

Observations on the ossification centres in the skull of *Cyprinus carpio* Linn.

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Abstract. The common carp, *Cyprinus carpio* Linn. has been studied for tracing the ossification centres in its skull. A large number of early developmental stages of the embryos and larval forms were sectioned at 10 micra and these sections were stained with Delafield's haematoxylin and counter-stained with eosin for studying the bones. Alizarine transparencies of various stages were also made. Five stages have been investigated and described.

Keywords. *Cyprinus carpio*; ossification centres; fish skull.

1. Introduction

The study of the ossification centres in fish skulls has been practically neglected in India, excepting for the work of Ramaswami (1948), Marathe (1959), Marathe and Bal (1960) and Pashine and Marathe (1973). The present investigation was therefore undertaken to examine the centres of ossification in the skull of *Cyprinus carpio* Linn., belonging to the family Cyprinidae. The development of the chondrocranium of the same fish has already been worked out by the authors (Pashine and Marathe 1977).

2. Materials and methods

A large number of fertilized eggs of *C. carpio* were collected from a local fish farm and were reared in the laboratory. A large number of stages were fixed in acetic-alcohol and 5% formalin. The serial sections of different stages were cut at 10 micra and were stained with Delafield's haematoxylin and counter-stained with eosin. Alizarine transparencies of the various stages were also made for noting the ossification centres.

3. Observations

The ossification centres in 7.5, 8.0, 10.0, 11.5 and 15.0 mm embryos and larval stages of *C. carpio* were noted by examining the serial sections and alizarine preparations.

3.1. Stage 1, 7.5 mm (figure 1, a, b)

The parasphenoid (pasp) is noticed as a single centre of ossification in the orbito-temporal region. Behind this, is noticed the centre of ossification of the basioccipital (boc). The premaxilla (pmx) is a thin centre of ossification. The maxilla (mx) is ossified as an elongated piece. In the lower jaw, only dentary (den) is noticed as a single centre of ossification. It is narrow in front and broad behind. A distinct ossification centre is noticed in the opercular region. It is the opercular (op) which is in the form of a large, triangular bone. In the branchial skeleton, ossification has set in the fifth arch for the inferior pharyngeal bone (ba-5). It bears few conical teeth (tba-5).

3.2. Stage 2, 8.0 mm (figure 2, a-d)

The parasphenoid (pasp) has increased in size, extending anteriorly up to the ethmoid region. It is narrow at the anterior end and broad posteriorly. In the auditory region, the first pair of otoliths (ot-1) has appeared. Small ossification centres of the post-temporal (ptl) are noticed. In the occipital region, the exoccipital (exo) is seen as a flap-like membrane bone, separated from the basioccipital. The basioccipital (boc) anteriorly shows two thin, flap-like structures and posteriorly a pair of lateral upgrowths. The basioccipital anteriorly extends over the parasphenoid. The notochordal sheath is being ossified into the free vertebral centra (ce).

Premaxillary and maxillary bones are better ossified. The premaxilla (pmx) is an elongated bone with a small knob-like structure at the anterior end. The maxilla (mx) is larger than the premaxilla. The lower jaw shows a large dentary, a small angular and a very small retroarticular. The dentary (den) shows a caronoid process at the posterior end. The angular (an) is a small bone and its articulating facet with the quadrate is being formed. Below the angular, a small centre of ossification of the retroarticular (rar) is seen. In the palate region, only two centres of ossification are noticed. They are the entopterygoid and the quadrate. The entopterygoid (enp) is an elongated, wedge-shaped bone. The quadrate (qu) is a small piece. Its condyle is being ossified. Posteriorly it bears a narrow, pointed spine (qpp).

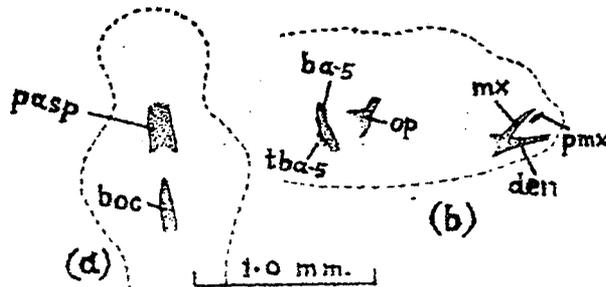


Figure 1. (a) Ventral view of the cranium of *Cyprinus carpio*, 7.5 mm long (stage 1), showing ossification centre (visceral arches are not shown). (b) Lateral view of the same, showing ossification centres in jaws, opercular and branchial regions.

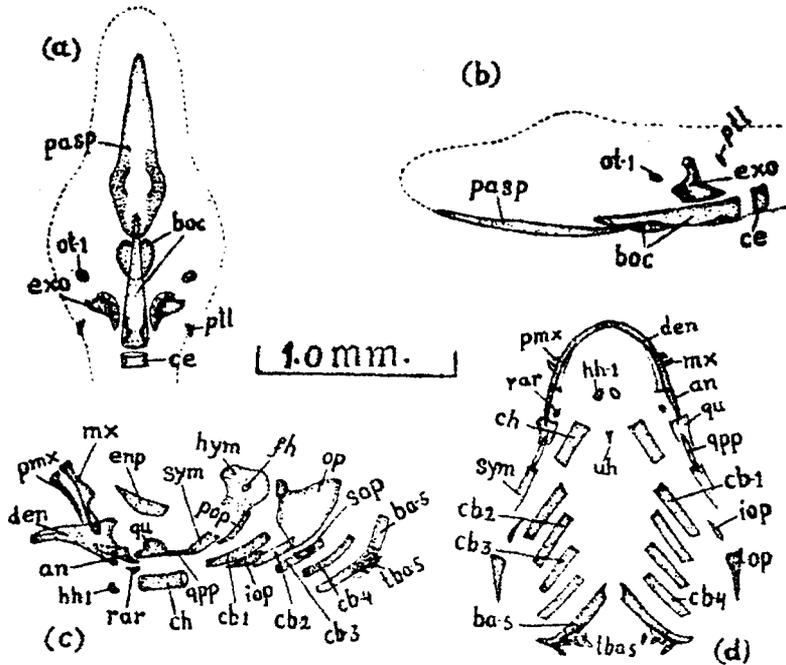


Figure 2. (a) Ventral view of the cranium of *C. carpio*, 8.0 mm long (stage 2), showing ossification centres. (b) Lateral view of the same, showing ossification centres. (c) Lateral, outer view of the same, showing ossification centres in jaws, hyopalate, opercular and branchial regions. (d) Ventral view of the visceral arches of the same, showing ossification centres.

In the hyoid arch, the following centres of ossification are noticed—hyomandibular, symplectic, hypohyals, ceratohyals and the urohyal. The hyomandibular (hym) is separate from the symplectic and its head is well ossified with a foramen (fh) for the exit of the hyomandibular branch of the VII cranial nerve. The symplectic (sym) is a small centre of ossification and it is connected with the quadrate spine (qpp). The first pair of hypohyals (hh-1) have appeared as small ossification centres. The ceratohyal (ch) is in the form of an elongated bar. The urohyal (uh) appears as a small dermal ossification. The opercular region shows all the four bones. The opercular (op) has increased in size and shows a well formed socket anteriorly for articulation with the hyomandibular. The preopercular (pop), subopercular (sop) and interopercular (iop) bones are seen as small centres of ossification. The ossification centres of the first-four ceratobranchials (cb 1-4) have appeared as elongated bars. The inferior pharyngeal bone (ba-5) is better developed and its dentigerous pad shows five small, conical, ossified teeth (tba-5).

3.3. Stage 3, 10.0 mm (figures 3-4, a-f)

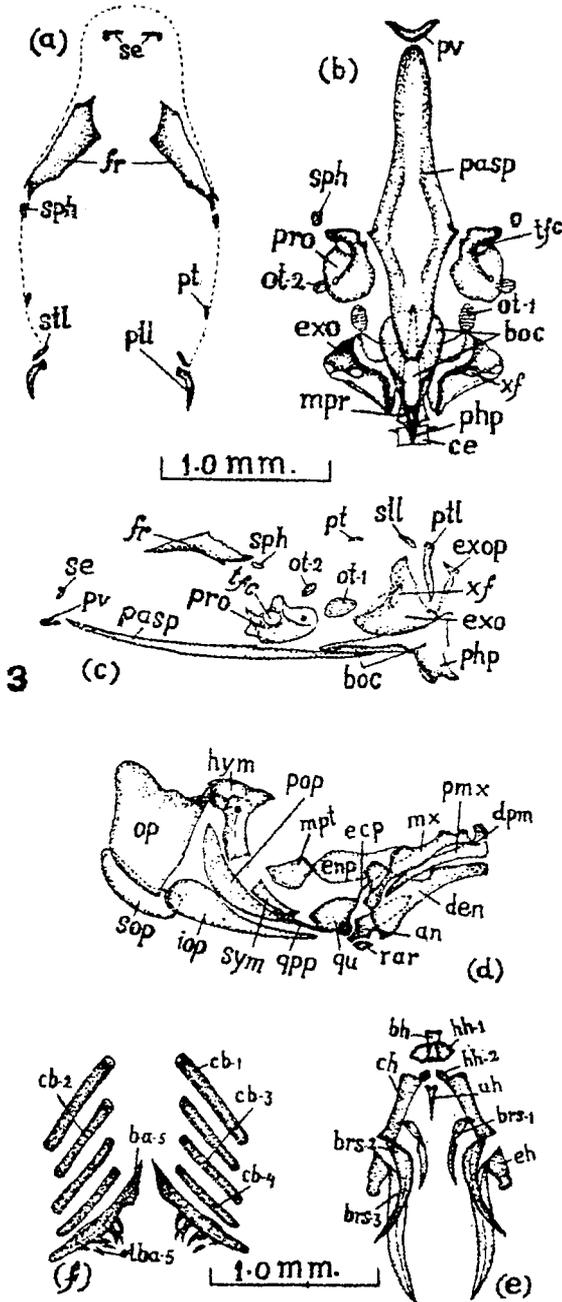
In the ethmoid region, the prevomer and the supra-ethmoid are noticed. The prevomer (pv) is ossified in front of the parasphenoid in the form of a crescentic

structure. It arises from a single centre of ossification. The supra-ethmoid (*se*) arises from two small centres of ossification. In the orbito-temporal region, the frontal (*fr*) has appeared as a prominent centre of ossification. A very tiny centre of ossification is noticed behind the frontal for the sphenotic (*sph*). Some distance behind this, the pterotic (*pt*) appears as a tiny ossification centre. A supra-temporal (*stl*) has appeared as a small ossification centre in front of the post-temporal (*ptl*). The parasphenoid (*pasp*) shows a pair of small lateral processes. The pro-otic (*pro*) has appeared as a broad, single ossification centre. Its anterior part arches over to enclose a large trigemino-facial foramen (*tfc*) for the passage of the V and VII cranial nerves. A second pair of otoliths (*ot-2*) have appeared behind the pro-otics. From the posterior side of the exoccipital (*exo*) a thin, elongated flap-like exoccipital process (*exop*, figure 3-c) is developed and it is directed upwards. The basioccipital (*boc*) develops a postero-ventral extension, which forms the masticatory (*mpr*) and the pharyngeal (*php*) processes.

The premaxillary process of the maxilla (*dpm*, figure 4 d) is ossified as a conical, pointed process. The dentary (*den*), angular (*an*), quadrate (*qu*) and the retro-articular (*rar*) are better ossified. In the pterygo-quadrate region, the metapterygoid and the ectopterygoid have appeared as new centres of ossification. The metapterygoid (*mpt*) is ossified behind the entopterygoid (*enp*) and is connected with it. The ectopterygoid (*ecp*) appears as a slender centre of ossification, extending from the anterior end of the quadrate. The hyomandibular (*hym*) and the symplectic (*sym*) are better ossified and the hyomandibular foramen is covered by bony part. The opercular bones are well formed. The opercular socket comes in contact with the head of the hyomandibular. The basihyal (*bh*) has appeared as a single centre of ossification, bordered by the first pair of hypohyals (*hh-1*). A second pair of hypohyals (*hh-2*) have appeared in front of the ceratohyal (*ch*). The epihyal (*eh*) is noticed. Three pairs of branchiostegal rays (*brs 1-3*) have appeared. The ceratohyal (*ch*) connects the first two branchiostegal rays (*brs-1-2*), while the epihyal (*eh*) gives attachment to the third branchiostegal ray (*brs-3*). The urohyal (*uh*) has increased in size. The ceratobranchials (*cb 1-4*), the inferior pharyngeal bones (*ba-5*) and its dentigerous pad (*tba-5*) shows better ossifications.

3.4. Stage 4, 11.5 mm (figures 5-7, a-g)

The ethmoid region shows the centres of ossification of the rostral and the lateral ethmoids. The rostral (*r*, figure 6 d) has appeared as a small centre of ossification, located postero-dorsally to the rostral processes of the premaxillae (*rpp*). Lateral ethmoid (*let*) is better represented in the ventral view. The two ossification centres of the supra-ethmoid (*se*) have joined to form a single bone. The prevomer (*pv*) is triangular in shape and its posterior end extends over the parasphenoid (*pasp*) ventrally. In the orbito-temporal region, the ossification centres of the parietals, pleurosphenoids and the lachrymals have appeared. The parietal (*par*) is quite large and is located behind the frontal. The pleurosphenoid (*pls*) is seen ossifying in front of the pro-otic, in the form of a small, curved ossification centre. The lachrymal (*lc*) has appeared as a dermal ossification, located antero-laterally to the frontal. The sphenotic (*sph*) has a well ossified socket for articulation with the hyomandibular. The pterotic (*pt*) is located postero-laterally to the parietal. The pro-otic (*pro*) is better ossified and forms internally ridges and depressions.



Figures 3 and 4. 3. (a) Dorsal view of the cranium of *C. carpio*, 10.0 mm long (stage 3), showing ossification centres. (b) Ventral view of the cranium. (c) Lateral view of the same. 4. (d) Lateral, outer view of the jaws, hyopalate and opercular regions of stage 3, showing ossification centres. (e) Ventral view of the hyoid arch of the same stage, showing ossification centres. (f) Ventral view of the branchial arches of the same, showing ossification centres.

The lateral process of the parasphenoid (pasp) is connected with the pro-otic. In the occipital region, the supra-occipital (so) has been added as a dermal, crescentic ossification, between the two parietals. The exoccipital process extends upwards and fuses with the postero-dorsal extension of the bone, enclosing an

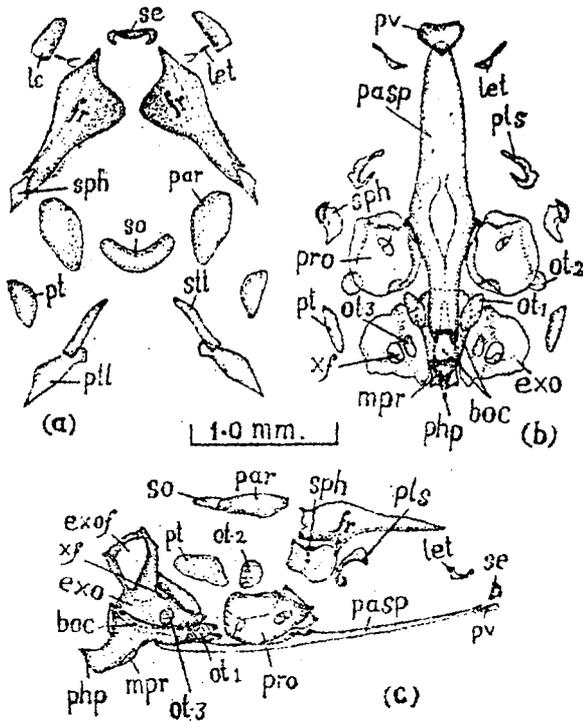


Figure 5. (a) Dorsal view of the cranium of *C. carpio*, 11.5 mm long (stage 4), showing ossification centres. (b) Ventral view of the cranium. (c) Lateral view of the cranium.

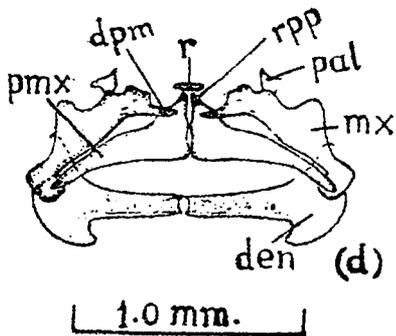


Figure 6. (d) Front view of the jaw region of stage 4, showing ossification centres.

exoccipital foramen (exof, figure 5c). A third pair of otoliths (ot-3) is seen in between the exoccipital bones.

In the palato-quadrates region, the palatine (pal) appears as a small ossified piece, having an anteriorly directed process. The ectopterygoid (ecp) has increased in size. The hyomandibular (hym) shows an ossified, thin, flap-like structure towards the anterior end. The opercular socket now forms into a distinct anterior opercular process (apo) which extends over the head of the hyomandibular. The basihyal (bh) has increased as an elongated, cylindrical rod. The first pair of

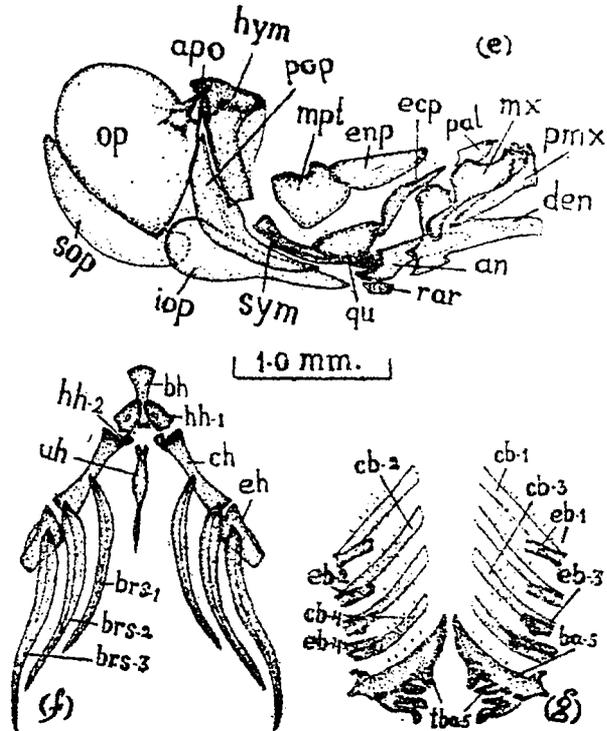
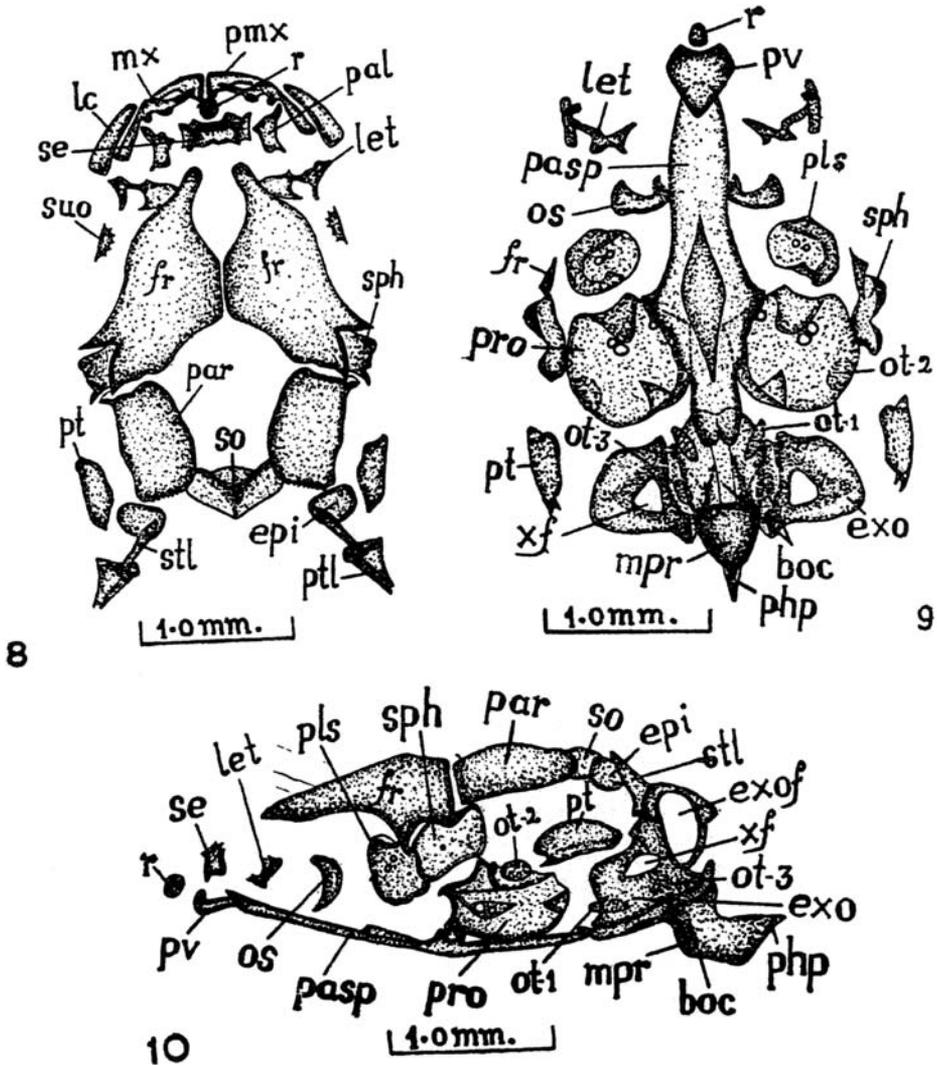


Figure 7. (e) Lateral, outer view of the jaws, hyopalate and opercular regions of stage 4, showing ossification centres. (f) Ventral view of the hyoid arch of the same stage, showing ossification centres. (g) Dorsal view of the branchial arches of the same, showing ossification centers.

hypohyals (hh-1) show a distinct foramen. The second pair of hypohyals (hh-2) is in contact with the ceratohyals. In the branchial region, four pairs of epibranchials (eb 1-4) are noticed as new ossification centres, giving attachment to their respective ceratobranchials (cb 1-4). The inferior pharyngeal bone (ba-5) has enlarged and it bears 7-8 well ossified teeth.

3.5. Stage 5, 15.0 mm (figures 8-12)

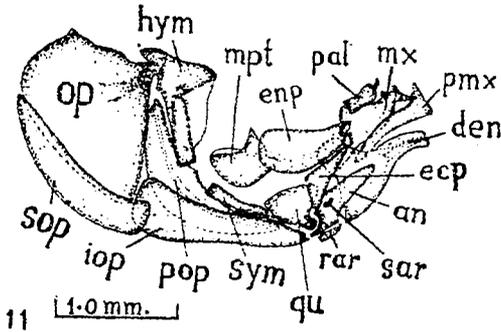
The supra-ethmoid (se) is better ossified and its lateral flaps are formed. The lateral ethmoid (let) has increased in size. The rostral (r) is ossified as a small, rounded bony piece. The prevomer (pv) shows a distinct curve anteriorly. In the orbito-temporal region, the supra-orbital and the orbitosphenoid have appeared. The supra-orbital (suo) is a small centre of ossification, located laterally to the frontal. The orbitosphenoid (os) appears as a single centre of ossification, located in front of the pleurosphenoid. The pleurosphenoid (pls) is better ossified as a circular plate. Frontals (fr) are better represented. The parietals (par) posteriorly overlap the supra-occipital and anteriorly they extend nearly up to the frontals. In the auditory region, the epiotic (epi) appears as an oval centre



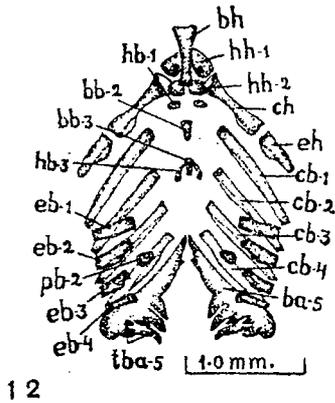
Figures 8-10. 8. Dorsal view of the cranium of *C. carpio*, 15.0 mm long (stage 5), showing ossification centres. 9. Ventral view of the cranium of the same stage. 10. Lateral view of the same, showing ossification centres.

of ossification. All the remaining otic bones, the basioccipital (boc), supra-occipital (so) and exoccipitals (exo) are better ossified.

In the lower jaw region, a small sesamoid angular (sar) has appeared as a tiny centre of ossification, on the inner side of the angular (an). The dentary (den) shows a mandibular sensory canal opening at its tip. The palatine (pal), all the three pterygoids and the opercular bones are better ossified. The preopercular (pop) shows the preopercular sensory canal. In the branchial region, the basi-branchials, two pairs of hypobranchials and one pair of pharyngobranchials have appeared as new centres of ossification. The second and third basi-branchials (bb-2 and bb-3) have appeared as small rod-shaped pieces. The first and the



Figures 11-12. 11. Lateral, outer view of the jaws, hyopalate and opercular regions of stage 5, showing ossification centres. 12. Dorsal view of the hyobranchial skeleton of the same stage, showing ossification centres (the hyomandibular and symplectic are not shown).



third pairs of hypobranchials (hb-1 and hb-3) have appeared as small ossified structures. The second pair of pharyngobranchials (pb-2) have appeared as small, oval bony pieces, in front of the second and third epibranchials. Remaining elements are better ossified in this stage.

4. Discussion

In the teleost skull the various bones may arise from more than one centre of ossification. The supra-ethmoid arises from a single centre of ossification as noticed in a 10.0 mm, larva of *Trichopodus* (Marathe and Bal 1960), 13.0 mm, larva of *Brachydanio* (Pashine and Marathe 1973) and 18.0 mm, larva of *Eleutheronema* (Marathe 1959). But in a 12.0 mm, larva of *Amia* (Pehrson 1922), the supra-ethmoid arises from four groups of osteoblasts. In a 10.0 mm, larva of *Cyprinus*, two small centres of ossification were noticed for the supra-ethmoid. These two centres join to form a single bone in a 11.5 mm, larva.

Fishes like *Acipenser* (Sewertzoff 1922), *Trichopodus* (Marathe and Bal 1960), *Eleutheronema* (Marathe 1959) and *Brachydanio* (Pashine and Marathe 1973) show a single centre of ossification for the prevomer (vomer). But in *Amia* (Pehrson 1922), paired rudiments for prevomers appear in a 10.00 mm, larval form. They are paired anteriorly and unpaired posteriorly in a 17.0 mm, larva of *Salmo* (de Beer 1937). In a 14.0 mm, larva of *Lepidosteus* (Aumonier 1941),

the prevomers appear as two separate centres of ossification and they remain separate in a 38.0 mm, stage. In a 10.0 mm, larva of *Cyprinus*, the prevomer appears as a single ossification centre.

The presence of rostral bone in the ethmoid region is not unique in all the teleosts. The rostral bone is not found in *Trichopodus* (Marathe and Bal 1960) and *Eleutheronema* (Marathe 1959). In a 10.0 mm, larva of *Brachydanio* (Pashine and Marathe 1973), a single centre of ossification is noticed for the rostral bone. But in a 38.0 mm, larva of *Lepidosteus* (Aumonier 1941), two separate pieces of rostral bones were found. In the fish under study, a single centre of ossification for rostral bone is noticed in a 11.5 mm, larval stage.

The appearance of the frontal bone is from a single centre of ossification, as noticed in *Lepidosteus* (Aumonier 1941) and *Trichopodus* (Marathe and Bal 1960), from two centres of ossification in *Brachydanio* (Pashine and Marathe 1973) and from three centres in *Eleutheronema* (Marathe 1959). In *Cyprinus*, the frontal bone arises as a single centre of ossification in a 10.0 mm, larva.

The supra-occipital bone is represented by single centre of ossification in *Eleutheronema* (Marathe 1969) and *Brachydanio* (Pashine and Marathe 1973) and by two centres of ossification in a 6.0 mm, larva of *Trichopodus* (Marathe and Bal 1960). In *Cyprinus*, a single centre of ossification is noticed for the supra-occipital in a 11.5 mm, larva.

The ceratohyal and epihyal jointly appear together in 4.0 mm, larva of *Trichopodus* (Marathe and Bal 1960) and in a 8.0 mm, larva of *Eleutheronema* (Marathe 1959). However, the ceratohyal and epihyal separate from each other in 7.0 mm, larva of *Trichopodus* (Marathe and Bal 1960) and 18.0 mm, larva of *Eleutheronema* (Marathe 1959). In *Brachydanio* (Pashine and Marathe 1973), separate, independent ossification centres are noticed for the ceratohyal and epihyal in a 10.0 mm, larval stage. In *Cyprinus*, independent ossification centre is noticed for the ceratohyal in a 8.0 mm, larva. In a 10.0 mm stage, the epihyal appears as a single free ossification centre.

5. Conclusions

The ossification centres in the skull of *Cyprinus carpio* Linn. have been described in five stages.

(a) In stage 1 (7.5 mm), the parasphenoid and the basioccipital have appeared in the cranium. The premaxilla, maxilla and dentary are noticed in the jaw region. In the opercular region, only the opercular is seen. The inferior pharyngeal bones of the fifth branchial arch have appeared. It bears a few teeth.

(b) In stage 2 (8.0 mm), the post-temporals, exoccipitals and the first pair of otoliths are added to the cranium. The angular, retroarticular, quadrate, entopterygoid, hyomandibular and symplectic are added in the jaw and palate regions. All the opercular bones are noticed. The first pair of hypohyals, ceratohyals, urohyal and first-four ceratobranchials are added to the hyobranchial region.

(c) In stage 3 (10.0 mm), the prevomer, supra-ethmoid, frontals, pro-otics, sphenotics, pterotics, second pair of otoliths and supra-temporals have appeared in the cranium. The metapterygoid, ectopterygoid, basihyal, second pair of hypohyals, epihyals and three branchiostegal rays are also noticed.

(d) In stage 4 (11.5 mm), the rostral, lateral ethmoids, lacrymals, parietals, pleurospenoids, supra-occipital and the third pair of otoliths are added to the cranium. The palatine and all the four epibranchials are noticed in the visceral skeleton.

(e) In stage 5 (15.0 mm), the following centres of ossification have appeared—the supra-orbital, orbitosphenoids, epiotics, sesamoid angular, second and third basibranchials, first and third pairs of hypobranchials and the second pair of pharyngobranchials.

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