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Osteological development of the vertebral column, paired, dorsal and anal fins in *Rutilus caspicus*, Pravdin (1927) (Teleostei: Cyprinidae)

S.H. Hasanpour, S. Eagderi*, B. Mojazi Amiri

Fisheries Department, Faculty of Natural Resources, University of Tehran, Karaj, Iran
* Corresponding author's E-mail: soheil.eagderi@ut.ac.ir

ABSTRACT

Study of the osteological development in fishes is important in fisheries, biology and aquaculture. It can be used as an early bio-indicator of non-optimal rearing conditions. The Caspian roach, *Rutilus caspicus* is a native cyprinid fish of the Caspian Sea basin that its artificial propagation is fulfilled in hatcheries to recruit its natural stocks. Hence, this study was conducted to provide early development of its vertebral column, paired and median fins from hatching up to 90-dph as basic biological information. For osteological examinations, the specimens were cleared and stained and a detailed description of the ontogeny of the post-cranial skeleton provided. The results showed that no osteological structure was present at hatching. The first observed skeletal structure was the vertebral column followed by the pectoral fins, caudal fins and almost simultaneously dorsal, anal and pelvic fins.

INTRODUCTION

Understanding the osteological development of fishes is important in fisheries, biology and aquaculture. From fisheries perspective, it helps in identifying fish larvae (Fritzsche & Johnson, 1980; Saka *et al.,* 2008). Study of this process also affects our interpretation about osteological characters of fishes in adults (Goslin 1961; Fritzsche & Johnson 1980; Koumoudorous *et al.* 1995, 1997, 2001). In addition, understanding the osteological ontogeny of fishes provides information about their taxonomic situation by providing valuable keys to distinguish homology of various skeletal elements (Peters, 1981; Fraser *et al.*, 2004; Javidan & Schilling, 2004;). Furthermore, monitoring of the skeletal anomalies on wild fish may be useful in evaluating pollution levels (Boglione *et al.* 2001). In aquaculture, high incidence of the anomalies and mortality associated with successive developmental stages is a significant bottle neck to their commercialization (Dasilao & Yamaoka 1998), as they reduced the market

value of produced fish by affecting their morphology, survival (Gavaia *et al.*, 2002), poor competition for food and increased susceptibility for both stressors, disease and growth depression (Boglione *et al.*, 2001; Lewis & Lall, 2006). Hatchery reared fishes have a malformation frequency ranging multiple times more than their wild counterparts (Koumoudorous *et al.*, 2001). Knowledge about normal development of the skeleton is crucial in addressing when and where abnormalities occurre under rearing conditions. It can be used as an early bio-indicator of non-optimal rearing conditions (Lewis & Lall, 2006) and also in determining the proper diet (Cahu *et al.*, 2003). The Caspian roach, *Rutilus caspicus* is a cyprinid fish and native to the Caspian Sea. This species is adapted to sea ranching with commercial value. Due to over fishing and deterioration of its spawning grounds, *R. caspicus* has experienced a remarkable decline in its fishing yields (Kiabi *et al.*, 1999). Therefore, its artificial propagation is fulfilled in hatcheries to recruit

its natural stocks during last two decades (Ghelichpour & Eagderi, 2012). Since in restocking programs, providing basic biological information is crucial for breeding and rearing of larvae as well as no information is available about larval development and skeletal calcification of this species. Hence, this study aimed to provide a detailed description of the ontogeny of its vertebral column, paired and median fins (except caudal fin) during early developmental stages from hatching up to 90 day post hatching (dph).

MATERIALS AND METHODS

A total of 20 adult Caspian roaches were obtained from the Sijval Restocking Center (Bandar-e-Turkmen, Iran) in Spring 2013 and introduced into an earthen pond at an ambient temperature. By semi-artificial propagation method, the broodstocks were bred, and the larvae produced. During rearing period, the larvae were fed by fertilizing the pond and a diet based on Fontagne and Silva (2009). The water temperature, DO and pH of the pond were 21.4 - 24.4°C, 6.5 - 8 ppm and 7.6 - 8.4 during rearing period, respectively. Fish were reared under the natural photoperiod. In addition, the semi extensive condition was applied to provide a natural habitat and producing high-quality larvae with low anomalies (Sfakianakis *et al.*, 2004, 2005; Lewis & Lall, 2006). After hatching, larvae were randomly sampled from hatching up to 90-dph prior to feeding in the morning, sacrificed by an overdose of MS 222 (Sigma-Aldrich) and preserved in 4% buffered formalin. From hatching till 20-dph larvae were sampled every day, then every 5 days up to 90-dph (n = 10).

The specimens were moved to 72% alcohol after 48 hours. Then, the specimens were photographed using a dissecting microscope equipped with a Cannon camera with 5 MP resolution and their Total Length (TL: from the tip of the snout to the end of the caudal fin) was measured using Imagej software (version 1.240) to the nearest 0.001 mm. TL was measured as the reference point in the description of the ontogeny because it is a proper measure of ontogenetic state than age (Saka *et al.*, 2008; Sfakianakis *et al.*, 2004, 2005).

For osteological examinations, the specimens of 0-40-dph were cleared and stained with alizarin red S and alcian blue according to Darias *et al.* (2010) and those of 45-90 dph based on the Taylor and Van dyke (1985).

Then, the specimens were studied using a stereomicroscope equipped with 13 MP Nikon cameras (Leica MC5); and their skeletal elements were dissected and scanned by a scanner equipped with a glycerol bath (Epson v600). Drawings of the skeletal elements were made using CorelDrawX6 software. The meristic characters, including total vertebrae (including the urostyle), anal, dorsal, pelvic and pectoral fin rays were studied under a stereomicroscopy (Leica MS5) (Boglione *et al.*, 2001).

The teleostean axial skeleton has 2 types of vertebrate in the abdominal region, pre-caudal vertebrate bear rib and caudal vertebrate has no rib.Nomenclature and abbreviations of osteological features fallowed and Lundberg & Baskin (1969), Peters (1981), Yuschak (1985), Dasilao & Yamaoka (1998), Rojo (1991), Sfakianakis *et al.* (2004) and Sfakianakis *et al.* (2005), presented in Tables 1 - 3.

Table1. Abbreviations of the vertebral column in *R. caspicus*

ABBREVIATIONS	STRUCTURES	ABBREVIATIONS	STRUCTURES
AR	Anal Ray	NC	Notochord
CN	Centrum	PP	Proximal Peterigiophore
CR	Caudal Ray	US	Urostyle
DP	Dorsal ray	PU	Preural Centrum
Ns	Neural Spine	Hs	Haemal spine
Pr	Pleural rib	Pp	parapophysis
NuA	Neural Arch	Bop	Basiocciptial articulating process
EP	Epural	PU1 + U1	Compound centrum formed the first preural and the first Ural centrum
RNA	Rudimentary neural arch	Hy	Hypural 1 - 6
EP	Epural	PH	Parhypural
Pls	pleurostyle	U1-2	Ural centrum 1, 2

Table 2. Abbreviations of the pectoral girdle in *R. caspicus*

ABBREVIATIONS	STRUCTURES	ABBREVIATIONS	STRUCTURES
Cl	Cliethrum	Prop	Propterigium
Fp	Fin plate	Poc	Postcliethrum
Co-Sca	Coraco- scapular	Mco	Mesocoracoid
Suc	Supracliethrum	Rd	Distal radial
R	Ray	P rx	Proximal pterigiophore
FPS	Pectoral fin spine	Pot	Post temporal

Table 3. Abbreviations of the pelvic fin in *R. caspicus*

ABBREVIATIONS	STRUCTURES	ABBREVIATIONS	STRUCTURES
R	Lepidotrichium	EVW	External ventral wing
Bp	Basipterigium	EDW	External dorsal wing
MEP	Metapterigium	S	Hard spine
POP	Protopterigium	CP	Central process

RESULTS

Pectoral fin (Figs. 1 - 2)

In the 4-dph larvae ($T_1 = 7.996 \pm 0.257$ mm), the pectoral fin was present as a small, rounded and transparent membrane. The first observed bony element of the pectoral fin was the cliethrum, presenting as a thin rod of the cartilage. The cliethrum is widened in its dorsal and ventral parts at 12.019 ± 0.571 mm (14-dph) and then begun to ossify. The cliethrum was fully ossified at 8.624 ± 0.267 mm (5-dph). Expansion of this bone was continued and articulated dorsally with the cartilaginous supracliethrum at 10.961 ± 0.731 mm (9-dph). Attachment of the cliethrum to the occipital region was accomplished by the supracliethrum and posttemporal.

The supracliethrum appeared as a slender-shaped cartilage in 9-dph, overlapping the cliethrum and possesses its adult shape at 12.019 ± 0.571 mm (14-dph).

The posttemporal appeared at 14-dph and connected the supracliethrum to the epiotic and intercalary at 12.389 ± 0.631 mm (18-dph). The posttemporal was ossified at 18-dph. The postcliethrum was formed under developing rays at 16.066 ± 0.513 mm (30-dph), as a thread-like cartilage and fully ossified after 20.762 ± 2.224 mm (40-dph).

The coraco-scapular appeared as a cartilage at 7.996 ± 0.257 mm (4-dph). The coraco-scapular bar showed a ventral extension of its anterior process alongside of the cliethrum. The coracoid and scapula were gradually separated concomitant with their ossification. Ossification of the scapula was begun at 16.066 ± 0.513 mm (30-dph) and completed at 40-dph. The scapular foramen was formed at 30-dph and fully developed at 39.539 ± 0.842 mm (70-dph).

The radials of the pectoral fin were present as a continuous sheet of the cartilage namely fin plate at 4-dph. The cartilaginous pectoral fin that supports first and second crevices, were present at 12.389 ± 0.631 mm (18-dph). The gap between radial 3 and 4 did not appear until 14.795 ± 1.034 mm (25-dph). At 40-dph, the ossification of the radials started and fully ossified at 29.639 ± 2.003 mm (50-dph). At 14-dph, the pectoral fin rays started to be formed, almost simultaneously with the appearance of the posttemporal bone. The mesocoracoid was developed at 40-dph. The adult structure of the pectoral girdle was observed at 40.21 ± 3.412 mm (90-dph).

Pelvic fin (Fig. 3)

The basipterigia appeared at 12.050 ± 1.232 mm (12-dph), as a pair of the crescentric cartilaginous structure lying ventrally. They were stained faintly with alcian blue. These bones were gradually expanded anteriorly and posteriorly, while their anterior tips were being converged.

The basipterigia were weakly ossified through the central process at 14-dph. The latero-dorsal and ventral wings of the pelvic fins were appeared at 40 and 50-dph and fully ossified at 50 and 60-dph, respectively (TL = 32.695 ± 2.393 mm). The first two rays and first spine were

present at 14 and 40-dph, respectively. The sequences of the fin rays development follow a median direction i.e. R1 of the pelvic ray forms first and R5 last. The full complement of the spine and 10 rays was attained at 50-dph. The rays were fully ossified, segmented and bifurcated at 90-dph. The cartilaginous metapterygium was formed at 14.063 ± 0.258 mm (20-dph). The protopterygium appeared as a cartilaginous bud at 30-dph and fully ossified at 50-dph.

Dorsal fin (Figs. 1 - 2)

At the early stages, the dorsal fin was present as a primordial marginal fin fold (TL = 11.305 ± 0.630 mm, 10-dph). The eight incipient soft-rays were observed at 14-dph. The number of rays increased to 11 at 18-dph. All 11 anterior soft-rays were partially ossified at 20-dph. The dorsal fin in the adult is comprised of 12 rays which are supported by 11 pterygiophores with the anterior most one supporting 2 rays. The pterygiophores form the base of the dorsal fin rays embedded in the epaxial muscles and are usually comprised of three fused bones viz. the proximal, medial and distal pterygiophores. The eight proximal pterygiophores are present as cartilage dorso-anteriorly over the neural spines at 14-dph. By 60-dph, final merestic counts of the proximal pterygiophores were achieved. Ossification of the proximal pterygiophores follow the same pattern as their formation. The distal and medial pterygiophores were first observed beneath the dorsal soft-rays, at 50 and 20-dph and then they attained their final numbers at 60 and 40-dph, respectively.

Anal fin (Figs. 4 - 5)

The soft rays of the anal fin appeared at 14-dph and proceeds both anteriorly and posteriorly. Their ossification followed the same pattern as their formation and the adult complement occurred at 40-dph. Five proximal pterygiophores were formed by a caudal direction i.e. their development were completed at 40-dph (12 in final meristic

counts). The formation of the medial and distal parts of the pterygiophores begun at 60-dph.

Axial skeleton (Figs. 1 - 2)

Newly hatched larvae were devoid of any trace of the neural and haemal arches and only anteriorly notochord segmentation was observed. They bear a straight notochord extending entire body length. Flexion of the notochord occurred between 8-dph (9.548 ± 0.482 mm) and 10-dph (11.305 ± 0.630 mm). Development of the vertebral centrum was initiated in parallel with the caudal fin structures.

The caudal fin development started at approximately at 8.624 ± 0.267 mm (5-dph) with the appearance of three cartilaginous plates beneath the notochord, including the hypural 1, 2 and 3, respectively, from left to right. The centra were faintly stained with alizarin red indicating the beginning of the ossification. The number of centra increased caudally while they were just weakly ossified.
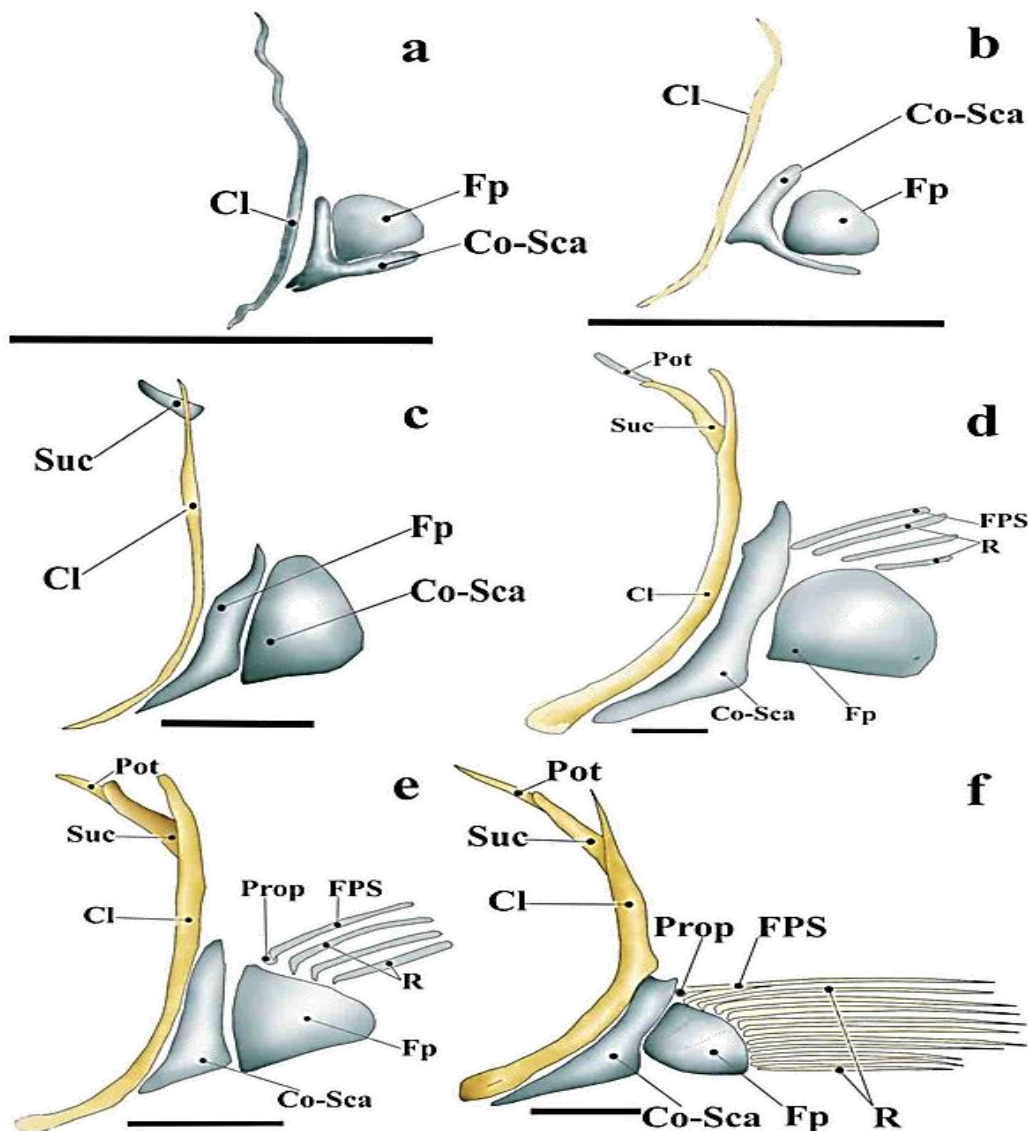
The parhypural appeared at 8.681 ± 0.356 mm (6-dph) as a cartilaginous bud at the ventral distal portion of the notochord. By the flexion stage, the hypural 4, 5 and two haemal spines were formed. The vertebral columns (centra) were fibrously ossified, whereas the neural and haemal spines were fully chondrified. By the late flexion stage (10-dph), a composite main caudal centrum comprising the first pre-Ural and Ural 1 were observed and the ossification of the PU1+U1 and PU2-3 were begun. At the dorsal face of the notochord, the neural spines of the preural 2 and 3 were appeared at 11.305 ± 0.630 mm (10-dph). The pre-Ural 2, 3 and their haemal and neural spines were included as a part of the caudal complex. Development of the haemal and neural arches of the caudal centra initiated with the buds formed ventrally and latero-dorsally by intra membranous ossification, respectively, while their formation progressed anteriorly. At the dorsal face of the abdominal vertebrae, the neural arches of the 3 - 9 were observed while their formation progressed posteriorly.

Secondary halves of the neural arch appeared at 30-dph. They are elongated dorsally until they joined together forming the arches. The spine was also appeared by intra membranous ossification and elongated dorsally.

The first abdominal vertebra was articulated with the basiocciptial articulating condyle. The ventral ribs first appeared at the pleural 5, and the formation of other ribs continued caudally. Their ossification followed the same pattern as their formation. The parapophysies, i.e. the vertebral processes were formed along with the formation of the pleural ribs. The parapophysies 5-8 were visible on the trunk centra and the calcification was extended from the base of the arches beginning to form the centra surrounding the notochord.

Configuration of the adult backbone in the adult was composed of 44 centra, 36 neural spines, 17 haemal spine and 19 pleural ribs. The vertebral column consists of 23 abdominal and 21 caudal centra, including the urostyle and U2. Each abdominal vertebra bears a neural arch and spine dorsally and a pair of the parapophysies and pleural ribs ventrally, whereas the caudal vertebra possesses a neural arch and spine dorsally and a haemal spine ventrally.

Fig. 1. Development of the pectoral girdle in *R. caspicus*. (a): 4-dph, (b): 5-dph, (c): 9-dph, (d): 14-dph, (e): 18-dph and (f): 20-dph. (Blue area: cartilage; and yellow area: ossification; bar = 0.50 mm).
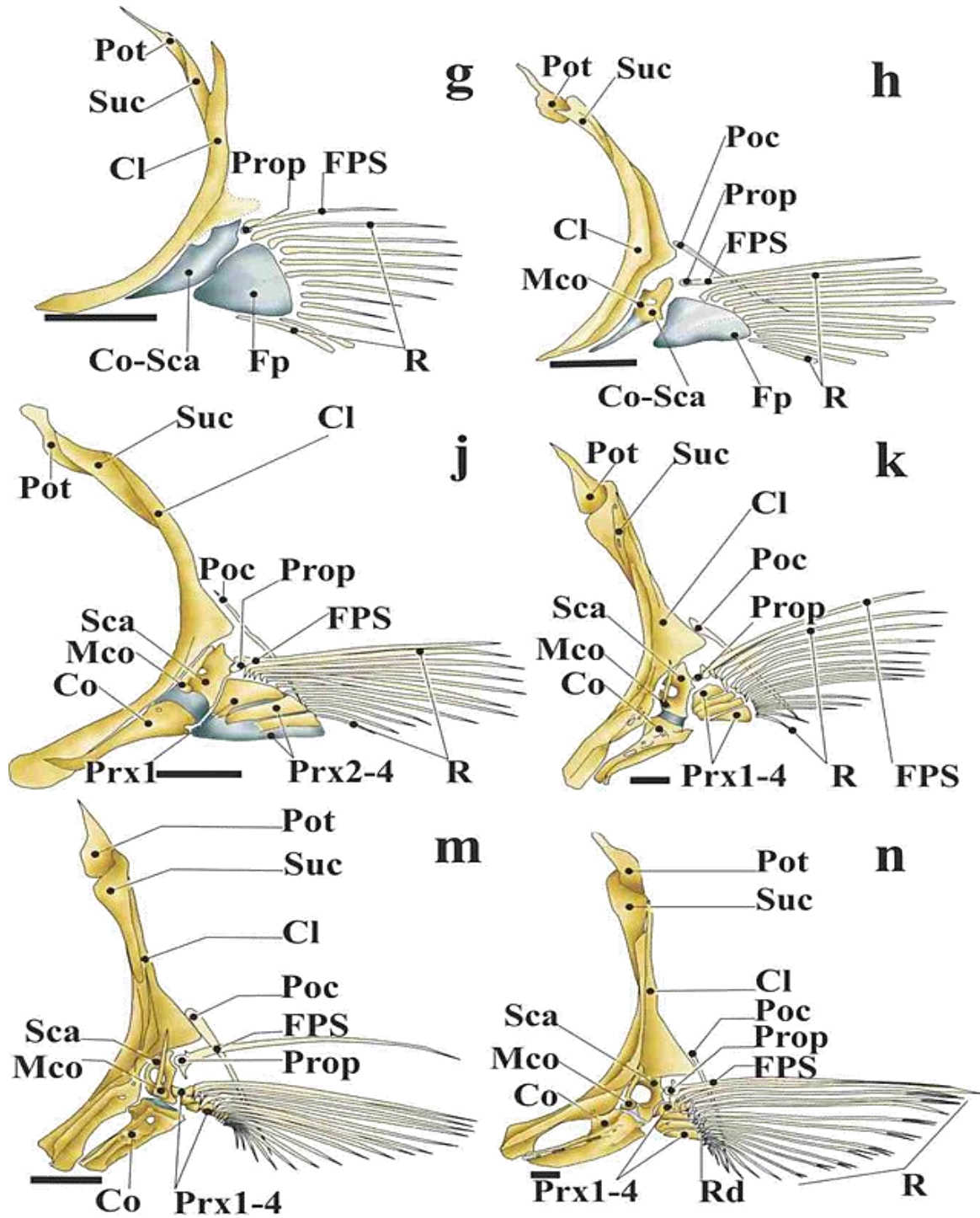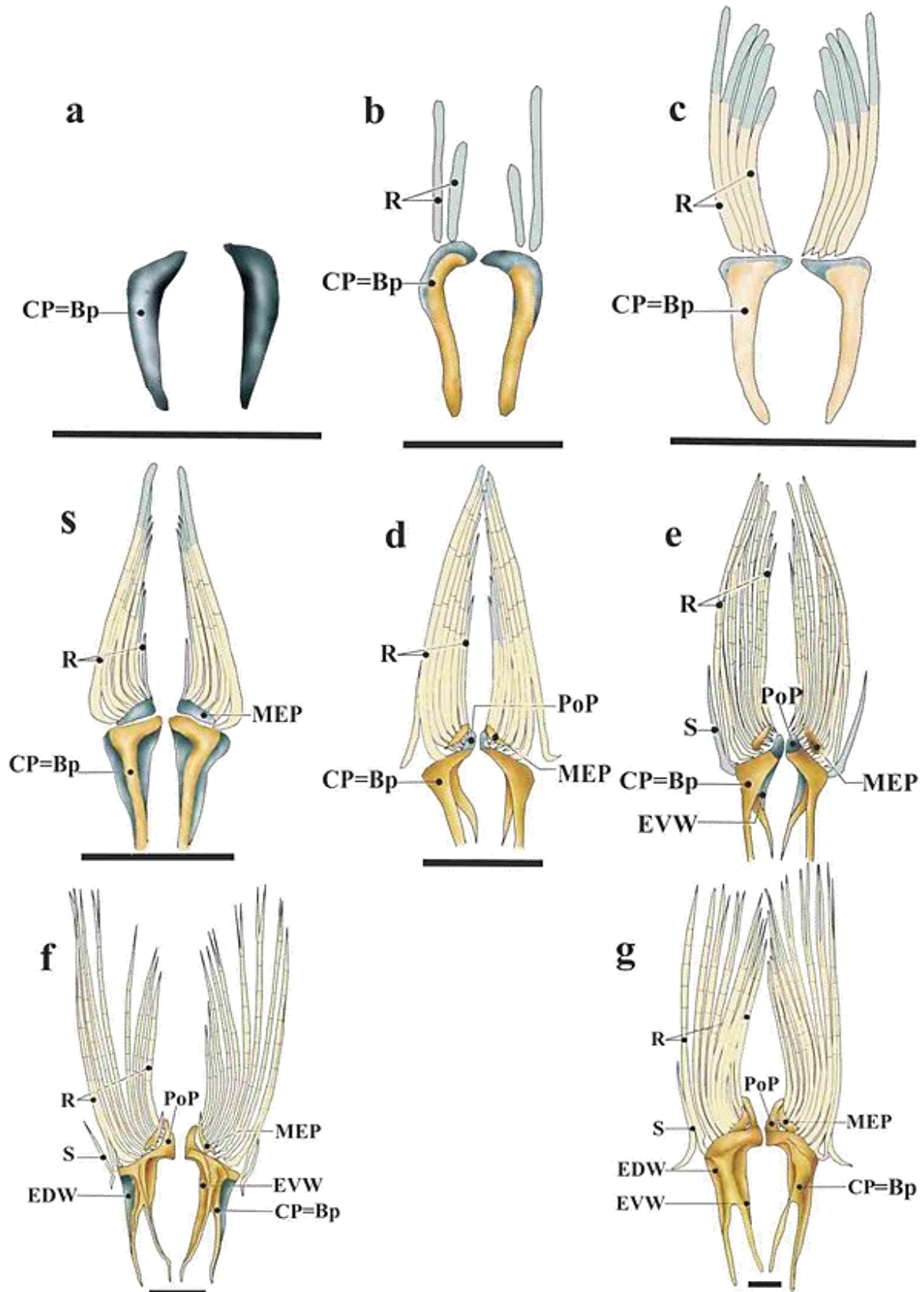
Fig. 2. Development of the pectoral girdle in *R. caspicus*. (g): 2-dph, (h): 30-dph, (j): 40-dph (k): 50-dph. (m): 70-dph and (n): 90-dph (Blue area: cartilage; and yellow area: ossification; bar = 0.50 mm).

Fig. 3. Development of the pelvic fin in *R. caspicus*. (a): 12-dph, (b): 14-dph, (c): 18-dph, (s): 20-dph, (d): 30-dph, (e): 40-dph, (f): 50-dph (g): 90-dph, (h): 10-dph, (I): 14-dph and (j): 25-dph (Blue area: cartilage; and yellow area: ossification; bar = 0.50 mm).
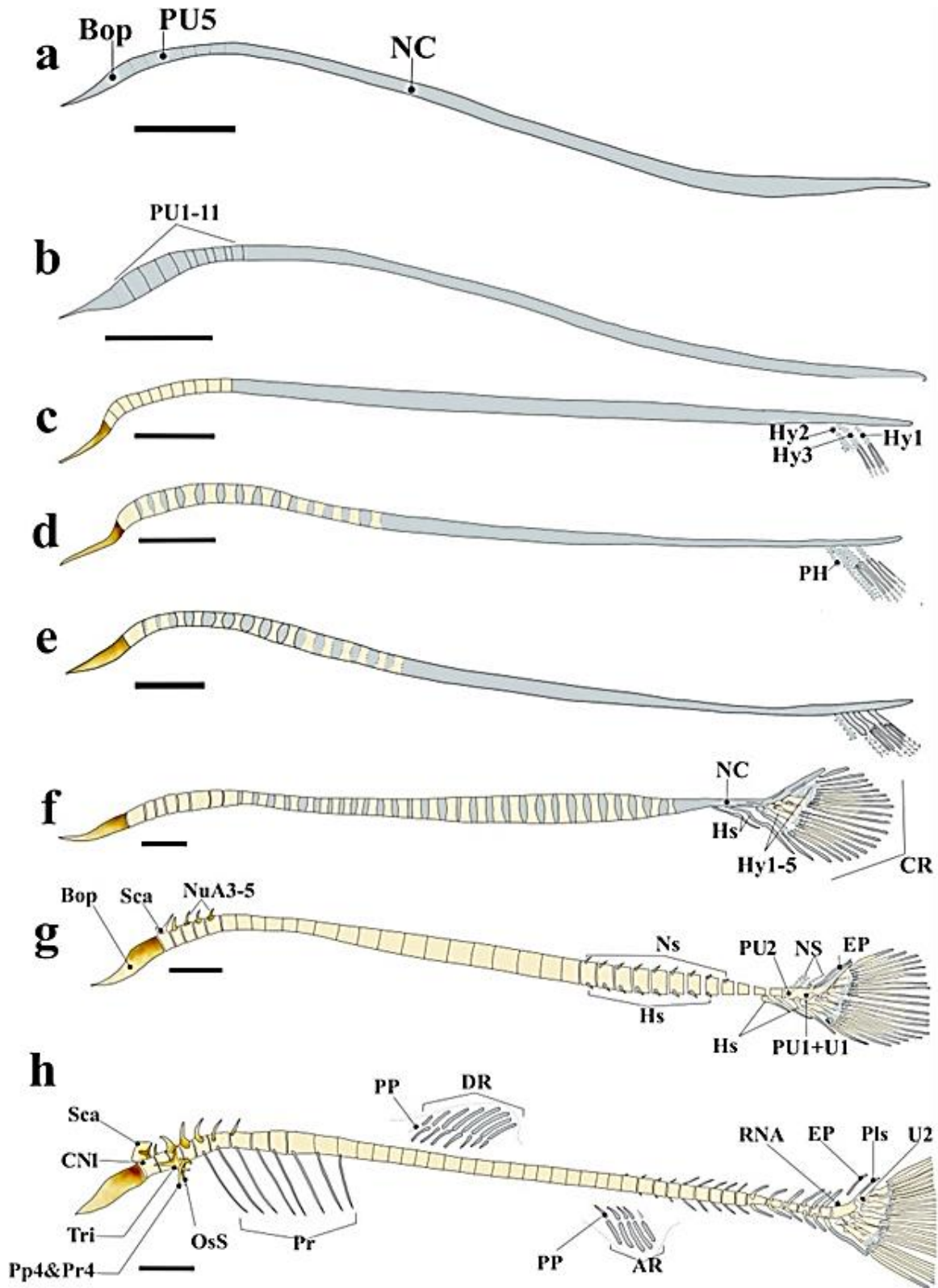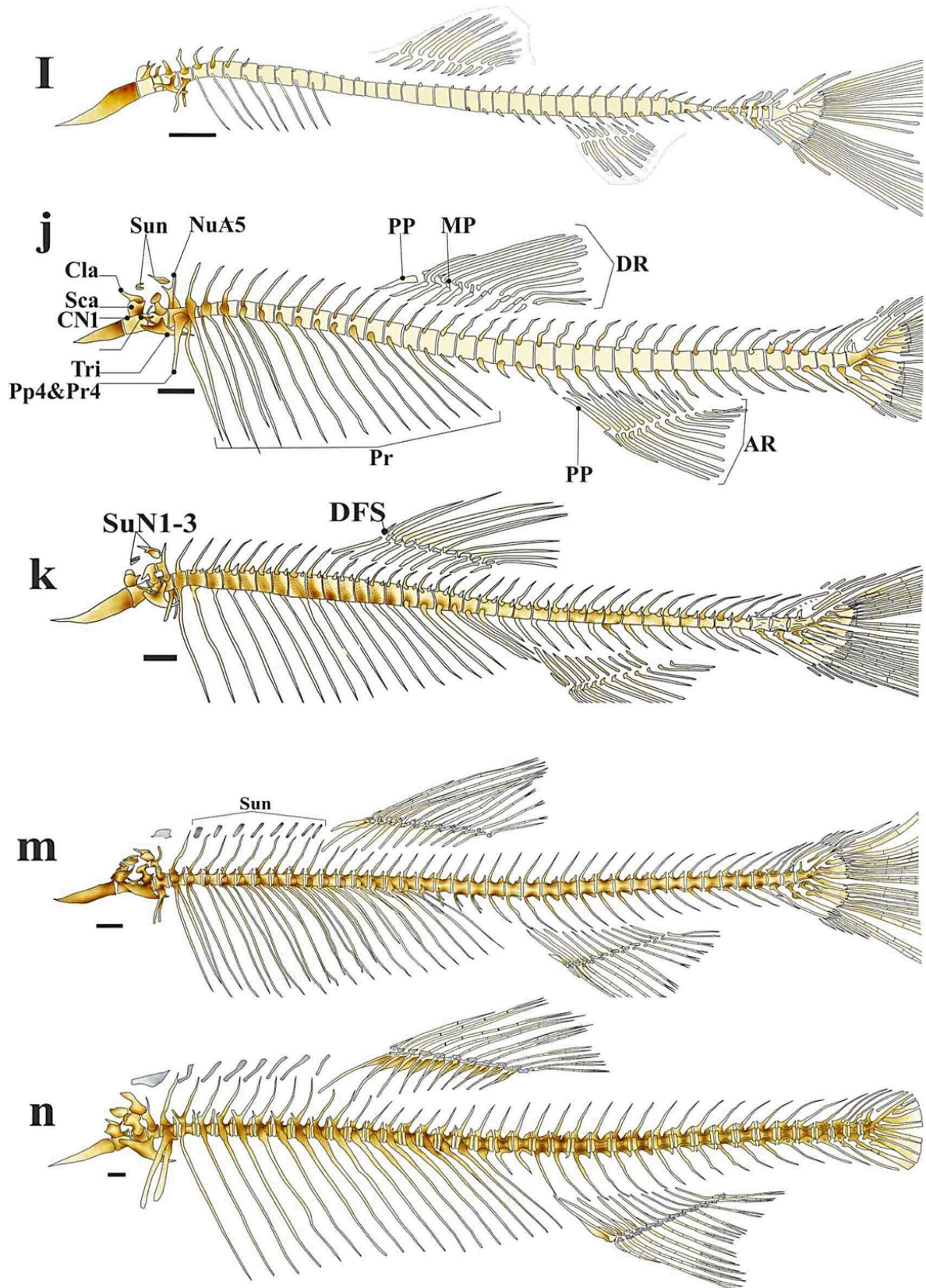
Fig. 4. Development of the vertebral column in *R. caspicus*. (a): 1-dph, (b): 3-dph, (c): 5-dph, (d): 6-dph, (e): 7-dph, (f): 8-dph, (g): 10-dph and (h): 14-dph (Blue area: cartilage; and yellow area: ossification; bar = 0.50 mm).

Fig. 5. Development of the vertebral column in *R. caspicus*. (I): 18-dph and (j): 20-dph (k): 40-dph, (m): 60-dph, (n): 90-dph (Blue area: cartilage; and yellow area: ossification; bar = 0.50 mm).

DISCUSSION

The development of the osteological structures in teleost is of demonstrable value in phylogenetic studies (Goslin 1967; Nibelin 1973; Koumoundouros *et al.*, 1995, 1997, 1999, 2001). These structures of the adult sometimes show a high degree of fusion, especially in advanced teleost, making it difficult correctly to identify (Matsuura & Katsuragava 1985). Hence, this study provided a detailed ontogeny of the post cranial skeleton (except caudal fin) that can be used in taxonomic studies of this taxon.

At hatching, teleost varies with respect to developmental stage of the skeleton (Koumoundouros *et al.*, 1999). In the Caspian roach, the sequence of the post cranial skeleton and fins develop relatively similar to those of *Sparus aurata* (Koumoundouros *et al.*, 1997) and *Dentax dentax* (Koumoundouros *et al.*, 1999). Osteological development in fish larvae is a detailed process that begins with the formation of the cartilage prior to ossification (Fraser *et al.*, 2004) as observed in *R. caspicus*.

There was no osteological development at hatching in the Caspian roach. The first observed skeletal structure was the vertebral column followed by the pectoral fins, caudal fins and almost simultaneously dorsal, anal and pelvic fins. Skeletal development allows progression in the muscle formation, which enable fish for faster and more complicate locomotion (Koumoundouros *et al.* 1999, 2001). The flexion of the notochord accompanies the development of the caudal complex and subsequently alteration in the locomotors ability, swimming mode, velocity, body shape and feeding behavior (Koumoundouros *et al.* 1999). This was occurred at 8-dph in *R. caspicus*. The second Ural is represented in the young of the most ostariophisian as a chorda centrum (Lundberg & Baskin 1969). It is generally believed that both U1 and U2 are co-ossified with the first pre-Ural as a typical condition of many advanced fishes (Lundburg & Baskin 1969), whereas the U2 was fused to the base of the hypural 3 in Cyprinidae as seen in the Caspian roach (Lundberg & Baskin, 1969).

Based on the results, the PU1+U1 (urostlye), the second and third pre-Ural contribute supporting of the caudal fin rays, similar to that of white perch (*Morone Americana*) and striped bass (*Morone saxatilis*) (Fritzsche & Johnson 1980). In the most Perciformes, the ossification of the vertebral centra mainly proceeds caudally up to pre-Ural and rostral from the urostlye to the anterior preural (Koumoundouros *et al.*, 2001). The Caspian roach follows the same pattern of the vertebral centra's ossification. In *R. caspicus*, the first centrum was ossified following the posterior centrum as observed in *Pagrus major* (Matsuoka, 1987).

Pectoral fin plays important role in the propulsion system and performs well for both high–speed cruising and high maneuverability in fishes (Westneat *et al.*, 2004; Thorsen & Westneat, 2005). The presence of the scapular foramen is typical characteristic of perciformes (Koumoundouros *et al.*, 2001) as it was present in *R. caspicus* at early developmental stage, i.e. 30-dph. Also, the presence of a metapterygium in the pelvic fin as a main feature of Perciformes (Koumoundouros *et al.*, 2001) can be found in the Caspian roach as well. Fundamentally, these facts indicate that Cypriniformes as fresh water fish are ancestors of the more at advanced marine fishes. The first pectoral fin ray is a short thick ray that articulates with the scapula in a synovial saddle joint (Westneat *et al.*, 2004) and these rays in the Caspian roach were appeared at 14-dph.

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بررسی روند تکوین ساختارهای اسکلتی ستون مهره، باله‌های زوج، پشتی و مخرجی در کلمه خزری

Rutilus caspicus, Pravdin (1927) (Teleostei: Cyprinidae)

ش. حسن‌پور، س. ایگدری*، ب. مجازی امیری

گروه شیلات، دانشکده منابع طبیعی، پردیس کشاورزی و منابع طبیعی دانشگاه تهران، کرج، ایران

چکیده

مطالعه و بررسی روند تکوین عناصر اسکلتی در ماهیان از نقطه‌نظر شیلاتی، آبزی‌پروری و زیست‌شناسی حائز اهمیّت است. روند تکوین عناصر اسکلتی می‌تواند به‌عنوان شاخص زیستی شرایط غیر بهینه پرورشی به کار گرفته شود. کلمه خزری یک گونه بومی از کپورماهیان دریای خزر است که تکثیر مصنوعی آن به‌منظور بازسازی ذخایر طبیعی در مراکز تکثیر انجام می‌پذیرد. از این رو این مطالعه به‌منظور مطالعه روند تکوین اسکلتی ستون فقرات، باله‌های زوج و میانی این ماهی از زمان تفریخ تا ۹۰ روز پس از آن، به‌عنوان اطلاعات پایه‌ای در مورد زیست‌شناسی آن به اجرا درآمد.به منظور بررسی عناصر اسکلتی، نمونه‌ها شفاف‌سازی و رنگ‌آمیزی شدند و تکوین اسکلتی ناحیه پساجمجمه‌ای به‌طور دقیق توصیف شد. نتایج نشان داد که هیچ ساختار استخوانی در زمان تفریخ در ماهیان وجود ندارد. نخستین ساختار استخوانی قابل مشاهده در ناحیه پساجمجمه‌ای، ستون فقرات بود که به دنبال آن باله‌های سینه‌ای و دمی نشانه‌هایی از استخوانی شدن را بروز دادند و سپس تقریباً به طور همزمان در باله‌های پشتی، مخرجی و لگنی علایم استخوانی شدن ظاهر شد.

*مولف مسئول