# Skeletal ontogeny of the caudal complex in Caspian kutum, *Rutilus kutum* (Kamensky, 1901) (Teleostei: Cyprinidae) during early development

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## ABSTRACT

Study of skeletal ontogeny during early development is crucial in a successful larviculture. In this study, ontogeny of the caudal complex skeleton in Caspian kutum, *Rutilus kutum*, was studied from hatching up to 50-day post hatching (dph). A total of 520 larvae and fry were randomly sampled, cleared and stained for osteological examinations. The results showed that development of caudal skeleton is started with appearing hypurals 1 and 2 on 4 dph and inflexion occurred on 7 dph with formation of eight cartilaginous plates, including two haemal spines, parhypural and five hypurals, below the notochord. The results also revealed that during post flexion from 13 to 50 dph other structures e.g. rudimentary neural arch, second ural and anterior half of the neural arch of PU3 were appeared. The inflexion point of tail growth pattern in *R. kutum* is in accordance with inflexion caudal complex, showing ontogeny of the caudal complex skeleton in agreements with its function demands during early development.

**Key words:** Tail, Ontogeny, Osteology, Deformity, Caspian kutum.

## INTRODUCTION

Description of the anatomical development during early ontogeny of fishes is crucial for understanding the patterns behind body formation, functional trends and environmental preferences (Koumoundouros *et al*. 2001). Among the various anatomical structures, development of skeletal structures involves a series of transitions between cell proliferation and differentiation i.e. transitions from cartilage to bone (Hogan 1996; Urist 1997; Chen *et al*. 2004; Tsumaki & Yoshikawa 2005). In the skeletal structures, development of fins is important during ontogeny of fishes, since it is closely correlated with changes in swimming mode, swimming velocity, as well as feeding techniques and preferences (Kendall *et al*. 1984). From aquaculture point of view, understanding the skeletal development of commercially-important fish species can assist early diagnosis of the skeletal abnormalities, since most of the skeletal deformities are occurred during early ontogenic stages (Koumoundorous *et al*. 1997; Boglion *et al*. 2001). Skeletal deformities are crucial in aquaculture since they decrease the quality of produced fish by affecting their external morphology, growth and survivals (Hasanpour *et al*. 2016). There are many reports regarding the skeletal deformities in various fishes (Koumoundorous *et al*. 1997; Gavaya *et al*. 2002; Fraser *et al*. 2004; Sfakianakis *et al*. 2005).

Caspian kutum, *Rutilus kutum*, (Kamensky, 1901), is a commercially-important cyprinid fish in the Caspian Sea basin (Ebrahimi 2001; Salehi 2002). Since there is no information available regarding the osteological structure during ontogeny of this species, hence this study was conducted to describe the ontogeny of its caudal complex skeleton as an important locomotor function element which in turn will help to better understanding of its swimming and feeding ontogeny.

## MATERIALS AND METHODS

Larvae specimens were obtained from propagation via mass combined spawning of 15 female and 30 male broodstocks in April-May 2012, from Dr. Yousefpour Fish Hatchery Center, Siahkal, Guilan Province, Iran. The eggs were incubated in 10 L vase incubators with flow-through freshwater system at 22 °C.

After six days of incubation, eggs hatched and then transferred to a large larval collector tank (200 L). After 3 days, at the beginning of exogenous feeding, 30,000 larvae (3 larvae L$^{-1}$) were transferred to a pond (0.1 ha) with a flow through freshwater system with a mean temperature, pH and DO of $25 \pm 2.2°C$, $8.1 \pm 0.5$ and $7.4 \pm 1.1$ mg L$^{-1}$, respectively. The natural water flow provided some natural prey but additional artificial feed was supplied when needed from seven-day post-hatch until the end of the experiment. The artificial feed was a specialized one designed for Caspian kutum larvae and juveniles based on a mix of protein and cereal meals.

A total of 520 larvae and fry were randomly sampled from hatching up to 50 dph (days post hatch). The sampling was performed initially from 0-20 dph every day, and then every five days up to 50 dph by sampling 20 specimens every morning from the same larval batch. Larvae were anaesthetized with 1% clove oil and fixed in phosphate buffered 5% formalin for 24 hours, then stored in 72% ethanol.

Ten specimens per each sampling time of 0-40 dph were cleared and stained according to Darias *et al.* (2010), whereas those of 45-50 dph larvae (n = 10) were cleared and stained based on the Taylor & Van Dyke (1985). The stained specimens were preserved in glycerol by adding a small piece of Thymol crystal to prevent fungi contamination (Gavaya *et al.* 2002). Osteological developments were examined using a stereoscopic dissecting microscope attached to a 13 MP Nikon camera. The cleared stained specimens were dissected and their skeletal elements were scanned by a scanner (EpsonV600) equipped with glycerol bath (Fig. 1). The skeletal elements were drawn from the obtained images using Corel DrawX5 software. Total lengths (TL) of larval specimens were measured to the nearest 0.01 mm and used as a reference point. Nomenclature and abbreviations of osteological features followed Hasanpour *et al.* (2016).
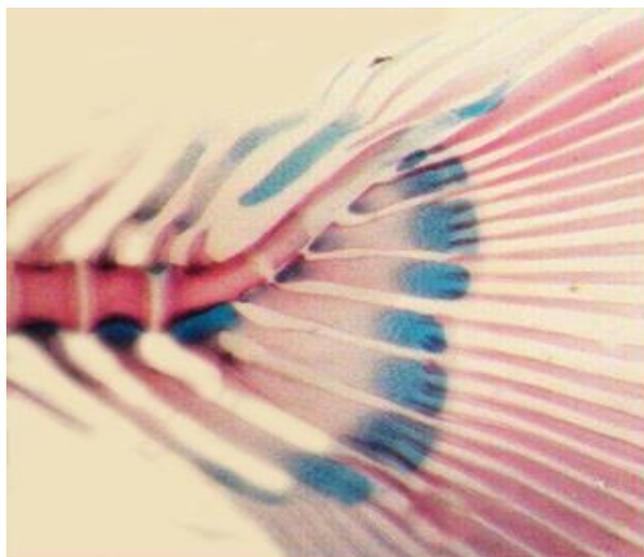


**Fig. 1.** Cleared and stained caudal skeleton of *Rutilus kutum* (50 dph, blue area, cartilage; red area, ossification).

## RESULTS
### Pre-flexion stage
At hatching, mean TL of the larvae was $7.11 \pm 0.48$ mm lacking any caudal elements (Fig. 2A-B). The notochord was straight and primordial marginal fin-fold was present (1-4 dph). The caudal fin was begun to develop approximately at $9.41 \pm 0.63$ mm TL (4 dph), with appearance of four cartilaginous elements beneath the notochord, including the hyporals 1-3 and parhypural, from left to right, respectively. The number of principal caudal rays (PCR) was four at 4 dph (Fig. 2C). The parhypural was developed at $9.74 \pm 0.36$ mm TL (5 dph) as a cartilaginous rod ventral to hyporals. The numbers of PCR increased to nine at this time (Fig. 2D).

### Early flexion stage
By the flexion stage at $9.98 \pm 0.63$ mm TL (7 dph), the notochord showed an upward curvature posteriorly. The hypurals 4 and 5, and two haemal spines were formed. The ossification of the hypural plates 1-3 and PCR were started (Fig. 2E).

Following formation of the epural at 10.32 ± 0.52 mm TL (8 dph), the parhypural, hypurals 4 and 5 and haemal spines were ossified partially (Fig. 2F).

**Late flexion stage**

The notochord flexion developed at 11.03 ± 0.44 mm TL (9 dph). At this stage, the caudal centrum was comprised, the 1$^{st}$ preural centrum fused to the 1$^{st}$ ural centrum (PU1 and U1) (Fig. 2G). Along with ossification of PU1+U1 and PU 2-3, the neural spines of the preural centrums 2 and 3 were appeared at 11.62 ± 0.57 mm TL (10 dph), as a part of the caudal skeleton (Fig. 2H).

**Post flexion stage**

During this stage, development of the caudal complex was occurred as following:

a) 12.89 ± 0.17 mm TL (13 dph): The epural cartilage was separated as neural spine of the PU1, while trace of its neural arch remained as rudimentary neural arch (RNA). The development of the cartilaginous neural spines continued (Fig. 2I).

b) 15.44 ± 0.67 mm TL (25 dph): The large pleurostyle covered the anterior margin of the hypurals. A second ural centrum was appeared (Fig. 2J).

c) 17.73 ± 0.19 and 21.84 ± 0.78 mm TL (30 and 40 dph, respectively): The anterior half of the neural spine of the PU2 was appeared at 30 dph. This structure further developed at 40 dph. The position of the U2 was shifted anteriorly i.e. toward the PU1+U1 (Fig. 2K-L).

d) 30.47 ± 0.43 mm TL (50 dph): The anterior half of the neural arch of the PU3 was appeared corresponding to development of the neural spines of the PU2 (Fig. 2M).

**DISCUSSION**

The teleostean caudal complex skeleton is a well-studied structure with respect to function, homology and phylogeny (Borden *et al*. 2013). In addition, study of the anatomical development is critical in understanding the functional trends and environmental preferences of the different developmental stages (Koumoundouros *et al*. 2001). Hence, the present study provided a detailed ontogeny of the caudal complex skeleton of *R. kutum* which can be used in this regard.

Similar to *R. kutum*, one epural bone has been reported in the caudal fin of Caspian roach, *Rutilus lacustris* (Hasanpour *et al*. 2016) and *Balistes capriscus* (Matsuura & Katsurava 1985), while in other species such as *Dentax dentax* (Koumondoros *et al*. 1999) and *Cypselurus heterurus doedeleini* (Dasilao & Yamaoka 1998), three epurals present.

In addition, no fusion of the caudal skeleton elements was observed in *R. kutum*. The cyprinids have six hypural plates (Buhan 1972) but five of them had been formed till 50 dph expecting another one appear until 90 dph similar to Caspian roach (Hasanpour *et al*. 2016). Based on the results, the flexion of the notochord was occurred at 7 dph with formation of 8 cartilaginous plates beneath the notochord corresponding to the two haemal spines, parhypural and five hypurals. This pattern was similar to those of *R. lacustris* (Hasanpour *et al*. 2016) except its timing (8 dph vs. 7 dph).

Observation of the osteological development process in fish larvae and juveniles is very important and close scrutiny of each skeletal region during the rearing period is necessary to understand normal development (Park *et al*. 2016). So that, tail development contributes to enhancing improved swimming and maneuvering capability for feeding and escape from predators, whilst decreasing the energetic costs of larval movements (Osse & Van den Boogaart 2004). The allometric growth pattern of tail in *R. kutum* during larval stages is positive till 7 dph (9.98 ± 0.63 mm), and isometric afterward (Ghorbanzaeh *et al*. 2014).

This external change is in accordance with our finding regarding notochord flexion. In addition, the notochord flexion corresponds with formation of the feeding apparatus, fins and gas-bladder (9.29 ± 0.36 mm TL) which started along with the exogenous feeding in 5-7 dph. This reveals improvement of the movement capability in Caspian kutum after notochord inflexion (7 dph) for searching food when larvae are capable of swimming and maneuvering to catch prey and escape from predators. Notochord flexion is followed by the quick development in fin-rays and body shape, as well as a consequent improvement in locomotor ability (Hoar 1988; Koumoundouros *et al*. 1999).
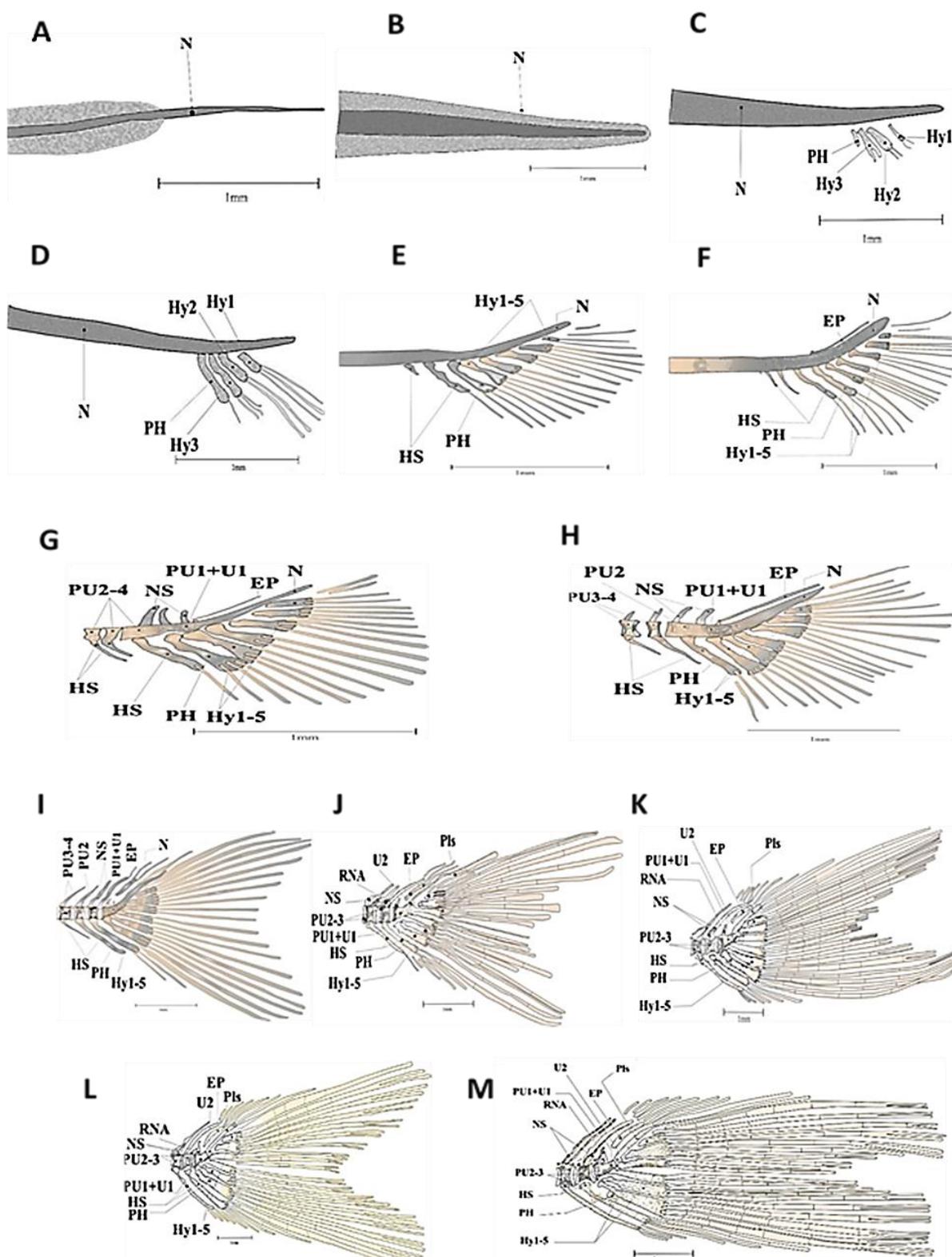
**Fig. 2.** Development of the caudal skeleton of *Rutilus kutum* from 1-50 dph. (A): 1 dph, (B): 2 dph, (C): 4 dph, (D): 5 dph, (E):7 dph, (F): 8 dph, (G): 9 dph, (H): 10 dph, (I): 13 dph, (J): 25 dph, (K): 30 dph, (L): 40 dph, and (M): 50 dph (Gray area, cartilage; orange area, ossification) (Abbreviation: EP: Epural, HS: Haemal spine, Hy: Hypurals 1-6, N: Notochord, NS: Neural spine, PH: Parhypural, Pls: Pleurostyles, PU1+U1: Compound centrum formed first preural and the first Ural centrum, PU1-2: Preural central 1,2, RNA: Rudimentary neural arch, U1-2: Ural centrum 1, 2).

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# تکوین ساختار اسکلتی دم در ماهی سفید دریای خزر (Kamensky, 1901) *Rutilus kutum*

# (ماهیان استخوانی حقیقی: کپورماهیان) در مراحل اولیه توسعه

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**چکیده**

مطالعه تکوین ساختار استخوانی در طی مراحل اولیه توسعه برای موفقیت در پرورش لارو ماهیان اهمیت بسیار بالایی دارد. در این مطالعه تکوین ساختار اسکلت دمی ماهی سفید دریای خزر (*Rutilus kutum*)، از روز تخم‌گشایی  تا ۵۰ روز پس از آن بررسی شد. برای این منظور تعداد ۵۲۰ لارو و بچه‌ماهی، به صورت تصادفی نمونه‌برداری و برای مطالعه استخوان‌شناسی، شفاف‌سازی و رنگ‌آمیزی شدند. نتایج نشان داد که توسعه اسکلت دمی با ظهور هیپورال‌های ۱ و ۲، در روز ۴ پس از  تخم‌گشایی شروع شده و خمیدگی نوتوکورد در روز ۷ پس از تخم‌گشایی  با شکل‌گیری هشت صفحه غضروفی در زیر نوتوکورد شامل دو خار خونی، پارهیپورال و پنج هیپورال به وقوع می‌پیوندند. نتایج همچنین نشان داد که ساختارهایی از قبیل کمان عصبی ابتدایی، دومین اورال و نیمه‌ی قدامی کمان عصبی PU3 در دوره بعد از خمش از روز ۱۳ الی ۵۰ پس از تخم‌گشایی ظهور می‌یابند. نقطه عطف الگوی رشد دم در ماهی سفید دریای خزر با خمیدگی اسکلت دمی در این گونه در انطباق بوده و نتایج نشان می‌دهد که تکوین استخوانی ساختار دمی در این گونه، همسو با نیازهای عملکردی آن در مراحل اولیه تکوین به وقوع می‌پیوندند.

\*مؤلف مسئول

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