CJES
Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Comparative skeletal ontogeny and allometric growth pattern in common and butterfly-tail goldfish, *Carassius auratus*

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ABSTRACT

The early stage of development is a vital period in the life cycle of fish in terms of achieving a suitable efficiency for survival. Knowing about fish growth patterns, including morphological changes and normal skeletal development, can be useful for optimizing hatchery production and monitoring and managing fish populations. This study aimed to survey allometric growth patterns and skeletal trait changes of the most popular variety of *Carassius auratus*, butterfly tail goldfish compared with its common strain during development. For the morphological part, specimens were photographed from the lateral view with a digital camera, and seven morphometric traits were measured. The allometric growth patterns were calculated as a power function of total length, and the inflexion points of growth curves were extracted. To study skeletal structure**,** specimens were cleared and stained with alizarin red for bone and alcian blue for cartilage. The positive allometric growth of the head, along with the onset of formation of its skeletal structures at the first-day post hatch that was observed in two studied strains, indicate the importance of the sensory system, nutritional and respiratory organs, which has a great effect on increasing survival rate. The most significant difference between common and butterfly goldfish was the formation and development of caudal fin. The formation process of tail skeletal elements was begun earlier in butterfly goldfish, and the growth pattern of TaL was approximately twice as fast as the common strain.

Keywords: Ornamental fish, Goldfish, Development, Osteology, Goldfish, *Carassius auratus.*
Article type: Research Article.

INTRODUCTION

The growth patterns reflect ontogenetic changes in morphology and phenotypic plasticity (Shingleton *et al*. 2007; Khemis *et al*. 2013), which can explain environmental conditions and generally fish health during growth stages (Dewiyanti *et al*. 2020). Genes and environment influence ontogenetic development (Gisbert *et al*. 2002), so this kind of data is valuable in assessing environmental impacts on the quality of the produced fish (Boglione *et al*. 2001; Kupren *et al*. 2016). The larval stage, because of the occurrence of morphological changes that make them achieve a suitable efficiency for survival, is considered a vital period in the life cycle of fish (Gisbert *et al*. 2002; Barriga & Battini 2009). Hence, the knowledge of fish growth patterns can be useful for optimizing hatchery production (Snyder *et al*. 2005; Kupren *et al*. 2016), as well as monitoring and managing fish populations (van Maaren & Daniels 2000). Ontogeny of skeletal structure is useful to identify the larvae of closely related species (Fritzsche & Johnson 1980; Saka *et al*. 2008). The high prevalence of bone deformities and the decrease in the survival rate during the larval stage is an important problem in the process of rearing larvae from an economic point of view in aquaculture (Koumoundouros *et al*. 1997; Dasilao & Yamaoka 1998; Boglion *et al*. 2001). Establishing a reference for normal skeletal development might be a useful tool for the early diagnosis of this type of deformities and reaching an optimized protocol through the modification of environmental factors and ratio

Caspian Journal of Environmental Sciences, Vol. 24 No. 1 pp.229-237 Received: May 14, 2025 Revised: Aug. 04, 2025 Accepted: Oct. 27, 2025
DOI: 10.22124/cjes.2024.7874

Publisher: University of Guilan,

optimization (Cahu *et al*. 2003; Lewis & Lall 2006). Furthermore, bony structures are valuable data in phylogeny and clarifying systematic relationships. The goldfish, *Carassius auratus*, belonging to the family Cyprinid is one of the most popular ornamental aquarium fish. At least 180 strains of this species have been known all over the world (Kon *et al*. 2020; Nasu & Ohuchi 2016). The unique variety of this species has fascinated many researchers. Since goldfish is economically valuable and is in increasing demand, therefore it is of particular importance to the breeding success and larvae production of this species (Sandford 2003). Various studies on the genetic and morphological characteristics of goldfish strains, especially common goldfish, were reported (Kodama *et al*. 2017; Kon *et al*. 2020). In addition, there are several studies related to allometric growth patterns and ontogeny of different parts of bones in other marine and freshwater fish, for instance, *Sparus aurata* (Boglione *et al*. 2001), *Chelon labrosus* (Khemis *et al.* 2013), *Epinephelus akaara* (Park *et al*. 2016), *Leuciscus leuciscus* (Kupren *et al*. 2016), *Polypterus senegalus* (Rizzato *et al*. 2019). However, to date, there is a lack of comprehensive information about ontogenetic changes in skeletal structures along with morphological changes in goldfish strains. In the present study, normal larval growth patterns, including morphological and skeletal trait changes of the most popular variety of *Carassius auratus*, butterfly tail goldfish, during development in comparison with those common strain were evaluated. The presented results, in spite of providing insight into the basic biology of this species during the initial development, can help in optimizing the breeding protocols of larvae of studied species, indicators of growth and evaluating the quality of produced larvae. In addition, it would be also useful for the taxonomic review of this taxon.

MATERIALS AND METHODS

Osteology

A number of 240 larvae of two goldfish varieties (*Carassius auratus*), common and butterfly tails, were studied. Breeders of two studied strains were purchased from a local fish store (Ancestors imported from Thailand) and were kept under constant conditions. Specimens from the first-day post hatch (dph) up to the 10th dph was randomly sampled daily and then every other day up to the 30th dph; afterward, sampling was continued every five days up to the 45th dph and every ten days up to the 90th dph. After euthanasia using 1% clove oil, five samples for each stage were fixed in phosphate-buffered 5% formalin for a day, and then were maintained in 72% ethanol. To survey the bone structure of larvae and adults, specimens were cleared and stained with Alizarin Red S for bone and Alcian blue for cartilage, according to Darias *et al*. (2010) and Taylor & van Dyke (1985) respectively. The cleared stained samples were scanned by means of a scanner (EpsonV600) equipped with a glycerol bath. Nomenclature and abbreviations of bon segments followed Hilton *et al.* (2011).

Morphology

Specimens were photographed from the lateral view (Left side) using a stereomicroscope (Leica MC5) equipped with a digital camera (Cannon) with a five MP resolution. The samples were stained with Toluidine Blue dye at a ratio of 1:1 with water to further contrast. Morphometric traits, including total length (TL), head length (HL), tail length (TaL), trunk length (TrL), eye diameter (ED), snout length (SnL), and body depth (BD) were measured by means of ImageJ software (version 1.240; Fig. 1). The allometric growth patterns were calculated as a power function of total length using non-transformed data, $Y = aX^b$. In that function X and Y are the independent and the dependent variables, respectively; a and b are the intercept and the growth coefficient, respectively. Positive, Isometric, and negative growth patterns are demonstrated by $b > 1$, $b = 1$, and $b < 1$, respectively (van Snik *et al*. 1997). The inflexion points of growth curves were extracted according to Fuiman (1983) and van Snik *et al.* (1997). The robustness of the regression was calculated by measuring the R^2 value and its significance level. Data analysis and drawing graphs were performed via Excel 2013 and Past 2.17 for Windows.

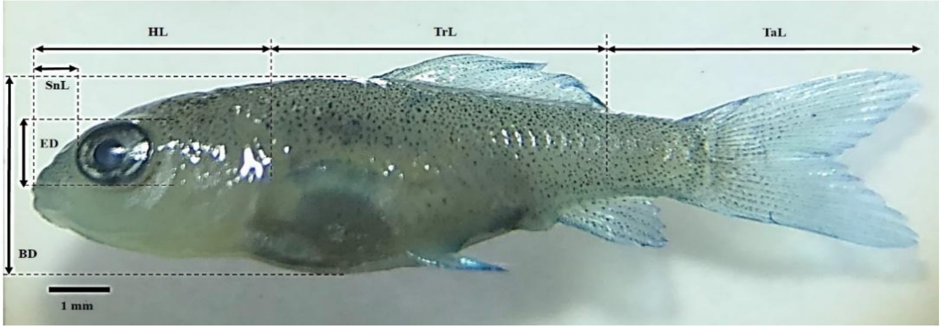

Fig. 1. Measured morphometric characters on 40th dph common variety of *Carassius auratus*.

RESULTS

Ontogeny of skeletal structure: The growth pattern of the bone structures in the butterfly tail goldfish was similar to common strain in nearly all parts. Some differences were visible between them, as follows:

Jaw

On the first day after hatch, the bones of the upper jaw were not visible unlike common goldfish (having semi-ossified maxillae and premaxillae on the 1st dph). The ontogeny of the jaw starts with the formation of the cartilaginous dentary of the lower jaw on the first dph. Cartilaginous maxillae and premaxillae were observed in the second and fourth dph, respectively. The development of the lower jaw continued with observing cartilaginous retroarticular in the third dph that was completely separated on 18th dph vs. in common strain. The dentary and retroarticular were observed as the bony structures on the first day after hatch. The process of ossification of upper and lower jaw elements was completed on the 5th dph, like what was observed in common goldfish.

Suspensorium

Ossified hyomandibular and symplectic were observed after hatching, whereas, in common goldfish, these two segments were visible as a semi-ossified unit that was approximately developed in its posterior part and was pointed in the intero-ventral side. The process of ossification was completed on the 9th dph, while metapterygoid and endopterygoid were still cartilaginous. This unit became ossified on 12th dph. In butterfly tail goldfish, on the 8th dph, suspensorium elements, including metapetrigoid, palatin, quadrate, endoptrygoid, ectoptrygoid were observed as an integrative cartilaginous structure which became separate and ossified on the 32nd dph. While in common goldfish, the formation of Plato-petrogo-quadrate plate was observed on the 18th dph and the cartilaginous ectopterygoid was visible on the 23rd dph, getting ossified six days later (on the 29th dph).

Opercular series

On the first dph, Opercular segments were not formed in butterfly tail goldfish. On the third dph, opercle was observed in butterfly tail goldfish, and on the 14th dph, other elements, including ossified praeopercle, interopercle, and subopercle, were visible. In common goldfish, the formation of opercle begins a day after hatch; it has an incomplete structure with a mixture of bone and cartilage tissue. The prompt process of ossification was completed on the second dph. Other segments of the opercular series were cartilaginous on the 22nd dph and were ossified at the 29th dph. This process was slow for other segments of the opercular series. They were cartilaginous and became ossified at the 22nd and 29th dph respectively.

Branchial apparatus and hyoid arch

On the first dph, the development process of hypohyal, ceratohyal, and epihyal was completed, whereas basihyal and urohyal were not still visible in the two studied strains. Cartilaginous basihyal and urohyal appeared on the 6th and 7th dph in common and butterfly goldfish, respectively. Ceratobranchial and inphrapharyngobranchial were cartilaginous, but in common goldfish, those structures were ossified on 1stdph. Epibranchial, hypobranchial, and basibranchial were not visible in the two varieties. These segments became visible on the 9th dph as cartilaginous structures and turned ossified at the 29th dph in common goldfish. This apparatus was developed on the 20th dph in butterfly tail goldfish.

Neurocranium

Neurocranium was osteocartilaginous, ossified part including parasphenoid and prootic and cartilaginous elements consisting of pterotic and Vomer that turned ossified 22 days post-hatch vs. in common goldfish, which was completely cartilaginous. Cartilaginous lateral ethmoid was observed at the 8th dph and were also ossified on the 22nd dph. While the bones of parasphenoid, vomer, and lateral ethmoid were ossified on the 29th dph in common strain. The occipital area, as well as epiotic, were observed on the 4th dph. Ossified pterosphenoid and orbitosphenoid were visible on the 8th dph, while parietal was still cartilaginous. In common fish, orbitosphenoid was bony on the 26th dph, the ossification of epiotic and supraoccipital were also slow and occurred on the 55th dph. The frontal was cartilaginous on the 24th dph and became ossified on the 32nd dph. The process of ossification of the frontal was continued up to the 60th dph in common fish.

Fig 2. Three views of the skeletal structure of common goldfish (a) on the 10th dph; (b) 29th dph; and (c) lateral view of the skeletal structure of butterfly tail goldfish from top to bottom respectively on the 12th, 32nd and 60th dph (Blue area: cartilage; red area: ossification).

Vertebral column

The development of the vertebral column of two varieties of goldfish was similar and began with the formation of anterior cartilaginous neural spines a day after hatching when the notochord was stretched directly along the entire length of the body. The development of the caudal fin was begun with the appearance of three cartilaginous paired elements, including 1-3 hypural under the notochord on the 3rd dph. The process of development was slow, and about eight days later, the sixth hypural appeared (on the 11th dph) while the all-formed hypurals became ossified. Notochord flexion as an important inflection point to develop of the caudal fin and the ability to swim occurred between the 6th and 8th dph. Pleurostyle and a narrow cartilaginous epural known as PU1, was formed on the 16th dph and became ossified on the 35th dph. Parahyporal as a cartilaginous bud was observed at the end of the notochord bending stage on the 18th dph. The process of development of the caudal fin and its supporting structures was complete on the 22nd dph, and neural spines were developed and fused on the 29th dph.

Paired and unpaired fins

In two studied strain fish, pelvic fin element was not observed after hatching; this element was cartilaginous on the 20th dph and became ossified on the 22nd dph in butterfly tail goldfish. The development process of this structure was slower in common goldfish, and ossification was complete with a 10-days delay (on the 32nd dph). Cartilaginous cleithrum, scapula, and coracoid were observed 24 after hatching, whereas supra cleithrum and post cleithrum were visible, respectively on the 29th and 32nd dph. The ossification process of supra cleithrum was more prompt in common goldfish and completed on the 14th dph. The ossification of scapula and coracoid were completed on the 23rd dph (unlike in common goldfish, scapula and coracoid were observed on the 3rd dph and were ossified at the 40th dph), when radial elements of pectoral fin appeared as an integrative cartilaginous sheet named fin-plate. Radials starts to separate on the 40th dph. Scapular foramen was begun to develop on the 45th dph, it was completely developed on the 60th dph. On the first day after hatching, the pterygiophores of the dorsal and pelvic fins were not visible, and they were begun to develop on the 3rd dph, unlike common goldfish. These segments were obviously visible as cartilaginous tissue on the 14th - 16th dph. The number of five cartilaginous rays of the dorsal fin on the 18th dph increased to 19 and 1/2 rays on the 32nd dph, while the number of five cartilaginous rays of the pelvic fin on the 20th day developed to the number of 6 and 1/2 bony rays on the 40th dph. In common goldfish, on the 29th dph, the dorsal fin's pterygiophore with a cartilaginous structure and a number of 15 rays was observed that developed to 22 after 2 months post-hatch. The anal fin's pterygiophore was visible on the 26th dph with a number of seven bony rays; at that time, the dorsal and anal fin's pterygiophore were 20 and 6, respectively.

Growth pattern of butterfly tail goldfish compared to common strain

In this section, the size of some important parts compared to the length of the whole body was evaluated. The allometric growth pattern of the HL displayed a positive allometry prior to its inflection on the 8th dph (TL = 6.318 mm), and after the inflection point, it was reduced and was close to isometric b = 1.1312 (Fig. 1).

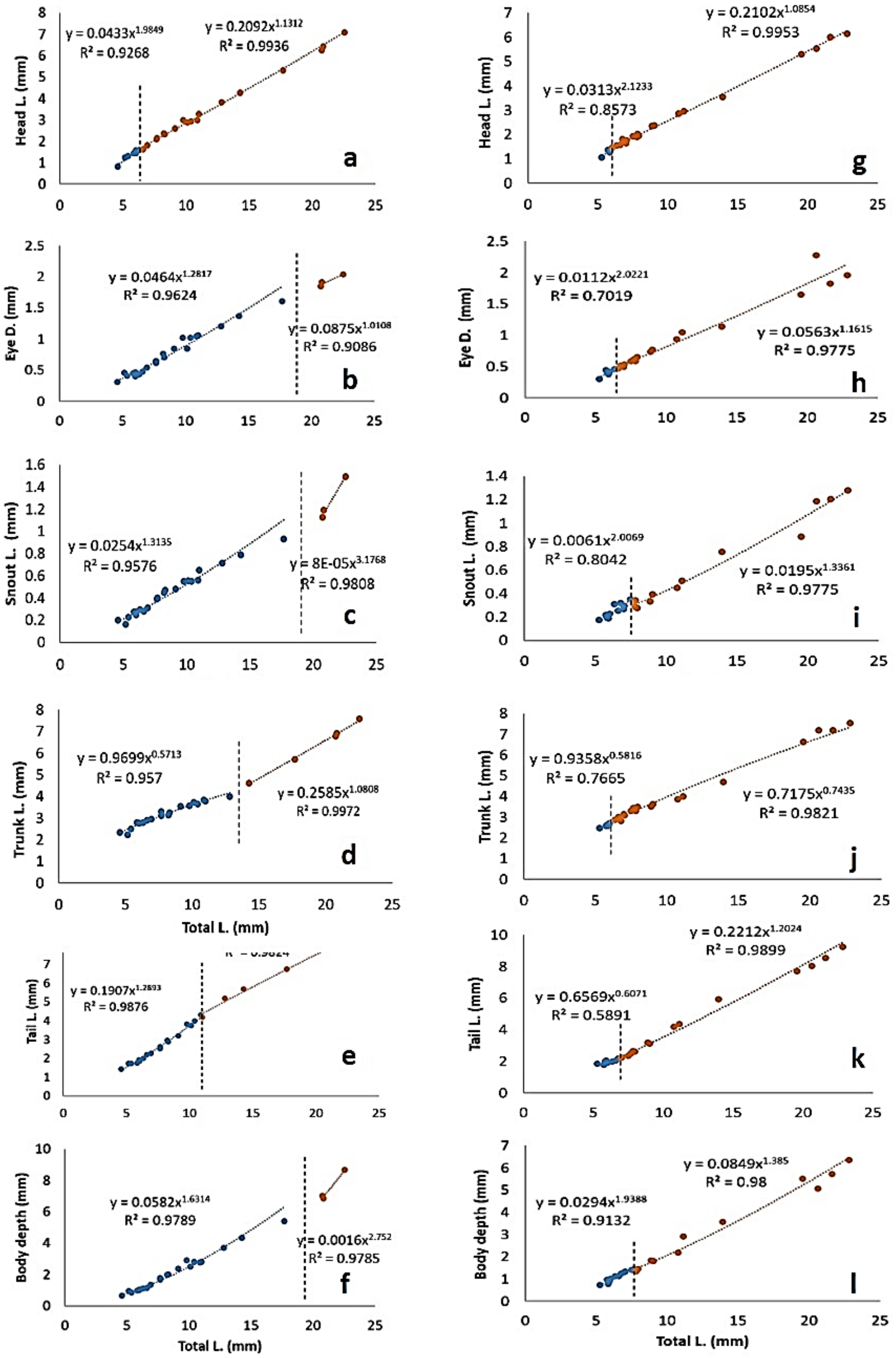

Fig. 3. Allometric growth pattern of the different body segments of butterfly tail (a-f) and common (g-l) varieties of goldfish (R^2 = correlated coefficient).

In addition, ED, SnL, and BD showed a positive growth pattern up to the 45th dph (TL = 17.6783 mm), then changed to isometric in ED (b = 1.01) and strongly positive allometric pattern in SnL (b = 3.1) and BD (b = 2.75). The growth pattern of TrL was relatively negative (b = 0.571) before its inflection point on the 35th dph (TL =12.808 mm). Afterward, it was changed to isometric. The allometric growth of TaL was positive prior to its inflection (on the 26th dph, TL=10.87 mm), whereas during post inflection-point, the growth was negative (b = 0.904). In a common variety of goldfish, the following segments of HL, ED, SnL, and BD have the same growth pattern as in butterfly tail goldfish with a difference of inflection points. Allometric growth of TrL and TaL has a negative pattern up to inflection point on the 5th and 12th dph, respectively. Afterward, they have negative and positive allometric growth pattern respectively.

Table 1. The results of linear regression of the measured morphological parameters (* indicates significance at the 5% level).

variables	slop	b	P value	R^2
HL	0.332	- 0.486	0.00^*	0.997
SnL	0.097	- 0.095	0.00^*	0.983
ED	0.064	- 0.115	0.00^*	0.979
TrL	0.277	0.95	0.00^*	0.982
TaL	0.399	- 0.374	0.00^*	0.989
BD	0.410	- 1.436	0.00^*	0.985

DISCUSSION

The process of formation and development of the upper jaw elements in the two studied goldfish followed the observed typical pattern in other Osteichthyes with a slight difference. In common goldfish, maxillary and premaxillary bones are formed immediately after hatching, while in butterfly goldfish, it appeared with one day delay on the 2nd dph. This outcome overlaps with the growth pattern of HL. According to the result, the growth pattern of HL was positive and faster than the other parts of the body, with a growth coefficient of 1.98 in butterfly tail and 2.13 in common goldfish during the first growth phase. The gained results are similar to others recorded in many species such as *Rutilus frisii kutum* (Ghorbanzadeh *et al.* 2014), blue cichlid *Sciaenochromis fryeri* (Mousavi-Sabet *et al.* 2019), zebra cichlid *Amatitlania nigrofasciatum* (Moshayedi *et al.* 2016), Benni fish *Mesopotamichthys sharpeyi* (Kiarsi *et al.* 2018) and bream, *Abramis brama* (Sahraian *et al.* 2015). The positive allometric growth of the head is related to the development of the brain, sensory system, nutritional and respiratory organs (Gisbert & Doroshov 2006). Starvation is one of the most serious and pervasive threats to the survival of fish larvae during early development, at the same time, with the active larval feeding after absorbing the yolk sac. Therefore, the formation and development of the jaw and other related structures in feeding is a preference for larval early life (van Snik *et al.* 1997; Koumoundouros *et al.* 1999; Gisbert *et al.* 2002). Other structures that are directly or indirectly involved in the feeding process include the hyoid arch, suspensorium, and some parts of the neurocranium. The hyoid arch, even before the completion of development, plays an effective role in the further expansion of the buccal cavity. Intensification of suction power and improvement of larvae hunter ability. The formation of this arch was almost matched to the onset of active feeding. In addition, the prompt development growth pattern of suspensorium elements provides the suction efficiency by a significant expand in the mouth volume (Ruck 1976). The ethmoid part in neurocranium also supports and strengthens the structure of the upper jaw as much as possible. In addition, cleithrum in the pectoral fin, as a point for muscle stabilization, plays an important role in the mouth opening and closing, while an indirect role in hunting (Hasanpoor *et al.* 2014). Since sensory structures, including eyes, chemical receptors, and feeding, breathing, and food organs, are located on the head (Fuiman 1983; Yufera & Darias 2007; Pena & Dumas 2009), such a growth pattern is also well understood in the head region in the studied species. The researchers believe that one of the reasons for the rapid growth of the head in the early stages of development is the reduction of yolk sac reserves and the larvae need to use useful nutritional supplements for external feeding. The change of cutaneous respiration to gill respiration is one of the important events that occurs during the larval period. Hence the development of the gill skeleton of the fish simultaneously by the elevation in the head volume compensates for the lack of oxygen and the need for the growing organs and increased body's metabolism. On the other hand, it improves swimming performance and reduces the mortality rate (Saka *et al.* 2008). The early observation of the opercular series and branchial apparatus in two strains of goldfish are also related to their great importance for breathing (Ruck 1976). The development growth pattern of the neurocranium started from the ventral part and was followed by the formation of weak dermal cartilages in its dorsal surface in the two studied strains. According to the fact that the muscles attached to the ventral area of the skull indirectly support the jaw and opercular series, it might be important in feeding and improving the breathing rate and volume (Ruck 1976). The TrL in both varieties has a negative pattern during the first phase of development. Afterward, the butterfly tail became isometric, which was similar to what was reported in other species (Ghorbanzadeh *et al.* 2014; Sahraian *et al.* 2015; Kiarsi *et al.* 2018; Nowosad *et al.* 2020). The negative allometry of TrL in the early developmental stage is another reason for the importance of the development of the functional organs of the head region (Gisbert *et al.* 2002). The isometric pattern of TrL in the second phase is evidence of the development in the internal and digestive organs of the trunk region after the growth of the head (Mousavi-Sabet *et al*. 2019). The negative allometric growth pattern of TrL segment in the butterfly tail variety, along with the positive allometric pattern of TaL indicated the importance of improving swimming and reducing the cost of energy consumption until day 35, while this trend was not observed in the common variety. In both varieties of common and butterfly goldfish, notochord flexion occurred along with the development of the caudal fin between the 6th and 8th dph, which caused changes in locomotor abilities, swimming pattern, body shape, and even feeding and hunting behavior (Koumoundouros *et al*. 1999). Simultaneously with notochord flexion, the ossification of the hypural plates was also observed as an ossified structure in two studied strains. Parhypural was also observed at the 8th dph, since the hypural and parhipural operate as a rudder provide space to connect muscles and consequently provide necessary force for movement. Obviously, by ossification and increasing strength (on the 8th dph), swimming performance improves significantly, which has a great effect on increasing the survival rate (Dasilao & Yamaoka 1988). It matched to growth pattern of tail length in butterfly tail goldfish. The positive allometry of the tail length helps the larvae to reduce the energy costs related to the movement and transmission of the larvae through the improvement of swimming and predator escape behaviours. The positive allometry of the tail during the period of internal and mixed feeding indicates the developmental preference of the structures related to swimming and feeding (Mousavi-Sabet *et al*. 2019). The growth pattern of the larval tail of common variety of goldfish in the early larval stages was negative, which is similar to the results reported for Benni fish (Kiarsi *et al*. 2018). Body depth had a positive allometric growth pattern in all stages of development. The increase in body depth can also be due to the development of the skeletomuscular structure and digestive system. In addition, it might help to avoid being hunted by other fishes (Pena & Dumas 2009). In general, the most obvious difference between common and butterfly goldfish was related to the formation and development of the caudal fin. The formation process of hypural plates was started earlier in butterfly goldfish, and the growth pattern of TaL was approximately twice as fast as the common strain. In butterfly-tail goldfish, the hypurals appear symmetrically on both sides of the vertebral column to give the tail a butterfly-like shape. Since this process occurs at the same time as the growth of the necessary organs located in the head and other parts of the body, it causes the reduction of the growth coefficient of another body segment of the butterfly tail compared to the common strain.

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Bibliographic information of this paper for citing:

Azimi, A, Mousavi-Sabet, H, Faghani-Langroudi, H, Eagderi, S, Abed-Elmdoust, A 2026, Comparative skeletal ontogeny and allometric growth pattern in common and butterfly-tail goldfish, *Carassius auratus*. Caspian Journal of Environmental Sciences, 24: 229-237.
