Larval Development of Himri, *Barbus luteus* (Cyprinidae: Cypriniformes) Reared in the Laboratory

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Abstract: Larval development of the himri barbel, *Barbus luteus* Heckel, reared at 19-22 °C in natural daylight is described. Morphological and some functional character appearances were similar to the main ontogenetic steps of development in most cyprinids. Small hatched larvae attained both metamorphosis and a length of 10.5 mm after almost 35 days. Relative growth of the selected body proportions demonstrated typical priorities in growth compared to the length. An ontogenetic index was inferred from applying age and length criteria at metamorphosis as a scale for ontogenetic events.

Key Words: *Barbus luteus*, Euphrates, Tigris, larva, ontogeny

Introduction

Morphological and functional development accelerates in fish during the larval period until most of the typical adult features appear. Early life ontogeny in fish has often been described morphologically as a system of developmental periods and steps (Vastnetsov, 1953; Peňáz, 1974, 2001; Balon, 1975, 1999; Peňáz and Gajdušek, 1979; Peňáz et al., 1986), or quantitatively scaling developmental events against a selected criterion (Rombough, 1985; Fuiman, 1994; Fuiman et al., 1998). Chronological descriptions of key developmental events during the early stages of fish life within their normal water temperature range, or those normally used in hatcheries, could improve the techniques used to rear progeny during this critical period, as well as provide information for related fisheries problems. Larval development in himri, *Barbus luteus*, has not been previously reported despite the importance of such studies for the management of natural fisheries in Mesopotamia (i.e. the Euphrates-Tigris Basin) and to conserve this endemic species. The aim of the present study was to provide a morphological and quantitative description of the larval period of himri under controlled conditions.

Materials and Methods

Brood stocks were propagated at the Mreaiya hatchery-farm in Deir ez Zor, Syria, according to Al Hazzaa and Hussein (2003), using carp pituitary extract injections. After hatching, 1000 free embryos were kept from mid-May to mid-June, in 400-l aquaria free of bottom substrata under a natural daylight period. The water was maintained at 19-22 °C and the dissolved oxygen rate was kept at 7-8 mg.l^{-1} by an injection aerator. The water was changed twice per week after first removing waste from each aquarium bottom with a siphon. At the start of exogenous feeding, larvae were fed *ad libitum* for the first day on finely ground hard-boiled egg yolk and then on commercial feed (Takara™, China), which contained 30% crude protein.

Ontogenetic development (of internal structures) was assessed twice per week through the examination of 5-7 live specimens under a light-microscope with a built-in digital camera attached to a monitor, and with a stereomicroscope with an ocular micrometer. Specimens were then treated with degraded ethanol (first 50%, then 75%) for the examination of external structures. The onset of larval development was considered to have

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begun when about 50% of the free embryos began exogenous feeding. As a starting point, the larval period was assessed using Peňáz's (2001) classification, which recognizes 6 larval steps ($L_1...L_6$) beginning with the onset of mixed feeding and ending with the transition to juvenile development (i.e. metamorphosis), as defined for many other teleosts (Copp and Kováč, 1996; Fuiman and Higgs, 1997; Balon, 1999; Pavlov, 1999). Thresholds during early development, such as oral feeding and squamation, were considered to be achieved when at least 50% of the specimens had attained the relevant character. Dorsal, anal, and caudal fins rays were counted without differentiation of smooth or serrated rays. Relative growth was examined in the following body characters: total body length (TL); body height (BH) from the dorsal fin bud to the base (vertically); head length (HL); pre-anal length (Pr-AL) from the rostrum to the anal orifice (i.e. digestive tract size); post-anal length (Pt-AL), body length anterior to Pr-AL, and eye diameter (ED). All measurements, except BH, were made parallel to the longitudinal axis of the larvae and rounded to the nearest 0.1 mm. Growth of BH, HL, Pr-AL, Pt-AL, and ED were expressed by Huxley's (1932) power function, which considers TL as the standard measurement using non-transformed data:

$$y = a\, x^b$$

where y is the independent variable, x is the dependent variable, a is the intercept, and b is the growth coefficient. Isometric growth occurs when b = 1. Allometric growth is positive when b > 1, and negative when b < 1. Daily growth until metamorphosis was calculated as:

$$G_d = S_m/T_{juv}$$

where G_d is the daily growth in the segment size in mm/day^{-1}, S_m is the segment size at metamorphosis, and T_{juv} represents the age at metamorphosis (in days). Biological ontogenetic events up to metamorphosis were scaled on logarithmically transformed data for TL and age calculated according to Fuiman's equation (1994):

$$O_L = \log (L)\, 100/\log (L_{juv})$$

where O_L is the calculated ontogenetic state based on TL, L_{juv} is the TL at metamorphosis, and O_L is 100 at metamorphosis.

Results

The main morphological and anatomical characters that appeared during the larval period were classified, within their time of occurrence, into the following 6 steps based on developmental state:

Step L_1, age 3 days: The start of exogenous food ingestion, yolk sac decreased slightly. Intestine contained swallowed organisms and ended in perforated anal orifice. Notochord was straight. The one-chamber air bladder filled with air. Pigmentation already started in eye lens. Blood was circulating through gill filaments and embryonic respiratory system.

Step L_2, age 7 days: Exclusive exogenous feeding started since depletion of the yolk sac. External erythrophores and melanophores spread dorsally on the head and body. A patch of melanophore appeared at the junction (point) of the hypural plate and mesenchymal lepidotrichia in the caudal lobe of the finfolds. Capillary rudiments in the air-bladder share gill filaments in circulation. Small anterior chamber in air-bladder is formed and filled. Kidney and liver are active in appearance. Larvae can swim actively.

Step L_3, age 12 days: The posterior end of the notochord is slightly bent upwards. Ten articulated ossified rays appeared on the homocercal caudal fin. Dorsal, anal, and pelvic lobes contained mesenchymal lepidotrichia and developed into fins. Bending intestines bumping its contents actively in ventral and anal direction (Figure 1a). The ability to swim and maneuver developed.

Step L_4, age 15 days: Slight development of incised homocercal-caudal fin with small upper membranous lobe with 10 fan-wise-arranged ossified rays directing backwards. The patch of melanophores on the caudal fin increased in size (Figure 1b). Ossification was observed in the dorsal, anal, and pelvic fin rays, with 5 articulated rays in the dorsal one. Anlagen of ventral fins appeared. The finfold's remaining parts in the caudal fin have vanished. Jaws and corniform teeth are fully developed (Figure 1c).

Step L_5, age 17 days: Six ossified rays in the anal fin are seen. Caudal fin is incised deeply. Rudiments are apparent in caudal and pectoral fins. Melanophores, erythrophores, and xanthophores spread externally in series, dorsally on the head and along the dorsal fin base, and ventrally in the trunks. Small patches of pigmentation variably distributed are seen in the caudal peduncle,
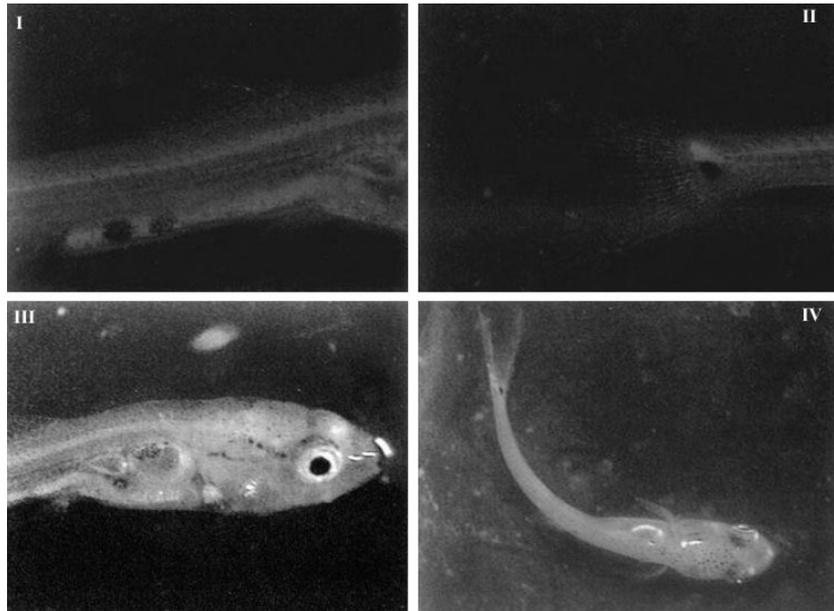
Figure 1. Different morphological steps during the development of himri larvae. (a) digested food is pushed through the intestines by peristalsis. (b) Large melanophore patch in the junction of the caudal fin plate. (c) Developed jaws and 2-chamber air bladder. (d) Dorsal view showing erythrophores over larva body giving reddish color.

notochord tip, and around the anus. Internal dermal erythrophores spread dorsally and ventrally along the notochord and over the air-bladder, producing the specific reddish color of himri (Figure 1d). Expandable anal orifice can flare.

Step L₆, age 33 days: Many of the body parts were still translucent. Interlaced rudiments rete is well developed in the gills. Finfolds completely disappeared. Scaling started on the body surface. Most larvae resembled the definitive adult morphology, measuring about 10.5 mm in length.

Body proportions changed considerably compared to total length (TL) during the larval period (Figure 2). Growth of eye diameter and head length was positively allometric (b = 2.72 and 2.50, respectively). Growth of body height and postanal length was nearly isometric (b = 0.92 and 1.25, respectively), whereas pre-anal length growth was negatively allometric (b = 0.85). Daily incremental growth in body segments showed the following different values: ED, 0.023 mm.day^{-1}; BH, 0.026 mm.day^{-1}; HL, 0.065 mm.day^{-1}; Pt-AL, 0.128 mm.day^{-1}; Pr-AL, 0.171 mm.day^{-1}.

Quantification of selected ontogenetic events compared to total length and age at metamorphosis raised an ontogenetic index profile for himri, as illustrated in Figure 3. Compared to total length or age at metamorphosis, succession in attaining ontogenetic events showed considerable difference within the experimental temperature range when plotted. No interspecific variability in the timing of the appearance of developmental events affected by ambient conditions was attributable to the fixed rearing conditions.

Discussion

Larval development in himri was typical of most cyprinids, while there were some specific characteristics that have not been observed in many other studied cyprinids; however, these may have been due to the controlled rearing conditions. Eye pigmentation in himri occurred early in the larval period rather than during the embryonic or free-embryonic phase, as in most cyprinids (Peňáz, 2001). Early eye pigmentation has also been reported in other endemic species of similar water bodies, such as gattan *Barbus xanthopterus*, romy *Barbus grypus*, and bunni *Barbus sharpeyi* (Al-Nasih, 1992; Pyka et al., 2001). The pattern of pigmentation was not constant, but developed during larval ontogeny and disappeared shortly after metamorphosis, taking on the
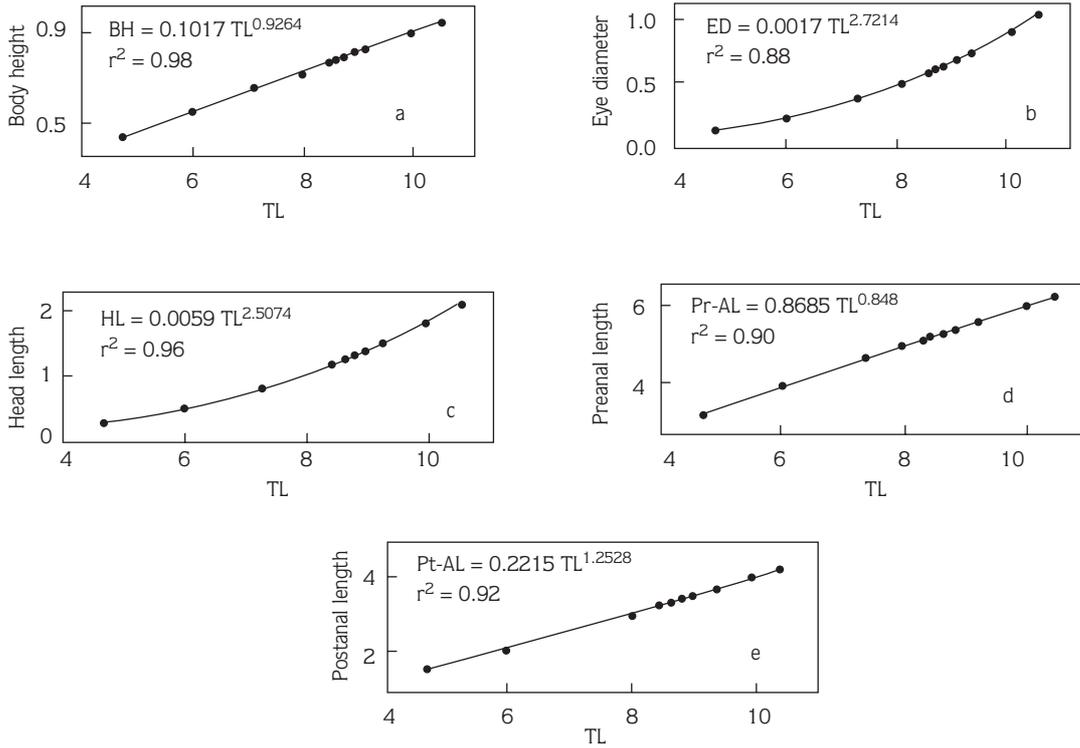
Figure 2. Power relationships in growing himri larvae between total length (TL) and: a) body height (BH); b) eye diameter (ED); c) head length (HL); d) pre-anal length (Pr-AL); e) postanal length (Pt-AL).
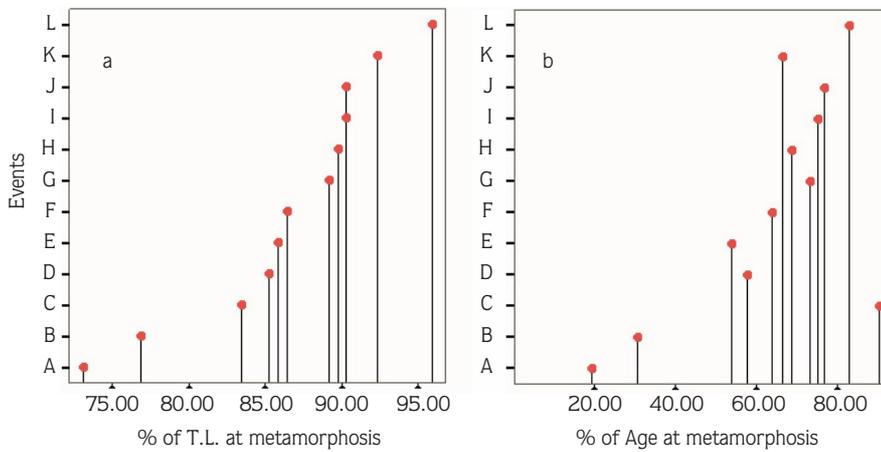
Figure 3. Ontogenetic index of observed selected ontogenetic events in himri, with respect to percentages of total length and age at metamorphosis. a) Compared to total length at metamorphosis. b) Compared to age at metamorphosis. (A) Pigmentation in eye. (B) Exogenous feeding. (C) External chromatophores along the body. (D) Anterior chamber in the air-bladder. (E) Caudal fin rays. (F) Melanophore patch on the caudal fin. (G) Articulation in caudal fin rays. (H) Jaws. (I) Dorsal fin. (J) Corniform teeth. (K) Pelvic fins. (L) Anal fin.

more complex pattern of adults (Urho, 2002; Parichy and Turner, 2003), but the melanophore patch in the caudal-fin plate of the himri larvae and other melanophores over the head did not vanish immediately following metamorphosis. These melanophores, however, were not present in adults, which indicated a limited elimination of the larval pattern of pigmentation during metamorphosis and the existence of a juvenile pigmentation pattern that preceded the adult pattern. This transitional pattern of pigmentation may have an ecological role affording protection against predators (Fuiman and Magurran, 1994). Chronologically, strips of different chromatophores occurred dorsally among myomers and over the head from day 4 of larval development. Denser erythrophores then dominated, resulting in the reddish color of adult himri, which appeared to be similar to the appearance of this ontogenetic pattern during the larval development of chub, *Leuciscus cephalus* (Çalta, 2000) and common carp, *Cyprinus carpio* (Peňáz et al., 1986), within the larval stage. Pigmentation in fish is highly correlated with metabolism, specific hormones, and growth factors that accelerate metamorphosis (Christensen and Korsgaard, 1999; Solbakken et al., 1999; Bolker and Hill, 2000), nutrition and food items (Bolker and Hill, 2000; Diler and Dilek, 2002), genes and genetic environmentally-sensitive factors (Toyoda et al., 2000; Parichy and Turner, 2003), as well as habitat (Urho, 2002).

Absorption of the yolk sac, which occurred over approximately 1 week, was slower than in other species under natural conditions (Peňáz, 2001) possibly due to the *ad libitum* availability of good quality food. This raises a question about the limits of the secondary buffering role of yolk sac contents in nourishing yolk-feeding larvae when an external good-quality substitute exists. In a similar case, laboratory-raised chub exhausted the yolk sac within 8 days, but only 30% of the initial population of larvae could establish exogenous feeding (Çalta, 2000). Depletion of the yolk sac may coincide with some significant improvements in the respiratory system, buoyancy ability, and swimming activity.

Maneuvering and routine swimming performance in himri larvae was highly correlated with development. It may be ascribed to increased mechanical power produced by muscles, such as in the common carp (Wakeling et al., 1999), and increased functioning of swimming organs. In the larval period, swimming activity and performance

seem to be the best when compared to the overall life course, and are the result of the rapid morphological changes in larvae that level-off towards adulthood (Fuiman and Webb, 1988).

As some characters related to the final larval stage still remain during the initial stages of the juvenile period (e.g., translucent body parts and exceptions in pigmentation pattern observed in larvae), the transition of himri from larvae to juvenile appears to progress slowly, although this can be influenced by a variety of factors, including environmental conditions and genetic heterogeneity, as in many other species (Copp and Kováč, 1996; Fuiman et al., 1998; Gozlan et al., 1999; Vilizzi and Walker, 1999).

As with the other endemic barbel species, gattan, romy, and bunni, himri are relatively short in TL at hatching, but at metamorphosis no other barbel species appear to have as small an average length, except bunni (Al-Nasih, 1992; Pyka et al., 2001). Small size at the onset of juvenile development reflects the relatively small ultimate size of himri adults relative to other cyprinids (Szypuła et al., 2001; Al Hazzaa, 2005).

Negative allometric growth in the digestive tract size of himri is not an unusual feature and does not imply that digestion is a secondary priority in ontogeny. Increased bending of the intestines coincided with increased functionality; however, the intestines grew at a slower rate compared to the rest of the body. Development in the digestive tract in many fish species has been found to improve considerably in structure and function during the larval period (Kjørsvik et al., 1991; Rønnestad et al., 2000; Cuvier-Pérez and Kestmont, 2002).

Variation in the ontogenetic index, depending on size and age, is not surprising. Development can be accelerated or hindered by extrinsic (temperature, oxygen, etc.), intrinsic (egg size, paternal effects, etc.), or combined actions of these major factors (Kamler, 2002). Size may be used in explaining ontogenetic state more than age (Fukuhara, 1986; Fuiman et al., 1998). On the other hand, size can vary considerably for any given developmental state, and so size is also less reliable than an ontogenetic assessment (G.H. Copp, personal communication).

Investigating the early life development of himri, and other fishes, in different ambient conditions can clarify

chronological ontogenetic process and enable us to quantify and compare development more precisely. Furthermore, the effects of major factors influencing development and viability can be understood.

Acknowledgments

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