Original Article

Developmental morphology and growth patterns of laboratory-reared giraffe cichlid, *Nimbochromis venustus* Boulenger, 1908

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Abstract: The giraffe cichlid *Nimbochromis venustus* Boulenger, 1908 is a well-known species in aquarium trade. The laboratory-reared electric giraffe cichlid was studied in terms of early morphological development and allometric growth pattern from hatching until the juvenile stage i.e. 51 dph. Total length increased linearly from hatching until 51 dph, with a high regression coefficient. The yolk sac exhaustion completed throughout 15 days. Significant morphological and morphometric variations occurred before the yolk sac absorption and early juvenile stage by evolving the anterior and posterior body section which improve swimming ability and food capturing. Positive growth coefficient of the body and trunk lengths were occurred after inflexion point coinciding with development of digestive system function. Eye diameter showed negative allometry at the inflexion point of 9.3 mm, continuing with positive growth rate until day 51. Body shape variation in early life history revealed consequent development in anterior and posterior body section in preflexion phase and development of body and trunk length in post flexion phase to support high survival of larvae. The present study emphasizes ontogenic study of different species to interpret biology and ecology of fish in association with evolutionary biology.

Introduction

The Giraffe Cichlid, *Nimbochromis venustus* (Boulenger, 1908), is a large cichlid endemic to Lake Malawi in Africa (Bangerter, 2007). Juveniles of this species are found in shallow water near rocks while adults forage in deeper regions with sandy substrate. They usually fed small fish and invertebrates. Half-grown individuals have been observed to remain motionless, partially buried in the sand, waiting for small fishes to come within reach. *Nimbochromis venustus*, as a mouth brooding fish (Konings, 1990; Snoeks and Manuel, 2004), prefers waters with pH, depth and temperature 7.2-8.8, 6-23 m and 25-27°C (Riehl and Baensch, 1996).

Fish morphological transformation from larvae to a juvenile or young adult form is occurred during early developmental stages in a relatively short period of time with completion of their functional systems as a survival strategy (Osse and Van de Boogart, 2004). In addition, study of the allometric growth patterns during early developmental stages of different teleost species helps to corroborate the importance of morphological development and growth patterns of young fish, by providing a better understanding of early life events (Fuiman, 1983; Khemis et al., 2013), their priorities during the early growth, and size-related behavior and ecology (Gisbert, 1999). In this regard, this study aimed to describe early morphological development and allometric growth pattern of *N. venustus.*

Materials and Methods

The broodstocks of *N. venustus* were obtained from a local ornamental fish farm in February 2015 and transferred to a rearing glass aquarium at the fisheries laboratory of University of Guilan, Guilan Province,

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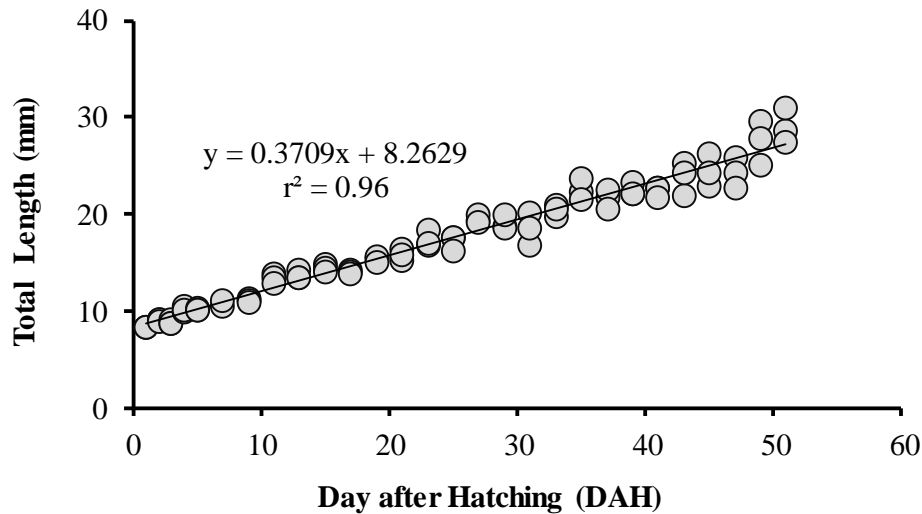E-mail address: mousavi-sabet@guilan.ac.ir

Figure 1. Linear regression between total length (mm) and days after hatching of laboratory-reared *Nimbochromis venustus* from days 0 to 51 after hatching.

Iran. A total of 20 breeding pairs were transferred to breeding aquaria for spawning. The eggs were kept in glass aquaria with water temperature of 26-28°C and after hatching, larvae were transferred to new aquaria for rearing. Rearing conditions were 27±0.8°C, 6.49±0.15 mg/l dissolved oxygen, 12L:12D artificial photoperiod and light intensity of 500 lux at the water surface (Mousavi-Sabet, 2011). Newly hatched larvae were fed with *Artemia* nauplii and micro-worms from 1-5 dph, then with a mixture of nauplii and commercial food pellets (Biomar A/S; 58% protein, 15% lipid) twice a day from 6-51 dph.

Daily sampling were randomly carried out from 1-5 dph (day post hatching), followed every other day till 51 dph from the same larval batch. Larvae were sacrificed with an overdose of MS222 (35 mg ml⁻¹) and fixed in 5% buffered formalin solution. Then, their left sides were photographed using a stereomicroscope equipped with a Cannon camera with a 5 MP resolution. The following morphological characteristics were measured from the digital images to the nearest 0.01 mm using Image-J software (version 1.240): 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). Measurement method followed Leis and Trnski (1989). Ontogenic development followed criteria as described by Balon (1977, 1986, 1999). Specimens were examined for general morphology, pigmentation, and fin development under a Leica MC5 Stereozoom microscope.

The allometric growth patterns were calculated as a power function of total length using non-transformed data: $Y=aX^b$, where (Y) was the dependent variable, (X) the independent variable, (a) the intercept and (b) the growth coefficient. Isometric, positive and negative allometric growth patterns 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 graphs was performed in MS-Excel 2013 (Microsoft Corporation) and data analysis in Past (ver 2.17).

Results

General morphology: Total length of *N. venustus* increased linearly from hatching up to 51 dph (Fig. 1). BL of day 0 larvae ranged 8.23-8.32 (mean±SD: 8.28±0.04) mm (n=5) and reached 27.39-30.92 (mean±SD: 28.93±1.08) mm on day 51 (Fig. 2, Table 1). The mouth and anus were closed at hatching. Newly hatched larvae had a large oval yolk sac (with horizontal length of 3.05±0.10 mm).

The head initially was separated from the yolk sac (Fig. 2a) at hatching, upper and lower jaws formed in day 1, mouth opened, and anus hardly seen on day 2. Formation of the anal-fin with visible rays and starting
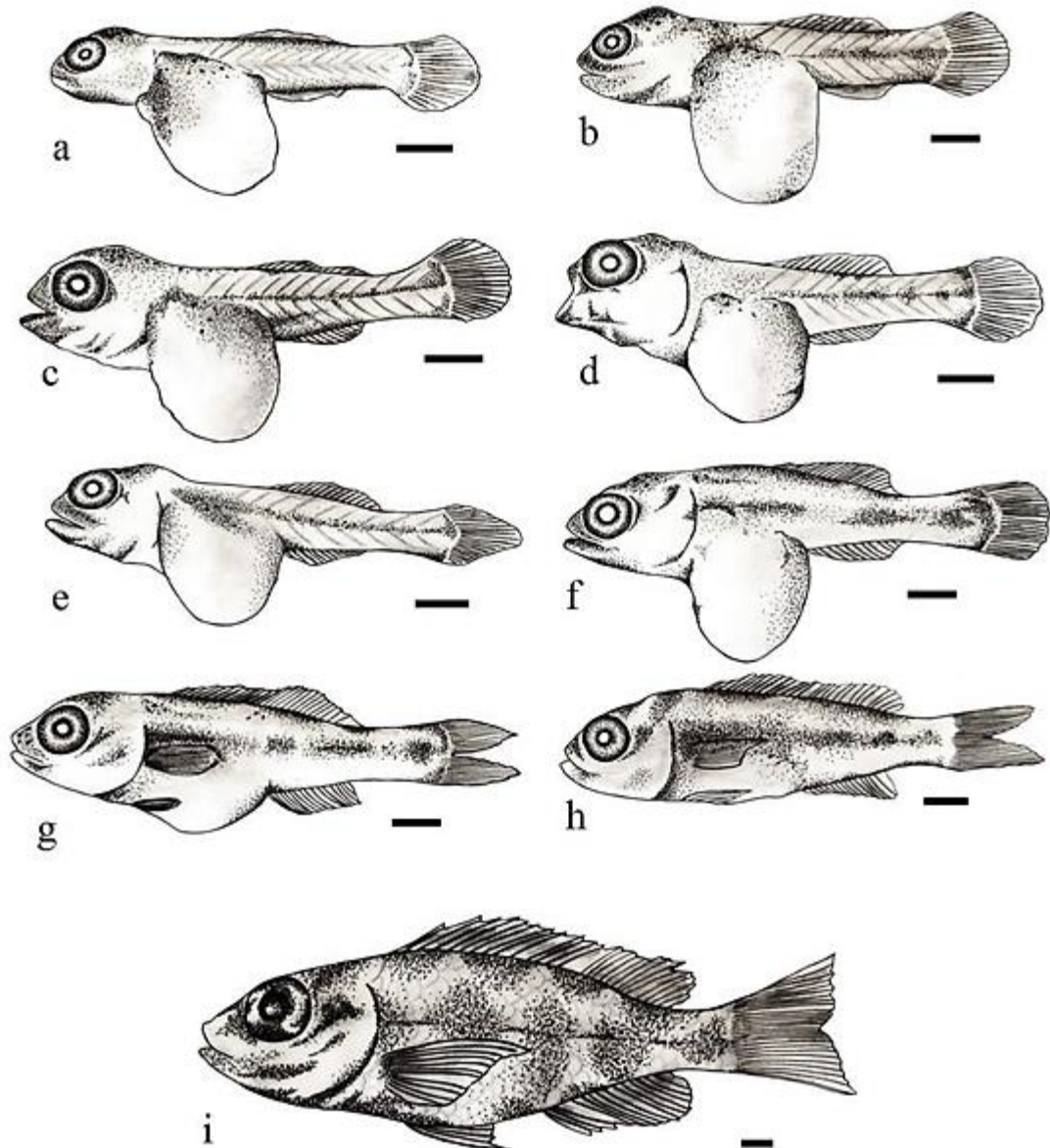
Figure 2. Early developmental stages of giraffe cichlid *Nimbochromis venustusa*. (a) Newly hatched embryo 1 day after hatching, (b) two day after hatching (dah), (c) 3 dah, (d) 4 dah, (e) 5 dah, (f) 7 dah, (g) 11 dah, (h) 15 dah, and (i) 51 dah; Completion of fin rays and juvenile formation after day 15. Scale bar=1 mm.

notochord flexion were observed on day 3 (Fig. 2c). Operculum appeared on day 4 (Fig. 2d), myomeres were visible from hatching to day 11. Lateral line was appeared on day 15, yolk moved dorsally to above abdominal cavity beside upper base of the pectoral fin (Fig. 2h), completely absorbed by day 15.

Head length calculated 22-25 %BL in day 1 and increased with growth, reaching 28–30 %BL on day 51 (Fig. 3a). The proportion of body depth, regardless of the yolk sac depth, increased continuously to over 27 %BL on day 51 from 12 %BL at hatching (Fig.3b).

Trunk length was nearly constant at all stages (40–44 %BL) (Fig. 3c). Eye diameter slightly decreased by day11 (11- 8 %BL) and thereafter reached to 11%BL on day 51 (Fig. 3d). Snout length increased continuously up to day 51 from 2 to 7 %BL (Fig. 3e). Tail length showed increasing trend as 24-32% by day 9 then decreased around 29% on day 51.

The soft rays of the caudal fin formed from hatching (with about 16 rays) (Fig. 2a), and finalized with 26 soft rays on day 51(Fig. 2i). The other fins not observed on day 1. Dorsal-fin soft rays appeared on

Table 1. Linear growth of larvae of the *Nimbochromis venustus* in the experiment. Above line: the mean value and standard deviation (in parentheses); under the line: limit of variations of the parameter, (TL: Total length n: the number of specimens).

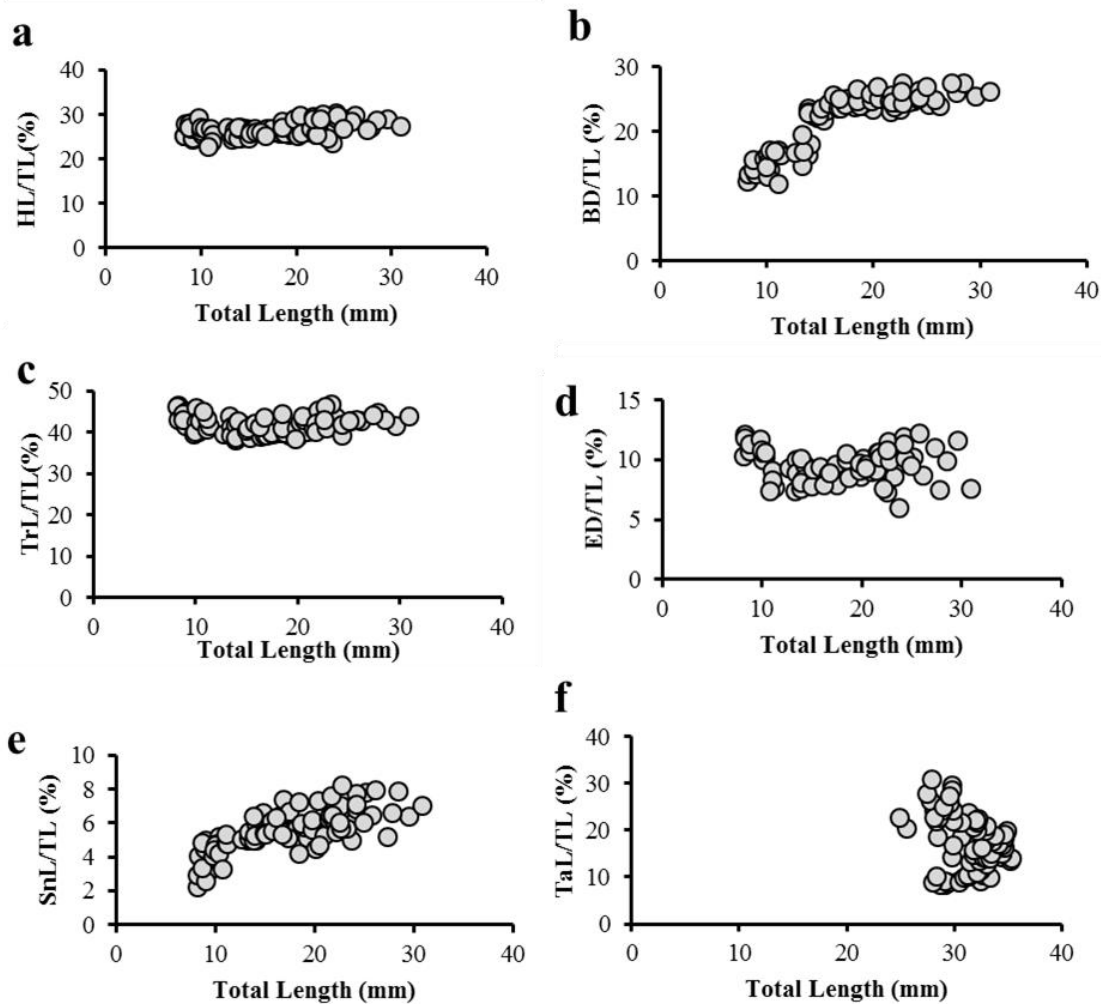| Age, days	TL±SD/min-max (mm)	n
1	8.28 (0.04)	5
	8.23 − 8.32	
11	13.31 (0.56)	5
	12.74 − 13.78	
21	15.78 (0.64)	5
	15.10 − 16.40	
31	18.50 (1.70)	5
	16.78 − 20.19	
41	22.39 (0.53)	5
	21.77 − 22.73	
51	28.93 (1.80)	5
	27.39 − 30.92	

Figure 3. Proportions of (a) head length (HL), (b) body depth (BL), (c) trunk length (TrL), (d) eye diameter (ED), (e) snout length (SnL), and (f) tail length (TaL) to Total length (TL) in young laboratory-reared *Nimbochromis venustusa*.

day 2 (Fig. 2b), attained 13 spines in anterior part on day 11 (entirely 26 rays) (Fig. 2g), and it was constant thereafter. Anal-fin soft rays appeared on day 3 (Fig. 2c) attaining 3 spines in anterior part on day 9 (entirely 12 rays) and was constant thereafter. Pelvic and

Pectoral find soft rays appeared on day 11 with 13 and 10 rays, respectively (Fig. 2g).

Allometric growth: The head length allometric growth pattern was negative (b= 0.84, R^2=0.68) up to 9 dph, then turned to positive (b=1.125, R^2=0.96) up to day
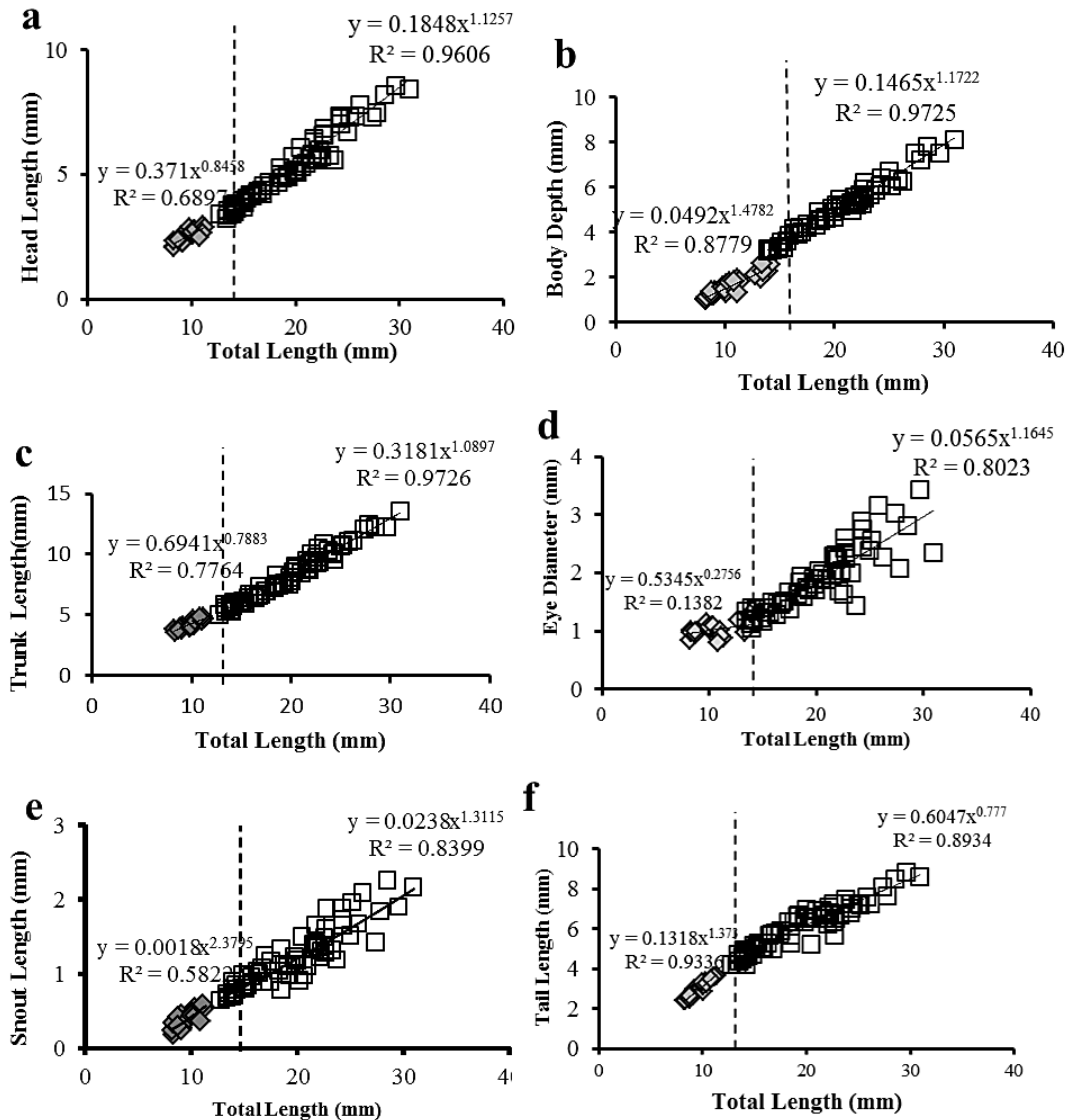
Figure 4. Allometric growth equations and relationship between different measured body proportions with total length in *Nimbochromis venustusa* during early stages of development (from hatching up to day 51). (a) Head length, (b) body depth (BL), (c) trunk length (TrL), (d) eye diameter (ED), (e) snout length (SnL), (f) tail length (TaL). The dashed line represents the inflexion point of growth. Power regression equations are displayed ($P<0.05$ for all regression equations; exception was the first phase of eye diameter R^2=0.138; $P>0.05$).

51 (Fig. 4a). The body depth growth coefficient was positive throughout the entire study period with decreasing trend, separated with an inflexion point on day 9 (Fig. 4b). The growth pattern of the trunk length (TrL) was allometrically negative ((b=0.78, R^2=0.77) until the ninth day at the 10.8 mm of total length (Fig. 3b). The growth pattern of TrL positively increased until day 51 (Fig. 4c). The growth of eye diameter (ED) showed negative allometric growth pattern (b=0.27 R^2=0.13) by day 11 followed positive allometric growth pattern (b=1.16 R^2=0.8) by day 51 (Fig. 4d). Snout length biphasic pattern of the growth

pattern showed a strong positive allometry in relation to total length (b=2.37, R^2=0.58), following days displayed positive growth with decreasing gradient to the previous phase (b= 1.31, R^2=0.83) (Fig. 4e). Tail length grew negatively by day 9 and positively until day 51 (b=1.37, R^2=0.93; b=0.77, R^2=0.89, respectively) (Fig. 4f).

Pigmentation: The newly hatched fry are transparent, grayish in color and slender with melanophores on head. Melanophore deposition in eyes was observed on 1 dph (Fig. 2a). The yolk sac in the anterior part, operculum, dorsal part of the body and upper surface

of eyes had a bit dispersed punctate pigment on day 1 (Fig. 2a). Melanophres emerged some dispersed melanophores on lateral line (on myomeres) with low density on day 2 (Fig. 2b). Melanophores initially were few and increased in number with growth in the following days. Melanophores were seen as colony in some area on lateral line and appeared around anal fin on day 7 (Fig. 2f). Few melanophores observed on snout on day 11(Fig. 2g) and then increased for the following days. Moreover, the concentration of melanophores increased on operculum and lateral body on day 13.

Discussion

In the present study, the newly-hatched free embryos of *N. venustus* were transparent, grayish and slender thereafter with increasing chromatophores. The pigmentation provides camouflage against predators or distracting flickering effect during swimming (Moser, 1981). Also, there has been shown in many species such as *Pleuronectes platessa* larvae and Atlantic halibut, *Hippoglossus hippoglossus* that pigmentation could be positively related to metabolism, some hormones, and growth factor (Christensen and Korsgaard, 1999; Solbakken et al., 1999), genetic and environmental factors (Urho, 2002). Pigmentation pattern in *N. venustus* like other Cichlidae showed differentiation compared to other species (Maan and Sefc, 2013; Ahmadi et al., 2013). Furthermore, it is assumed that identical pigmentation can imply similar function while body coloration is inextricably referred to diversification as well as responding to both natural and sexual selection (Meyer, 1993; Kocher, 2004; Maan and Sefc, 2013).

Yolk sac depletion was completed on 15 dph in the present study. It is assumed that *N. venustus* has 15-day preparatory period for shifting from endogenous to exogenous energy dependent periods. The previous studies have showed longer period for shifting from endogenous to exogenous feeding as observed in *Clarias gariepinus* (Matsumoto et al., 2001), climbing perch, *Anabas testudineus* (Morioka et al., 2009a) and snakeskin gourami, *Trichogaster pectoralis* (Morioka et al., 2009b). In the current study, complete

exhaustion of yolk sac on the day 15 coincided with transformation of free embryo to juvenile supporting its survival in environment conducting the importance of evolutionary ecology during early life history (Kamler and Keckeis, 2000).

Based on the results, the lateral line was appeared on day 15 coincident with yolk depletion and exogenous feeding. It is supposed that lateral line plays a critical role in the prey/ predator detection (Coombs and Montgomery, 1994; Coombs et al., 1996) and recognition of the low frequency movement of water (Harries and Van Burgeijk, 1962) in fish during early life history especially with the beginning of exogenous feeding which need further tools for survival against predators to interact better with their environment. In addition, as the yolk sac depletion increases, external morphological efforts manage to optimize the localization and uptake of prey in favor of fish survival in the early life stage (Comabella et al., 2013).

Food capturing and predator avoidance are critical issues contributed to essentially related organogenesis for feeding (Porter and Theilacker, 1999; Makrakis et al., 2005) and swimming (Murphy et al., 2007; Huysentruyt et al., 2009). Development of organs associated with these functions must occur in a mutual balance (Osse et al., 1997; Rodríguez-Mendoza et al., 2011; Saemi-Komsari et al., 2018). *Nimbochromis venustus* larvae exhibited positive growth in tail length by day 9 followed by negative allometric growth pattern later on. The positive allometry of tail has been reported in sturgeon (Gisbert and Doroshov, 2006), croaker (Shan and Dou, 2009) and catfish (Huysentruyt et al., 2009). Tail length positive growth coefficient in the early life history could enhance swimming capacity considering the changes in swimming style from anguilliform to subcarangiform (Van Snik et al., 1997; Osse and Boogaart, 1999; Osse, 1990). In our study, the pelvic and pectoral fins developed after unpaired fins. In more developed fishes, including teleost, the pelvic fin with trimming function decreases the pitching and up-ward body displacement in fish effort of braking (Murata et al., 2010). In many other fish species, the maneuverability

function of the effective swimming in the favor of feeding has been reported (Comabella et al., 2013).

The trunk and body allometric growth pattern of *N. venustus* was negative by day 9 and positive till 51 dph. The negative growth pattern of the trunk during the first stages of development in *N. venustus* has been reported for most fish species to develop primary functions (feeding, respiration and locomotion) related to the anterior and posterior body sections prior to the abdominal region (Osse and van den Boogart, 1995). Trunk allometric growth increases after the development of the head and tail (Osse et al., 1997; van Snik et al., 1997; Gozlan et al., 1999) that is considered as the priority of digestive system development.

The head growth showed negative pattern by day 9 in *N. venustus*. Thereafter, when the larvae were less dependent on endogenous feeding, positive growth coefficient was observed. It is assumed that positive growth of head can describe the development of nerve, sensory and respiratory organs as well as feeding systems essential for exogenous feeding (Gisbert and Doroshov, 2006). Negative growth pattern (b=0.2) of eye diameter by day 11 followed by positive growth pattern afterward. The morphological variation toward positive growth of eye diameter could explain visionary essence after shifting to exogenous feeding as vision provide better spatial orientation in swimming (Gisbert, 1999; Petereit et al., 2008).

In conclusion, the present study highlights the preference of morphogenesis as energy demanding transformation to improve the strategy of survival considering the respective importance of functionality.

Acknowledgments

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