to caudal area at 6.8 – 8 mm TL, whereas the common carp, *Cyprinus carpio,* the larvae have yellow pigments and a silver string at this length (Hoda & Tsukahara, 1971; Economou *et al.,* 1991), and in crusian carp, *Carassius carassius,* pigmentation is specific with only two rows of melanophores from head to tail along the dorsal edges of the body (Laurila & Holopainen, 1990). The presence of stellate melanophore, therefore, could be used as a characteristic to identify the species. As in the most cyprinids, eye pigmentation in bream takes place in the early larval period. Mouth opening and eye pigmentation are events that appear almost simultaneously (Lasker *et al.,* 1970). In bream larvae, eye pigmentation started in the beginning of the yolk sac stage at about 4.9 mm TL, while the mouth developed in the late period of this stage at 7 mm TL, indicating their ability to apprehend exogenous food items in the late yolk sac stage. In conclusion, a clear acceleration in growth and main morphological changes were observed in postflexion stage. This may be due to the main development of fins, which would suggest enhanced swimming capabilities and also prey capture efficiencies. The results of this work might contribute to a better understanding of the larval development of bream to explain some aspects of the early life history at hatchery conditions and help identify some wild cyprinids in larval stages.

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تغییرات مورفومتریک و مریستیک در ماهی سیم (*Abramis brama orientalis*, Berg, 1949) در دوره تکامل لاروی

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چکیده

این مطالعه اطلاعاتی در مورد ویژگی‌های شمارشی و ظاهری، در کنار الگوی رنگدانه‌ها در لارو ماهی سیم (*Abramis brama orientalis*) در طول ۴ مرحله توسعه لاروی ارائه می دهد. ویژگی‌های مورفولوژیکی شامل طول کل، طول استاندارد، طول نوتوکورد، طول سر، عرض سر، قطر چشم و عرض دهان، از زمان تخم‌گشایی تا ۳۰ روز پس از تفریخ بررسی شدند. نتایج حاصل، تغییرات مورفومتریک را در مراحل مختلف لاروی نشان داد. تکامل و توسعه باله‌ها اساسا در مرحله post flexion و با تکمیل باله دمی در طول ۹ میلی‌متری طول کل و تکمیل باله شکمی در طول ۱۳/۳۳ میلی‌متری طول کل اتفاق افتاد. در کل مراحل لاروی، بیشترین تجمع رنگدانه‌ها به ترتیب روی قسمت پشتی، شکمی و تا حدودی در دو طرف بدن دیده شد. بیشترین تجمع ملانوفورها در لارو دارای کیسه زرده، در کیسه زرده و پشت ناحیه سر مشاهده شد در حالیکه در مراحل preflexion و flexion ملانوفورها بیشتر در پشت سر و چین‌های پوستی که در نتیجه جذب زرده ایجاد شده بودند مشاهده شدند. در مرحله post flexion بیشترین تجمع رنگدانه‌ها در پشت سر، پایه باله‌ها و باله دمی بوده، در حالی که تجمع کمتری از آن‌ها در دو طرف بدن قابل رویت بود. به عنوان نتیجه‌گیری نهایی، یک تحول مشخص در رشد و تغییرات مهم مورفولوژیکی در مرحله post flexion مشاهده شد که ممکن است در نتیجه رشد کامل باله‌ها و متعاقب آن تسهیل در شنا کردن و افزایش کارایی شکار باشد.

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