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Growth and morphological development of guppy *Poecilia reticulata* (Cyprinodontiformes, Poeciliidae) larvae

[1]Hamed Mousavi-Sabet, [1]Hoda Azimi, [2]Soheil Eagderi, [1]Sepideh Bozorgi, [3]Bagher Mahallatipour

[1] Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran; [2] Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Alborz, Iran; [3] Department of Fisheries, Tonekabon Branch, Islamic Azad University, Tonekabon, Mazandaran, Iran. Corresponding author: H. Mousavi-Sabet, mousavi-sabet@guilan.ac.ir

Abstract. Morphological developments, including fins, body proportions and pigmentation in *Poecilia reticulata* larvae, were described under rearing conditions. Body length of larvae were 4.5 to 5 mm just after birth (day-0), reaching 11.9 to 13.5 mm on day-30 after birth. The anal fin modified on day-23 in males and females. The caudal fin coloured on day-28 after birth in males. Male and female distinguished by gonopodium, body colour pattern and size on day-40. The majority of both sexes were sexually mature and fins are fully developed on day-50 after birth.
Key Words: livebearer, gonopodium, aquarium, ontogeny.

Introduction. Early life history of fishes has been studied in a number of different perspectives (Ahlstrom & Moser 1976). These studies are important tools to understand differences in reproductive strategies among populations under different environmental conditions (Reznick & Endler 1982; Reznick & Miles 1989; Reznick et al 1990, 1997; Downhower et al 2000; Johnson & Belk 2001). Also, understanding the early developmental stage under laboratory conditions is essential for successful aquaculture (Pyka et al 2001). Some studies on early development of fishes are directly associated with embryology and ontogeny, while the others have emphasized on functional morphology of larval structures (Kendall et al 1984).

At the time of birth, larvae can be in various states of development, which is largely dependent on the size of yolk (Wourms 1981). While in viviparity, which nourishment is supplied by maternal structures, the larval has evolved many times (e.g., Poeciliids) and the fish is born as juveniles (Wourms 1981).

The Poeciliidae family contains about 200 species in 22-29 genera and is widely distributed in America and Africa (Lucinda 2003). Fishes of the family Poeciliidae inhabit in fresh and brackish water (Nelson 2006). These species are characterized by viviparity (with the exception of *Tomeurus gracilis*) and internal fertilization (Rosen 1964). The popular aquarium fish, *Poecilia reticulata* Peters, 1859 is commonly known as ‘guppy’ (Mousavi-Sabet et al 2012). The guppy is native to the northwestern South America and one of the most frequent viviparous species in the aquarium industry (Bisazza 1993). The females retain their fertilized eggs within the follicle during gestation (Turner 1940; Lambert 1970), also they can store the sperm in their mates (Shahjahan et al 2013). The males show a modified anal fin with an intermittent organ (gonopodium) for transferring sperm (Mousavi-Sabet et al 2012). Unfortunately, studying the early development of viviparous is more complicated than that of oviparous species, due to the unavailability of developing embryos for examination (Martyn et al 2006). However, Haynes (1995) has described the embryonic development of Poeciliids using specific developmental stages derived from *Gambusia*. Also, a few studies performed on the larval development stages of guppy (Kunz 1963; Kunz & Ennis 1983; Goodrich et al 1944; Shahjahan et al 2013). Therefore, the present study was carried out on the alometric growth pattern and morphological development of *Poecilia reticulata* in a controlled aquarium condition.

Material and Method. The females of *P. reticulata* were bought from local ornamental fish farm in February 2014 and transferred to a rearing glass aquarium at the fisheries laboratory of University of Guilan (Guilan Province, north of Iran). Females were clearly distinguished by having swollen abdomen and the absence of gonopodium (Mousavi-Sabet et al 2012). A total of twenty ripe females of *P. reticulata* were randomly sampled and were transferred to breeding aquarium. After giving birth, the newly born fish (n = 6) were randomly collected, fries on days 1–5 were randomly sampled daily, and from day 7 till 51 every other days. The collected specimens were preserved in 5% formalin immediately. The left sides of specimens were photographed by a stereomicroscope equipped with a Cannon camera with a 5 MP resolution.

Specimens were examined for observations on general morphology, pigmentation, fin development and the following morphometric measurements in mm: body length (BL), head length (HL), head depth (HD), trunk length (TrL), Tail length (TaL), maximum body depth (BD), eye diameter (ED) and snout length (SnL). Some of the specimens were cleared and stained in alizarin red S and alcian blue based on Taylor & Van Dyke (1985) for observations of fin ray formation. All measurements were taken along lines parallel or perpendicular to the horizontal axis of the body from obtained images using ImageJ software (version 1.240). Abnormal specimens were excluded from the study.

A comparison of measured morphometric charactrestics was performed between two sexes during 25-51 days after birth using Manova analysis in PAST software. Allometric vs. standard method was used to remove size-dependent variation in morphometric characters (Elliott et al 1995) using following formula: Madj = M (Ls/L0)b, where *M* is the original measurement, *Madj* the size adjusted measurement, *L0* the standard length of the fish, *Ls* the overall mean of the standard length for all fish from all samples in each analysis, and *b* was estimated for each character from the observed data as the slope of the regression of log M on log L0 using all fish in any group.

The allometric growth patterns were calculated as a power function of total length using non-transformed data: $Y = aX^b$, where (Y) was the independent variable, (X) the dependent variable, (a) the intercept and (b) the growth coefficient. Isometric growth, positive and negative allometric growth are indicated by b = 1, b > 1, b < 1, respectively. The inflexion points of growth curves were determined according to Fuiman (1983) and Van Snik et al (1997). Drawing plates and data analysis were performed in MS-Excel 2013 (Microsoft Corporation). Data analysis was performed in Past (ver 2.17) for Windows.

Results and Discussion. The newly hatched fries are transparent, blackish or grayish in colour and slender with melanophores on head. Most of the newly hatched fries, absorbed their yolk sac completely, while the rest born with a little of yolk sac. Also born with developed jaws on the mouth, so they can take the food immediately after birth, similar to the guppy specimens described by Martyn et al (2006) and Shahjahan et al (2013). Also, it is observed that at the moment of birth, each fry was fully capable of swimming, eating and avoiding danger like to other livebearers (Shikano & Fujio 1997; Shahjahan et al 2013).

Pigmentation. Melanophores present on eyes in newly hatched larvae. Many of melanophores present on the yolk sac surface, particularly on ventral face and decreasing in number with yolk absorption. Small punctate melanophores are covering dorsal surface of head and behind opercle on day-1 after birth. Many of the punctate melanophores are appearing on caudal peduncle in day-5. Decreased melanophores scatter on ventral surface in day-10. Appearance melanophores on the dorsal and ventral fins ray on day-15 after birth. Many melanophores are appearing on caudal fin in day-20 larvae, and increasing in number with growth. Caudal fin is coloured in the males on day-

28 after birth in agree with Shahjahan et al (2013). The base of anal fin is dark, and presents a dark spot on the base of dorsal fin on day-35 after birth. Male clearly distinguished from female by developed gonopodium (vs. absence), colour pattern on body and caudal fin (vs. commonly colorless) and smaller size on day-40 after birth while, a sooner time (day-35) is reported for appearing of these sexual dimorphisms (Shahjahan et al 2013). In some of the female a dark spot surrounding the anus has seen on day-50. The majorities of male and female are sexually mature on day-50.

Fin development. The guppy larvae are born with all fins such as other poeciliids (Wourms 1981), which the fins are clearly observable. In one day after birth, pelvic fin is smaller than pectoral fin, and caudal fin has a rounded edge. The caudal fin has more development and clearly appears as a tail in day-5, while Shahjahan et al (2013) observed it in day-7. Pelvic and anal fins have more developments, which are similar with Shahjahan et al (2013) observation, confirmed that the anal fin in both sexes is still similar until day-15. The anal fin changes in both sexes of *P. reticulata* observed in day-23, while Shahjahan et al (2013) reported these changes on day-21. The anal fin in males is elongated and tube shaped, while in the females it is small and rounded on day-23. One month after birth, the base of caudal fin is dark and abdomen becomes swollen in females, while these characters were reported on day-28 by Shahjahan et al (2013). Fins are fully developed on day-50.

Proportions. Our observation showed that the BL of newly hatched larvae (day-0) ranged from 4.5 to 5 mm, reaching 8.5 mm on day-15, 10.5 to 11 mm on day-25, 14 mm on day-35 and 18.5 to 19 mm on day-51 (Figure 1) while, Shahjahan et al (2013) reported that larval length 6.8~8.5 mm on day-1 and 8~9.5 mm a month after birth.

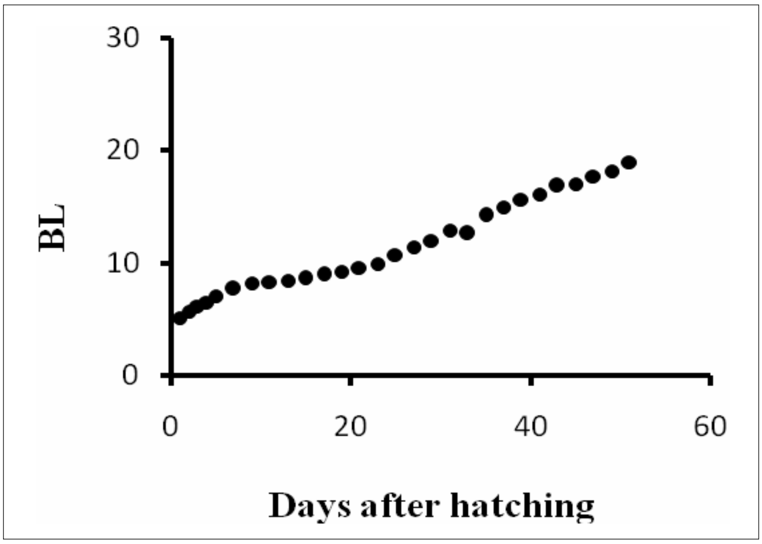

Figure 1. Changes in body length (mm) of laboratory-reared guppy *Poecilia reticulata* larvae from days-0 to 51 after birth.

Head length (HL) 18% BL in 1-day old fry, proportion subsequently increasing with growth, reaching 26-28% BL in 21-day fry, 31% BL in 41-day and 29-30% BL on 51-day after birth. Maximum body depth (BD) initially is 14-16% BL, proportion increasing with growth, reaching 20-25% BL in day-41 and then decreasing with body extension in day-51. Eye diameter (ED) is initially 9–10% BL, proportion increasing with growth, reaching 12-14% in 22-day old larvae and suddenly proportion, reaching 16-20%, in 45 to 51-day old larvae. SnL is 3–4% BL on day-1, increasing to 6–8% BL in 35-day old fry and proportion increasing with growth, reaching 11-17% on 45 to 51-day after birth.

The results showed there is no significant difference between the two sexes in terms of measured morphometric features, therefore the data of two sexes were pooled and analyzed. Analysis of body characteristics showed that growth of all body segments can be divided into two phases (Figure 2). Allometric growth of the head length (b = 1.4784), head depth (b = 1.623) and snout length (b = 1.6474) showed positive allometric growth prior their inflection points, at 16.09, 16.84 and 16.84 mm TL (41, 43 and 43 day after birth), respectively. During post inflection point, allometric growth pattern of head length was negative, whereas those of head depth (b = 5.6558) and snout length (b = 6.739) were strongly positive. In addition, eye diameter showed the isometric growth pattern up to 37 day after birth (14.91 mm TL), then changed to strongly positive allometric growth (b = 4.2352).

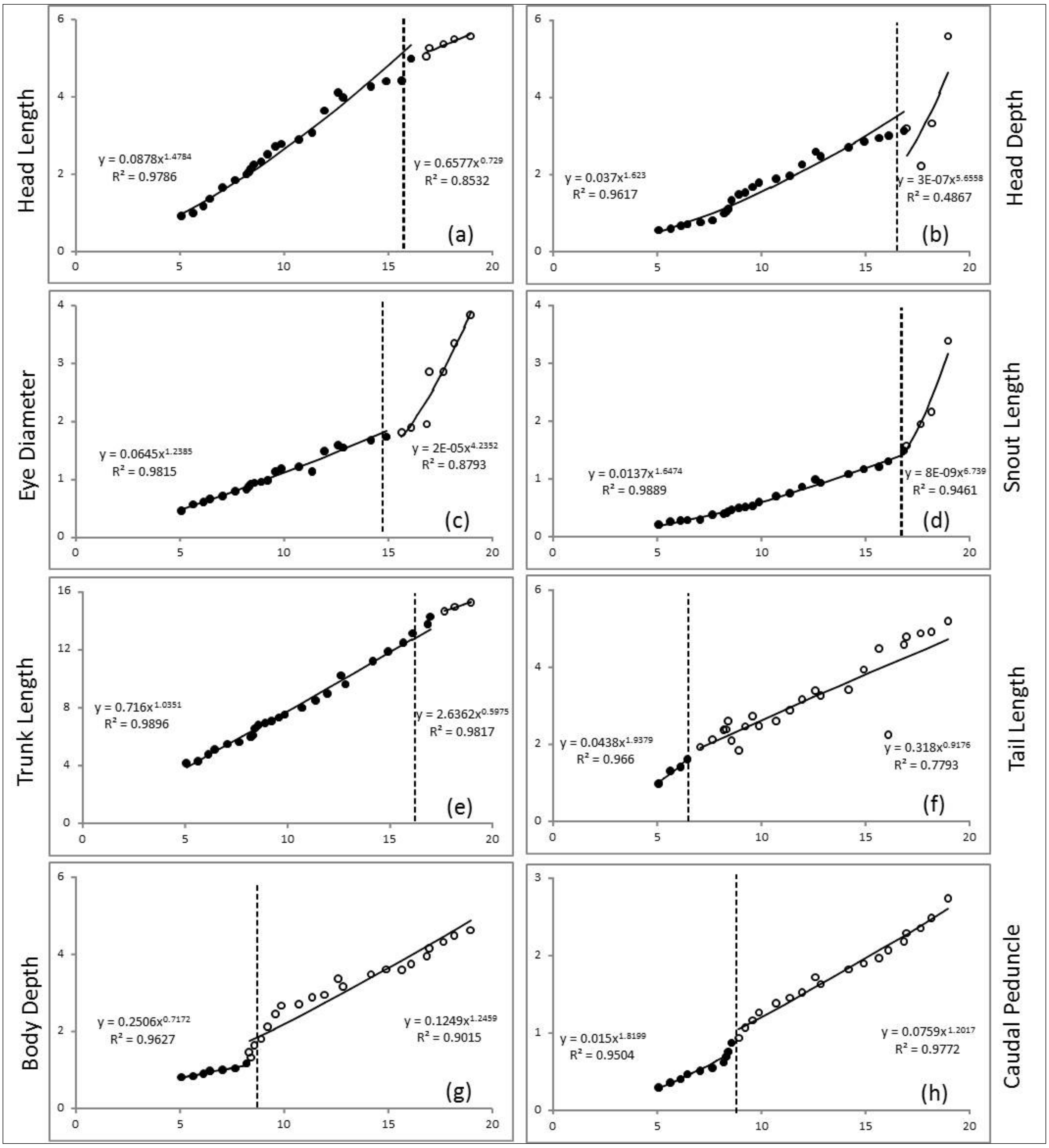

Figure 2. Growth allometries of the different body segments in laboratory-reared guppy *Poecilia reticulata* larvae (R^2 = correlated coefficient) (n = 140).

Despite oviparous species, guppy is a livebearer and many of its biological system are functional at its birth. A strong positive allometric growth pattern of head area including eye diameter, snout and head depth during early development may reflect the ecological and biological demands and functional performance in this species (Peña & Dumas 2009).

This morphilogical alternation may be related to changing feeding habit i.e. foraging from water column to water surface in guppy. Hence, feeding shifts and morphological changes in this species are concomitant. To increase prey detection and ingestion ability, development and differentiation in sensory, feeding and nervous systems are necessary in head region to catch prey (Fuiman 1983). Also, the size of mouth has an important effect on prey catching (Hjelm et al 2003). As larvae hatches, head length growth and increasing in food particle size occur simultaneously (Osse et al 1997).

The growth pattern of caudal peduncle was positive during preflexion (b = 1.8199) and post flexion (b = 1.2017) periods. Allometric growth of tail length was positive (b = 1.9379) until 4 day after birth (6.43 mm TL), then nearly isometric (b = 0.9176) during post inflection. Allometric growth pattern of body depth showed a negative pattern (b = 0.717) and then a relatively positive pattern (b = 1.1459). The trunk length showed an isometric growth before inflection point and negative one (b = 0.5975) after inflexion point at 6.43 mm TL.

Based on the results, the growth pattern of tail features were positive during early developmental stage up to 51 days after birth. Such a pattern of tail, enable guppy's fries to avoid from predator and may help to swim faster and catch preys. Tail growth improves swimming capability to catch prey and escape from predator (Fuiman 1983).

In juveniles the trunk length allometry was negative, while the tail and head allometry pattern were positive, showing importance of head and tail growth during occurrence of maturity. According to Osse & Van Den Boogaart (2004) an extensive shift in ontogenetic development occurs to increase the possibility of survival. In addition, the body depth had negative growth pattern during prefelexion point reflecting less importance of trunk growth during early development and seems in this species many systems related to its feeding are complete at the birth time

Conclusions. The results of this study revealed that guppy born with developed jaws, fins and eye that capable them to take the food, swim properly and avoiding predators immediately after birth. The allometric growth pattern of guppy showed that the inflection points of the most of body segments occur during 41-43 days after birth concomitant with mutation, showing a late morphological change in compare to oviparous species. These morphological changes are associated to head depth and snout length may be relating to change in feeding habit of this species. In addition, positive growth pattern of caudal peduncle and tail length occur earlier that head region at 4-15 days after birth that can be related to improve swimming capability to avoid predadors and catch preys. Apart from the aquaculture viewpoint, the information on morphology during the early life stages is essential, particularly for investigating mechanisms of survival. Additionally, by clarification of these stages, we can classify fishes according to ecologic, reproductive and ontogenetic (fish development from embryo to adult) factors.

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Authors:

Hamed Mousavi-Sabet, Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran; e-mail: mousavi-sabet@guilan.ac.ir

Hoda Azimi, Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran; e-mail: azimihoda@yahoo.com

Soheil Eagderi, Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Alborz, Iran, e-mail: soheil.eagderi@yahoo.com

Sepideh Bozorgi, Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran; e-mail: sepidehbozorgi@yahoo.com

Bagher Mahallatipour, Department of Fisheries, Tonekabon Branch, Islamic Azad University, Tonekabon, Mazandaran, Iran; e-mail: smahalatipor@gmail.com

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