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Early development of the chondrocranium in *Salmo letnica* (Karaman, 1924) (Teleostei: Salmonidae)

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The ontogenetic development of the chondrocranium of Ohrid trout *Salmo letnica* was studied from hatching until 92 days post-hatching (dph). Most of the samples were *in toto* trypsin cleared and stained, some specimens were used for serial histological sectioning. The serial histological sections of fish specimens at the age of 92 dph were used for a graphical reconstruction of the cartilaginous neurocranium. A chronological evaluation of the formation of the cartilaginous skull in the early development of *S. letnica* was performed. In order to investigate to what degree the ontogeny of the Ohrid trout is unique, the results were compared with data of the development of other salmonids, as well as some non-salmonid teleosts. The development of the cartilaginous structures of the Ohrid trout was found to be similar to that of other salmonids. Most of the cartilage structures of the neurocranium and the viscerocranium are present at the moment of hatching of this species. A fully developed chondrocranium was observed at the age of 92 dph, when the first signs of cartilage resorption could also be observed.

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Key words: chondrocranium; ontogeny; *Salmo letnica*; Salmonidae; Salmoniformes.

INTRODUCTION

Ontogeny during the embryonic and larval phases may be considered as a period of qualitative increase (*i.e.* structural development) within the ‘Bauplan’ of an organism, followed by a phase of a mainly quantitative increase (*i.e.* growth) during the juvenile and the adult phases. While the juvenile and adult phases mainly focus on maturation, the embryonic and larval periods can be considered as a reflection of a continuous struggle between the functional demands that have to be met and the presence of those structural elements that constitute the apparatuses that meet those demands. Hence, it should be underscored that early ontogeny should not be considered merely as a process leading to an adult morphology, with the latter being ‘a final episode of the chain of ontogenetic

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changes' (Alekseyev & Power, 1995). On the contrary, every interval during ontogeny, resulting in a surviving individual, is the result of an evolutionary response to natural selection. Besides, every interval generates a basis for further development.

Of all developmental periods of an organism, the embryonic and the larval periods are of special importance. These phases are characterized by critical periods, where the structural design might only just meet the functional requirements placed upon the structures (Galis *et al.*, 1994). Accordingly, individuals are more sensitive to external influences in these periods, which results in high mortality. Subsequently, any stage of head ontogeny can be expected to be adapted during its evolution in response to these functional demands. Consequently, a lower success at survival of a species or specimens can simply be the result of a bad timing of the emergence of the structural innovations that underlie functional adaptations. So, studying head ontogeny not only provides an insight in the formation of the adult structural design in the cranial 'Bauplan', but it also reflects phylogenetic and evolutionary relationships between species (in terms of character additions and character polarization: Nelson, 1978; Kluge & Strauss, 1985; Adriaens & Verraes, 2002).

The embryonic and larval development of the teleostean skull has been elaborated on in many studies. In some of them, traditional classic anatomy has been studied from a comparative or purely developmental point of view (de Beer, 1927, 1937; Srinivasachar, 1957*a, b*, 1958*a, b*, 1959; Bertmar, 1959; Hoedeman, 1960; Weisel, 1967; Paine & Balon, 1984; Kobayakawa, 1992; Vandewalle *et al.*, 1992, 1995*a*; Wagemans *et al.*, 1998; Kováč, 2000; Faustino & Power, 2001). Others have pursued research on the relation between the development of certain skeletal elements and their functions (Verraes, 1974*a*; Ismail, 1979; Verraes & Ismail, 1980; Aerts & Verraes, 1987; Casciotta & Arratia, 1993; Bartsch, 1994; Vandewalle *et al.*, 1995*b*; Hunt von Herbing *et al.*, 1996; Kohno *et al.*, 1996; Adriaens *et al.*, 1997; Adriaens, 1998; Wagemans & Vandewalle, 1999). Some studies, based on ontogenetic comparative examinations, have reconstructed phylogenetic relationships between groups, and have contributed to the understanding of the evolution of developmental mechanisms (Arratia, 1990, 1992; Arratia & Schultze, 1990, 1991; Mabee, 1993; Cabbage & Mabee, 1996; Mabee & Trendler, 1996; Patterson & Johnson, 1996).

Owing to its crucial role in understanding all of the interrelations between form, function and evolution, the early development of head structures has stimulated interest in the necessity of an ontogenetic trade-off between these factors, with regard to fishes in the waters of Macedonia, *i.e.* Ohrid trout *Salmo letnica* (Karaman). This trout is one of the best known fishes in Lake Ohrid. A large amount of information is available in the literature, pertaining to the biological importance of *S. letnica* as an endemic, relict and polymorphic species as well as its economic value (Karaman, 1926; Stefanovic, 1948; Spirkovski, 1994). No attention, however, has been paid to the early ontogeny of skeletal structures.

Despite many studies on the skeletal ontogeny of other teleosts, few have focused on embryonic and post-embryonic development in salmonid skulls. Some studies are concerned with the complete development of the osteocranium (Švob *et al.*, 1971; Jollie, 1983; Alekseyev & Power, 1995) or parts of the

osteocranium (Arratia & Schultze, 1990, 1991; Alexeev, 1993). Few investigations include the complete development of cartilage and bone in the head region of salmonids (Parker, 1873; de Beer, 1927, 1937; Verraes, 1973). Thus, the present aim was to describe the normal pattern of cranial development in *S. letnica*, and to evaluate to what extent cranial development in salmonids is similar or whether some interspecific differences occurs. Analyses of chondrocranial development in *S. letnica* are presented in this paper, whereas the ontogeny of the osteocranium will be dealt with separately.

MATERIAL AND METHODS

The samples of *S. letnica* were taken from the artificial spawning tanks located in the Hydrobiological Institute, Ohrid. The samples were raised in special incubators, with constant water temperature of 10.5° C corresponding to the lake environment. The material for the analysis was collected from the moment of hatching up to the age of 92 days post-hatching (dph). Three hundred and thirty specimens between the age of 1 day and 92 dph were processed. After hatching, up to the age of 30 dph, specimens were collected every day, after which samples were collected on every fifth day up to the age of 50 dph. The fry of 50–92 dph were collected on every tenth day (seven to 10 fry were taken at each spawning). The total length (L_T) was measured in mm by callipers for all specimens. The study of the chondrocranial development was conducted by using the following methods: *in toto* clearing and staining (alcian blue and alizarin red S), serial histological sections and manual graphical reconstructions. The specimens for *in toto* clearing and staining were anaesthetized, using an MS-222 overdose, fixed for 24–48 h in a 10% neutral formalin solution, and stained according to Balon & Flegler-Balon (1985). The head region of the specimens was studied by using a WILD M3C stereomicroscope equipped with a camera lucida. Fourteen specimens were selected for serial sections. These were fixed in Bouin, and embedded according to Verraes (1974b). Serial sections of 10 µm were cut on a rotation microtome (Microm HM360) and stained according to Mangakis *et al.* (1964). The sections were studied using a light microscope (Leitz Wetzlar). Serial sections from 92 dph Ohrid trout were used for making a manual graphical reconstruction according to Verraes (1974b).

RESULTS

DAY 1 POST-HATCHING (13.4–14.4 MM L_T) (FIG. 1)

As early as on the first day after hatching, all specimens examined have well developed cartilaginous elements of both the neurocranium and the splanchnocranium.

Neurocranium

The major part of the neurocranial base already comprises the trabecular bars, parachordal plates and otic capsules (capsula auditiva). Rostrally, the parachordal plates are fused to the posterior end of the trabecular bars (trabecula cranii), whereas laterally they are continuous with the base of the otic capsules and caudally with the pilae occipitales. The paired trabecular bars enclose the fenestra hypophyseae, and continue rostrally in a single trabecula communis. The caudal parts of these trabecular bars lie more ventrally than the other parts of the neurocranial floor. In the ethmoid region, the ethmoid plate (cartilago ethmoideum) already supports the lamina orbitonasalis at its postero-lateral

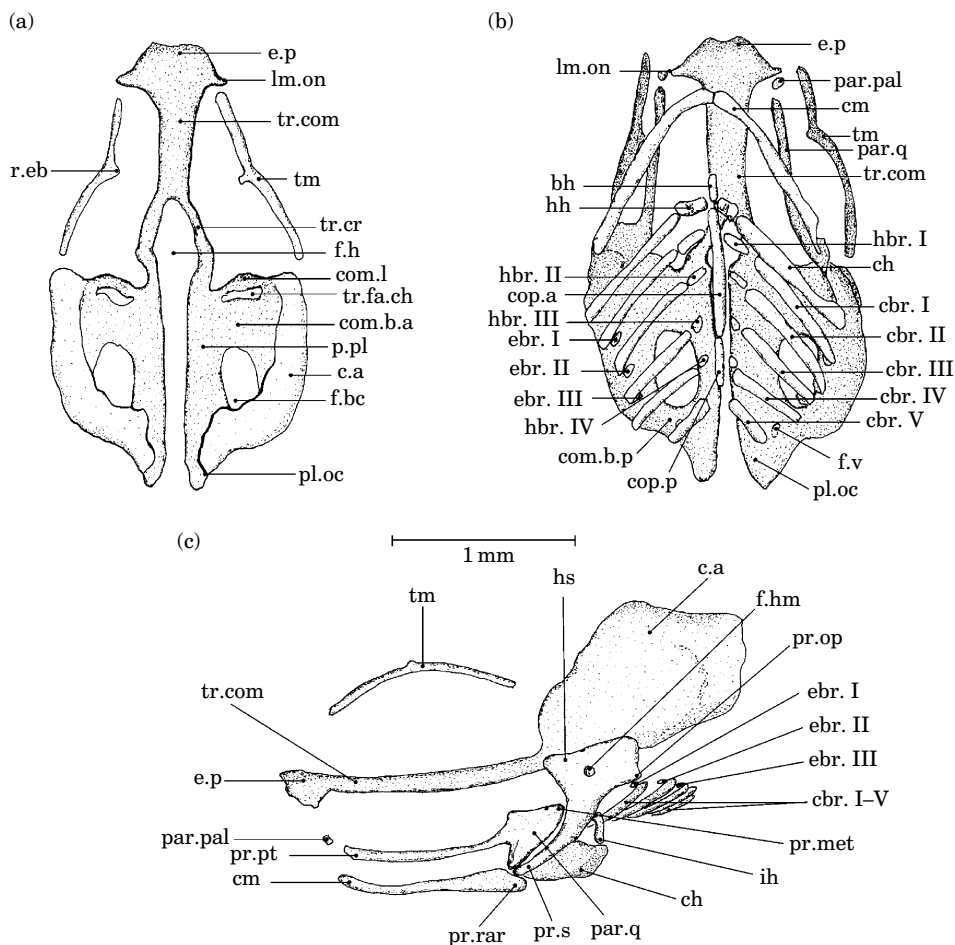


FIG. 1. Skull of *Salmo letnica* at 1 dph. (a) Dorsal view of the neurocranium, (b) ventral and (c) lateral view of the chondrocranium. bh, basihyal; c.a, capsula auditiva; cbr, ceratobranchial; ch, ceratohyal; cm, cartilago Meckeli; com.b.a, commissura basicapsularis anterior; com.b.p, commissura basicapsularis posterior; com.l, commissura lateralis; cop.a, copula anterior; cop.p, copula posterior; ebr, epibranchial; e.p, ethmoid plate; f.bc, fenestra basicapsularis; f.h, fenestra hypophyseae; f.hm, foramen hyomandibulare; f.v, foramen vagus; hbr, hypobranchial; hh, hypohyal; hs, hyosymplecticum; ih, interhyal; lm.on, lamina orbitonasalis; p.pl, parachondral plate; par.pal, pars palatina; par.q, pars quadrata; pl.oc, pila occipitalis; pr.met, processus metapterygoideus; pr.op, processus opercularis; pr.pt, processus pterygoideus; pr.rar, processus retroarticularis; pr.s, processus symplecticus; r.eb, rudiment of epiphyseal bridge; tm, taenia marginalis; tr.com, trabecula communis; tr.cr, trabecula cranii; tr.fa.ch, foramen of trigemino-facial chamber.

margin. No processus internasalis (septum internasale) is present. The commissura lateralis is present in all the samples at this stage, forming the ventro-lateral wall of the trigeminofacial chamber. On the roof of the orbito-temporal region, the taeniae marginales develop as independent cartilages, contacting neither the ethmoid cartilage nor the auditory capsules. The rudiments of the epiphyseal bridge are already present in the middle of the taenia's medial face. The

interorbital septum is not yet chondrified. The rostral and caudal parts of the otic capsule floor are connected to the parachordal plates by the commissurae basicapsulares anterior and posterior respectively, and separated by the fenestra basicapsularis. In the histological sections the n. glossopharyngeus was observed passing through this fenestra, whereas a small foramen vagum lies more caudally to it. The lateral walls of the auditory capsules are still only partially chondrified, leaving the major part of the membranous labyrinths uncovered. In the occipital region, the pilae occipitales do not contact each other medially, thus not forming a tectum posterius at this stage. The tectum synoticum that at later stages covers the brain is absent.

Viscerocranium

Almost all elements of the cartilaginous viscerocranium are present at this stage. In all specimens examined, the palatoquadrate consists of two cartilaginous parts: a small, rostral pars palatina and an elongate, caudal pars quadrata. A processus metapterygoideus is present in the postero-dorsal part of the pars quadrata. The paired Meckel's cartilage (cartilago Meckeli) already has a primordial processus retroarticularis. In 1 dph *S. letnica*, the hyoid arch comprises four paired cartilaginous elements: hyosymplectic, interhyal, ceratohyal and hypohyal. Ventro-medially, the unpaired basihyal connects the left and right hyoid arches. In the dorsal part of the hyosymplectic, known as the pars hyomandibularis, a small foramen hyomandibulare is present, through which passes the truncus hyomandibularis of the facial nerve. The hyosymplectic bears a primordial processus opercularis (posteriorly) and a rostro-ventral extension, *i.e.* the processus symplecticus. The interhyal is a small, independent, cartilaginous rod. Dorsally it articulates with the postero-ventral margin of the hyosymplectic and ventrally with the postero-dorsal margin of the ceratohyal. The hypohyal lies rostro-medial to the ceratohyal, and is already perforated by a small foramen transhyoideum. In the histological sections, the presence of a blood vessel could be observed, which by its location corresponds to the arteria pseudobranchialis afferens. The caudal margin of the basihyal is in contact with the anterior end of the copula anterior. All five branchial arches are formed, though they are not complete. They comprise large to small hypobranchials I–IV, well formed ceratobranchials I–V, and small epibranchials I–III. Hypobranchials I, II and III are in contact with the copula anterior, while hypobranchial IV is in contact with the copula posterior.

DAY 3 POST-HATCHING (13.4–15.2 MM L_T) AND 5 POST-HATCHING (14–15.2 MM L_T) (FIG. 2)

Neurocranium

Compared to the previous stage, significant changes are noticeable. At this stage, the caudal end of the taenia marginalis posterior is fused with the post-orbital process of the capsula auditiva. In some of the samples, a short vertical cartilaginous projection from the medio-dorsal surface of the ethmoid region starts to form, representing the future processus internasalis.

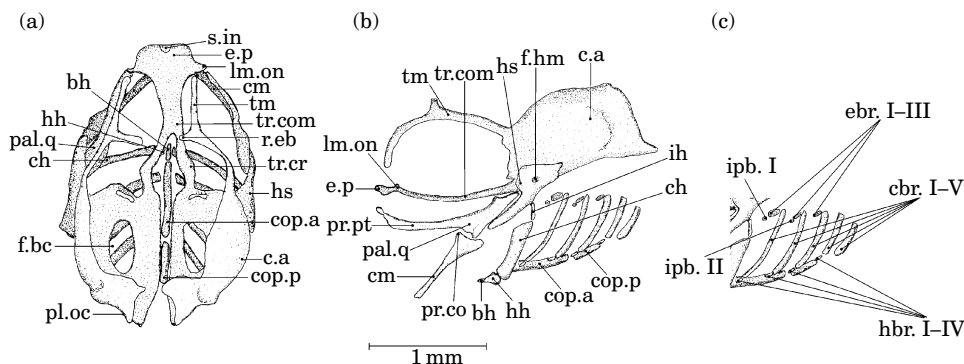


FIG. 2. Skull of *Salmo letnica* at 5 dph. (a) Dorsal and (b) lateral view of the chondrocranium. (c) Lateral view of the cartilaginous branchial arches. bh, basihyal; c.a, capsula auditiva; cbr, ceratobranchial; ch, ceratohyal; cm, cartilago Meckeli; cop.a, copula anterior; cop.p, copula posterior; e.p, ethmoid plate; ebr, epibranchial; f.bc, fenestra basicapsularis; f.hm, foramen hyomandibulare; hbr, hypobranchial; hh, hypohyal; hs, hyosymplecticum; ih, interhyal; ipb, infrapharyngobranchial; lm.on, lamina orbitonasalis; pal.q, palatoquadratum; pl.oc, pila occipitalis; pr.co, processus coronoideus; pr.pt, processus pterygoideus; r.eb, rudiment of the epiphyseal bridge; s.in, septum internasale; tm, taenia marginalis; tr.com, trabecula communis; tr.cr, trabecula cranii.

Viscerocranium

At 3 dph, the processus pterygoideus is extended and now contacts the pars palatina but they are not fused yet. At this stage infrapharyngobranchials I and II are present in all specimens. Serial sections indicate that these two structures are still procartilaginous (which stain with alcian blue but show no distinct chondrocytes on the serial sections). The cartilaginous copula posterior shows further elongation in the caudal direction, thereby extending almost to the anterior end of the ceratobranchial V.

A fusion between the pars palatina and the pars quadrata is first evident at 5 dph. Compared to 1 dph specimens, Meckel's cartilage now bears a distinct processus coronoideus. At 5 dph, the epibranchials I, II and III are more elongated. Epibranchial IV is absent.

DAY 7 (16.1–16.9 MM L_T) AND DAY 9 POST-HATCHING (16.6–17.3 MM L_T) (FIG. 3)

Neurocranium

The completion of the epiphyseal bridge was first observed at 7 dph. The taeniae marginales are now rostrally elongated and bifurcated forming the medial process which is directed toward the septum internasale, and the lateral process leading to the lamina orbito-nasalis. In all samples, the lateral process is fused with the lamina orbito-nasalis, thus generating the commissura sphenothmoidalis. The roofs of the otic capsules start to fuse with each other at 9 dph. As a result of this fusion, the fontanella postpinealis is formed. At the same stage, the prootic bridge is established between the parachordal cartilages on the floor of the neurocranium. The fenestra basicapsularis is still present but is relatively smaller than in the previous stages.

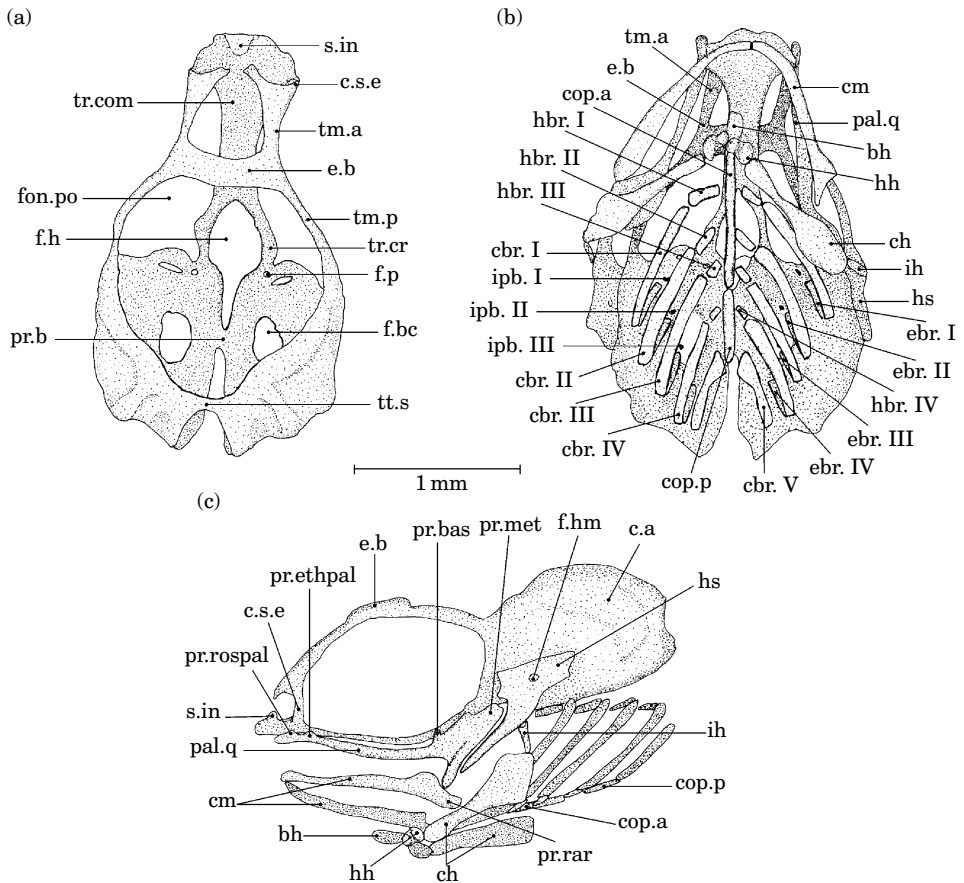


FIG. 3. Skull of *Salmo letnica* at 9 dph. (a) Dorsal view of the neurocranium, (b) ventral and (c) lateral view of the chondrocranium. bh, basihyal; c.a, capsula auditiva; cbr, ceratobranchial; ch, ceratohyal; cm, cartilago Meckeli; cop.a, copula anterior; cop.p, copula posterior; c.s.e, commissura sphenothmoidalis; e.b, epiphyseal bridge; ebr, epibranchial; f.bc, fenestra basicapsularis; f.h, fenestra hypophyseae; f.hm, foramen hyomandibulare; fon.po, fontanella postpinealis; f.p, foramen palatinum; hbr, hypobranchial; hh, hypohyal; hs, hyosymplecticum; ih, interhyal; ipb, infrapharyngobranchial; pal.q, palatoquadratum; pr.b, prootic bridge; pr.bas, processus basalis; pr.ethpal, processus ethmopalatinus; pr.met, processus metapterygoideus; pr.rar, processus retroarticularis; pr.rosPAL, processus rostopalatinus; s.in, septum internasale; tm.a, taenia marginalis anterior; tm.p, taenia marginalis posterior; tr.com, trabecula communis; tr.cr, trabecula cranii; tt.s, tectum synoticum.

Viscerocranium

The formation of the epibranchial IV was first observed at 7 dph. At 9 dph, the rostral part of the palatoquadratum has two processes: the processus rostopalatinus and processus ethmo-palatinus. A fully developed articulation between the palatoquadratum and the ethmoid plate is developed at the level of this rostopalatinine articulation in all samples at this stage. On the palatoquadratum, the processus basalis has developed at the base of the processus metapterygoideus. The processus retroarticularis of the Meckel's cartilage is larger and posteriorly relatively elongated. Infrapharyngobranchial III is present in 9 dph specimens.

DAY 14 (18.4–19.2 MM L_T) AND 16 POST-HATCHING (18.7–19.7 MM L_T) (FIG. 4)

Neurocranium

There are no significant changes in comparison with the previous stage, except that the medial process of the rostral part of the taenia marginalis fuses with the septum internasale, thereby forming the commissura sphenoseptalis.

Viscerocranium

Infrapharyngobranchial IV has formed. Even though *in toto* cleared and stained specimens showed that the cartilaginous branchial basket has almost completely formed, the serial sections reveal that the epibranchials III and IV, and infrapharyngobranchials III and IV are still procartilagenous. Ceratobranchial V is larger than the first four ceratobranchials.

DAY 19 POST-HATCHING (19.8 –20.9 MM L_T) (FIG. 5)

Neurocranium

At this stage, the processus sphenoseptalis and the septum internasale are fused in all samples. As a result the foramen olfactorium advehens and the fontanella praepinealis are present. Chondrification of the epiphyseal bridge continues in an anterior direction, thereby forming a significant part of the cartilaginous neurocranial roof. A complete chondrification is present at the level where the fenestra basicapsularis was present earlier on, leaving only a small foramen glossopharyngeum (visible in the histological sections). This cartilage, filling this basicapsular fenestra, lies more ventrally than the rest of the otic floor and accommodates the sacculus of the labyrinth. The tectum synoticum now becomes continuous with a procartilagenous structure, which may correspond to the tectum posterius, judging from its position.

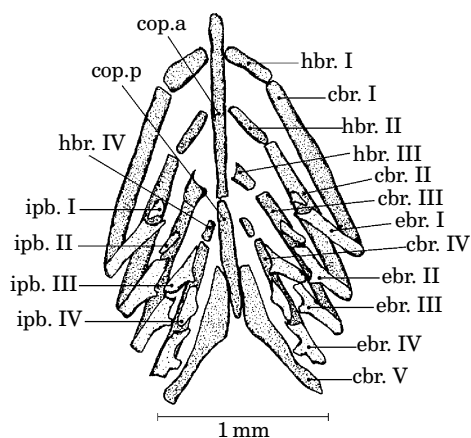


FIG. 4. Cartilaginous branchial basket of *Salmo letnica* at 14 dph (dorsal view). cbr, ceratobranchial; cop.a, copula anterior; cop.p, copula posterior; ebr, epibranchial; hbr, hypobranchial; ipb, infrapharyngobranchial.

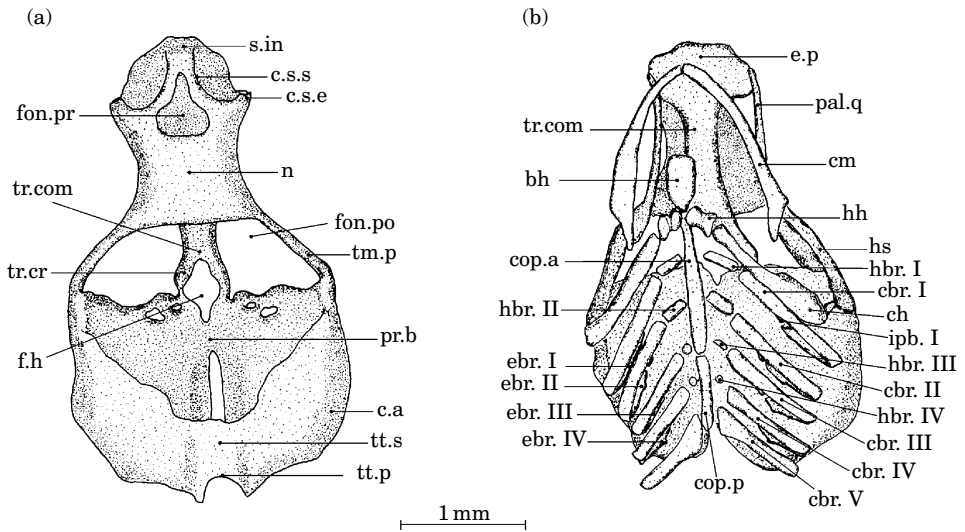


FIG. 5. Skull of *Salmo letnica* at 19 dph. (a) Dorsal view of the neurocranium, and (b) ventral view of the chondrocranium. bh, basihyal; c.a, capsula auditiva; cbr, ceratobranchial; ch, ceratohyal; cm, cartilago Meckeli; cop.a, copula anterior; cop.p, copula posterior; c.s.e, commissura sphenoseptalis; c.s.s, commissura sphenoseptalis; ebr, epibranchial; e.p, ethmoid plate; f.h, fenestra hypophyseae; fon.po, fontanella postpinealis; fon.pr, fontanella praepinealis; hbr, hypobranchial; hh, hypohyal; hs, hyosymplecticum; ipb, infrapharyngobranchial; n, neurocranial roof; pal.q, palatoquadratum; pr.b, prootic bridge; s.in, septum internasale; tm.p, taenia marginalis posterior; tr.cr, trabecula cranii; tr.com, trabecula communis; tt.p, tectum posterius; tt.s, tectum synoticum.

Viscerocranium

The first signs of the interbranchial IV are evident only in a few specimens of this stage. It appears as an independent cartilage between the ceratobranchial IV and the epibranchial IV.

DAY 25 POST-HATCHING (20.7–21.8 MM L_T) (FIG. 6)

Neurocranium

The ethmoid region is now relatively broad and extended further rostro-caudally. The septum internasale is enlarged. At this stage, the rostral part of the neurocranial roof is entirely 'chondrified', resulting in a completely closed fontanella praepinealis. A chondrification at the level of the interorbital septum is evident for the first time at this stage. This chondrification can only be observed in the histological sections since no alcian blue staining is evident. In the position of the trigemino-facial chamber, the foramen facialis is subdivided into two foramina. Laterally the otic walls are more pronounced at the level of the canalis semicircularis horizontalis of the membranous labyrinth.

Viscerocranium

In comparison with the 9 dph old Ohrid trout, the processus basalis is more conspicuous. The processus ethmo-palatinus articulates with the ethmoid cartilage. The basihyal is rostro-caudally elongated. The copula posterior is also

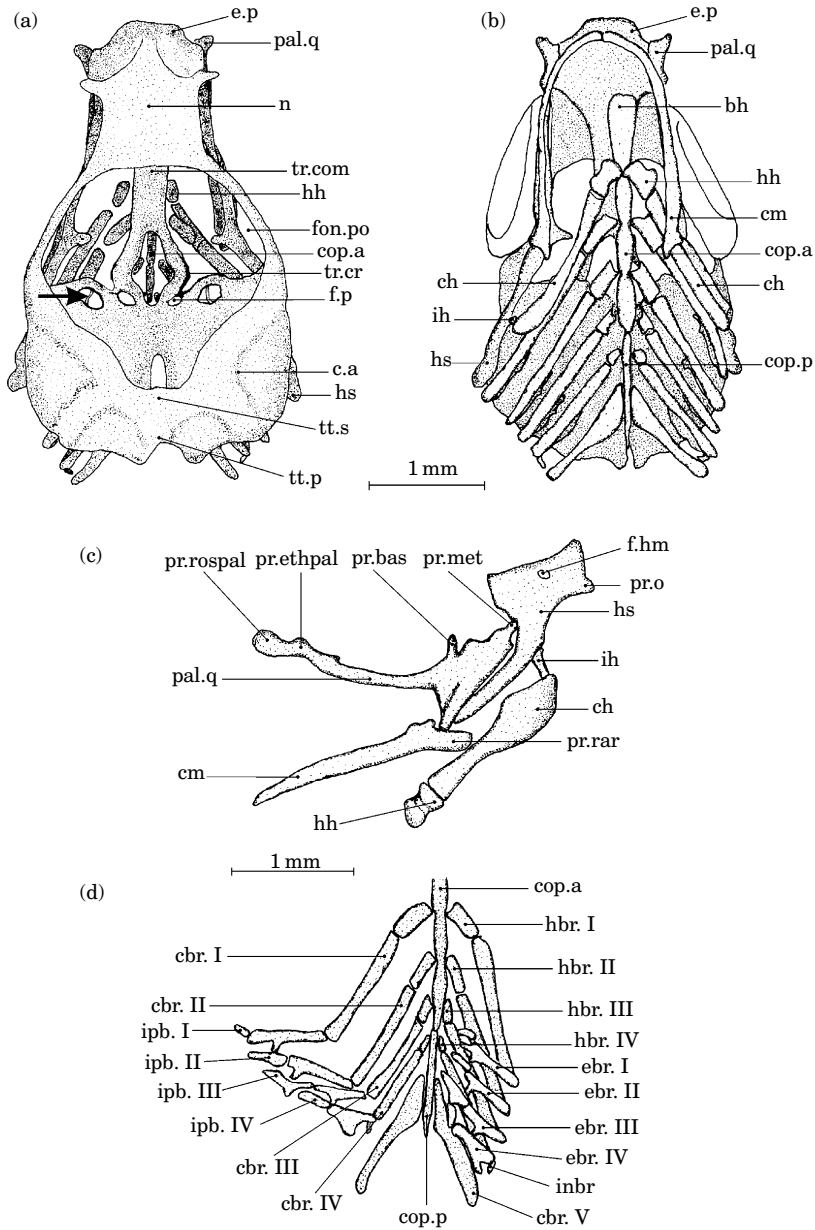


FIG. 6. Skull of *Salmo letnica* at 25 dph. (a) Dorsal and (b) ventral views of the chondrocranium, (c) lateral view of the mandibular and hyoid arch and (d) dorsal view of the cartilaginous branchial arches (dorsal elements of left side folded back). bh, basihyal; c.a, capsula auditiva; cbr, ceratobranchial; ch, ceratohyal; cm, cartilago Meckeli; cop.a, copula anterior; cop.p, copula posterior; ebr, epibranchial; e.p, ethmoid plate; f.hm, foramen hyomandibulare; f.p, foramen palatinum; fon.po, fontanella postpinealis; hbr, hypobranchial; hh, hypohyal; hs, hyosymplecticum; ih, interhyal; inbr, intrabranchial; ipb, infrapharyngobranchial; n, neurocranial roof; pal.q, palatoquadratum; pr.bas, processus basalis; pr.ethpal, processus ethmopalatinus; pr.met, processus metapterygoideus; pr.o, processus opercularis; pr.rar, processus retroarticularis; pr.rosal, processus rostopalatinus; tr.cr, trabecula cranii; tr.com, trabecula communis; tt.p, tectum posterius; tt.s, tectum synoticum. →, subdivision of the foramen facialis.

caudally elongated, reaching the level of the middle of ceratobranchial V. The interbranchial IV is present in all samples from this stage.

DAY 39 POST-HATCHING (22.4–23 MM L_T) (FIG. 7)

Neurocranium

The formation of the taenia tecti medialis posterior begins at 39 dph. It emerges as a medial process at the caudal margin of the epiphyseal bridge.

Viscerocranium

The fourth hypobranchial is fused with the ceratobranchial IV.

DAY 92 POST-HATCHING (28.5–31.2 MM L_T) (FIG. 8)

Neurocranium

The manual graphical reconstruction allows a complete and detailed picture of the architecture of the chondral neurocranium in 92 dph old *S. letnica* to be obtained. The taenia tecti medialis posterior, which appeared in 39 dph old Ohrid trout is now caudally elongated (reaching the anterior border of the os supraoccipitale, not depicted here). The chondrification of the interorbital septum is more extensive than in previous stages and the formation of the foramen

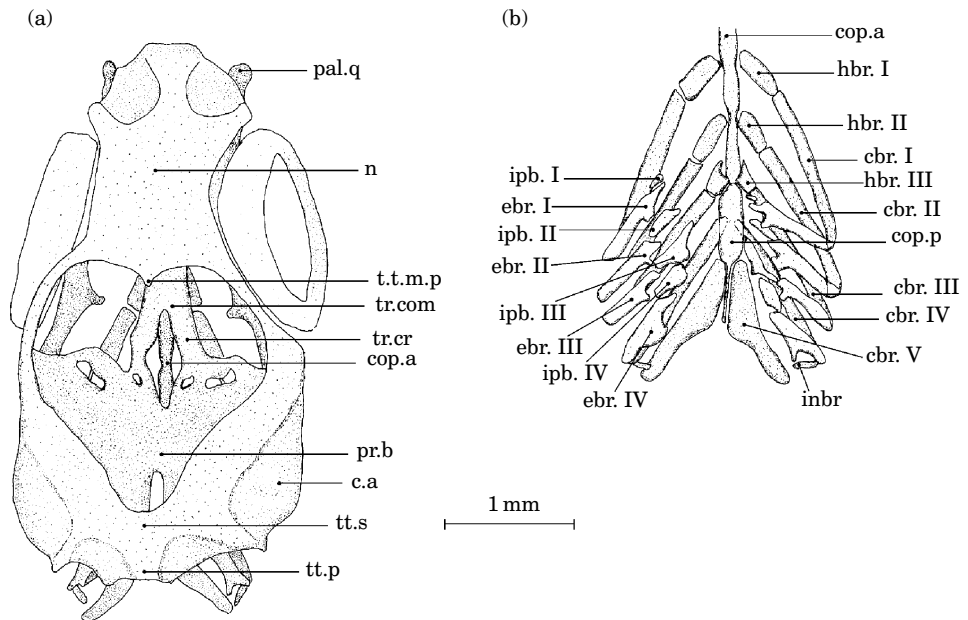


FIG. 7. Skull of *Salmo letnica* at 39 dph. (a) Dorsal view of the chondrocranium and (b) cartilaginous branchial arches. c.a, capsula auditiva; cbr, ceratobranchial; cop.a, copula anterior; cop.p, copula posterior; ebr, epibranchial; hbr, hypobranchial; inbr, intrabranchial; ipb, infrapharyngobranchial; n, neurocranial roof; pal.q, palatoquadratum; pr.b, prootic bridge; tr.com, trabecula communis; tr.cr, trabecula cranii; t.t.m.p, taenia tecti medialis posterior; tt.p, tectum posterius; tt.s, tectum synoticum.

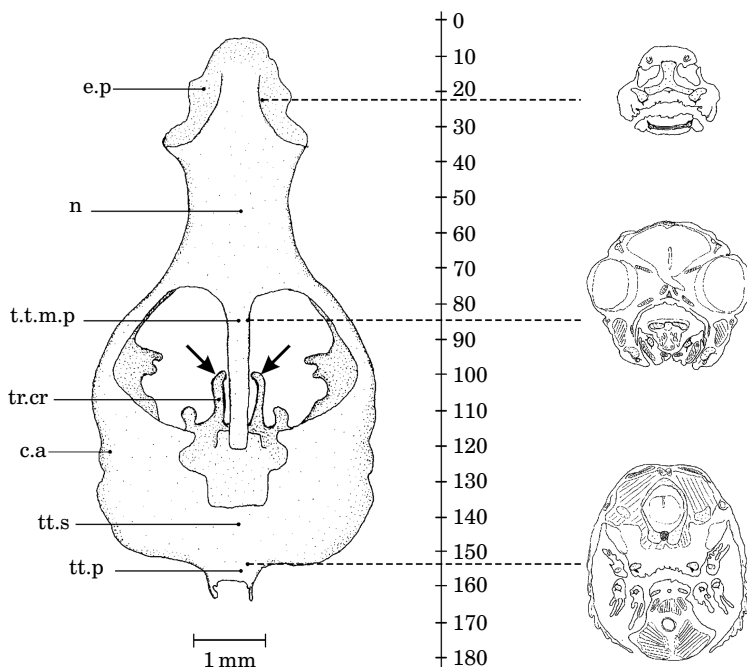


FIG. 8. Three dimensional – manual graphical reconstruction of the cartilaginous neurocranium of 92 dph old *Salmo letnica*, based on the serial sections, with the representation of the histological sections at three levels (reconstruction of a dorsal view). c.a, capsula auditiva; e.p, ethmoid plate; n, neurocranial roof; tr.cr, trabecula cranii; t.t.m.p, taenia tecti medialis posterior; tt.p, tectum posterior; tt.s, tectum synoticum; \rightarrow , resorption of the cartilage at the level of the anterior part of trabecula cranii. Vertical bar represents millimetre paper on which the measured points were plotted for the histological sections (not at the same scale as the dorsal view).

olfactorium evehens, through which the fila olfactoria passes, has started. The first signs of cartilage resorption are evident where the trabecula cranii are fused with the anterior part of the parachordal plates. The ethmoid cartilage is extended more rostrally than in the previous stages.

Viscerocranium

The most anterior part of the palatoquadrate cartilage has a well-developed process, which is 'hooked' over the head of the maxillary bone (Fig. 9).

DISCUSSION

In most teleosts, chondrification of the neurocranium starts simultaneously with that of the splanchnocranium (de Beer, 1937). Some published data, however, suggest that viscerocranial structures form prior to neurocranial elements (Srinivasachar, 1959; Vandewalle *et al.*, 1997; Adriaens & Verraes, 1997a). Usually, cartilaginous elements start to form a few days before hatching.

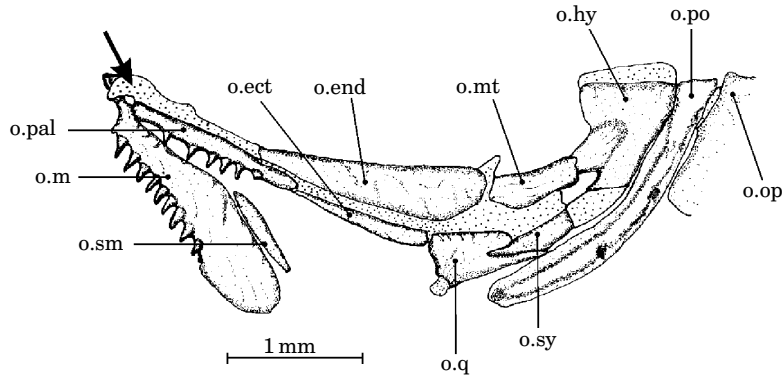


FIG. 9. Lateral view of the ossified suspensorium and upper jaw of *Salmo letnica* at 92 dph. o.ect, os ectopterygoideum; o.end, os endopterygoideum; o.hy, os hyomandibular; o.m, os maxilla; o.mt, os metapterygoideum; o.op, os opercular; o.pal, os palatinum; o.po, os preopercular; o.q, os quadratum; o.sm, os supramaxilla; o.sy, os symplecticum. →, a well-developed process, at the level of the anterior part of the palatine cartilage, which is 'hooked' over the head of the maxilla.

Nevertheless, the order of appearance and the course of development of cartilaginous elements are highly variable in teleosts. The complexity of the chondrocranium at hatching may also depend on the size of the eggs, because the amount of yolk material is frequently correlated with the duration of a prehatching development (Adriaens & Vandewalle, 2003). Genetic variability, as well as epigenetic influences, are also undoubtedly crucial of great importance.

In *S. letnica*, at the moment of hatching, most of the structures of the cartilaginous neurocranium and viscerocranium are present (however, in this study it was not possible to discern which structures arise first). In this respect, *S. letnica* does not differ from other salmonid species like *Salmo salar* L., *Oncorhynchus mykiss* (Walbaum) (Verraes, 1973) and *Salvelinus namaycush* (Walbaum) (Balon, 1980), even though some structures are still absent in *Salmo trutta* L. (*S. fario*, de Beer, 1937) (Table I).

An early development of the cartilaginous neurocranium and viscerocranium does not imply a faster chondrification. The subsequent development of these structures in *S. letnica* is slower compared to that of some non-salmonid teleosts, even though fewer structures may be present in those non-salmonids. For instance, although at the moment of hatching *Catostomus commersoni* (Lacépède) (Catostomidae) (McElman & Balon, 1985), *Heterobranchus longifilis* Valenciennes (Clariidae) (Vandewalle *et al.*, 1997), *Barbus barbus* (L.) (Cyprinidae) (Vandewalle *et al.*, 1992) and *Solea solea* (L.) (Soleidae) (Wagemans & Vandewalle, 1999) do not possess any cartilaginous structures, the further development of their chondral skull is faster than that of *S. letnica* (for comparisons made at 16, 10, 24 and 20 dph, respectively).

At 1 dph, a cartilaginous neurocranial base comprises the parachordal plates, which are anteriorly fused to the trabecular bars. The latter bars are fused rostrally, in this way forming a trabecula communis typical of the tropibasic skull, which is assumed to be related to large eye size (Corsin, 1961; Daget, 1964;

TABLE I. Timing of the appearance of the cartilaginous structures in *Salmo letnica*, *Salmo fario* (= *S. trutta*) and *Oncorhynchus mykiss*

Age (dph)	<i>Salmo letnica</i>	<i>Salmo fario</i> *	<i>Oncorhynchus mykiss</i> *
1	Presence of ethmoid plate, lamina orbitonasalis, trabecula communis, trabecular bar, parachordal plate, floor and part of the walls of capsula auditiva, commissura lateralis, commissura basicapsularis anterior and posterior, pila occipitalis, taenia marginalis, pars palatina, pars quadrata with well developed pterygoid process, processus metapterygoideus, Meckel's cartilage, hyosymplecticum, interhyal, ceratohyal, basihyal, copula anterior and posterior, hypobranchial I-IV, ceratobranchial I-III, copula anterior and posterior	Presence of ethmoid plate, trabecula communis, trabecular bar, parachordal plate, floor of the capsula auditiva, commissura lateralis, commissura basicapsularis anterior and posterior, pila occipitalis, taenia marginalis, pars quadrata with pterygoid process, proc. metapterygoideus, Meckel's cartilage, hyosymplecticum, interhyal, ceratohyal, hypohyal, basihyal, copula anterior and posterior, hypobranchial I-II, ceratobranchial I-IV	Presence of ethmoid plate, lamina orbitonasalis, trabecula communis, trabecular bar, parachordal plate, floor and part of the walls of capsula auditiva, commissura lateralis, commissura basicapsularis anterior and posterior, pila occipitalis, taenia marginalis, pars palatina, pars quadrata with well developed pterygoid process, Meckel's cartilage, hyosymplecticum, interhyal, ceratohyal, hypohyal, basihyal, copula anterior and posterior, hypobranchial I-IV, ceratobranchial I-V, epibranchial I-III, beginning of formation of epibranchial IV
3	Formation of internasal septum		
5	Infrapharyngobranchial I-II, fusion between taenia marginalis and capsula auditiva	Appearance of epibranchial III, ceratobranchial V, formation of the walls of capsula auditiva	
6			Formation of infrapharyngobranchial II-IV
7	Formation of epiphyseal bridge, fusion between commissura sphenothmoidalis and lamina orbitonasalis, appearance of epibranchial IV	Fusion between taenia marginalis and capsula auditiva, formation of lamina orbitonasalis and nasal septum	
8		Appearance of pars palatina	

TABLE I. Continued

Age (dph)	<i>Salmo letnica</i>	<i>Salmo fario</i> *	<i>Oncorhynchus mykiss</i> ♣
9	Formation of tectum synoticum, prootic bridge, processus rostralatinus, beginning of formation of processus ethmopalatinus and processus basalis, appearance of infrapharyngobranchial III	Formation of epiphyseal bridge	
10		Fusion between commissura sphenothmoidalis and lamina orbitonasalis, and fusion of internasal septum with commissura sphenoseptalis, beginning of formation of processus ethmopalatinus and processus basalis Appearance of hypobranchial IV and presence of hypobranchial III	
11			Fusion between commissura sphenothmoidalis and lamina orbitonasalis; and fusion of septum internasale with commissura sphenoseptalis, presence of epiphyseal bridge, presence of tectum synoticum and tectum posterius, formation of
12			infrapharyngobranchial I

14	Appearance of infrapharyngobranchial IV	Appearance of infrapharyngobranchial I–II, copula has been divided in copula anterior and posterior, formation of the cranial roof (tectum synoticum and tectum posterius), formation of the prootic bridge	
19	Fusion between commissura sphenoseptalis and internasal septum, formation of tectum posterius, beginning of formation of interbranchial IV		Formation of interbranchial IV
24			
25	Interorbital cartilage, presence of interbranchial IV		
39	Beginning of formation of taenia tecti medialis posterior, fusion between hypobranchial IV and ceratobranchial IV		
48			Beginning of formation of taenia tecti medialis posterior
92	Resorption of the cartilage at the level of the caudal part of trabecula communis and anterior part of the trabecula cranii		resorption of the cartilage at the level of the caudal part of trabecula communis and anterior part of the trabecula cranii

* According to de Beer (1927)

♣ According to Verreaes (1973)

Verraes, 1974c; Adriaens & Verraes, 1997b). Rostrally, the trabecula communis is markedly broad, forming the ethmoid cartilage. Even at this stage, in all samples of *S. letnica*, the formation of a lamina orbitonasalis was noticed. The presence of this cartilage was also discerned in *O. mykiss* (Verraes, 1973, 1974d) at 1 dph, while its presence in *S. trutta* (de Beer, 1927) was observed at only 7 dph (Table I). In *S. letnica*, the internasal septum appears early compared to the salmonids mentioned above. Nevertheless, even though structures of the ethmoid region in *S. letnica* appear a few days earlier than in *S. trutta* or *O. mykiss*, the whole chondrification of the ethmoid region in *S. letnica* is slowed down. At this point it remains unclear whether these differences are a result of evolutionary heterochronies or simply of environmental factors such as water temperature, density of fish in cultivation tanks, water quality, and the type and quantity of food administered to the fish at a particular point in their development.

Compared to the foramen olfactorium advehens, the formation of which could clearly be followed through the ontogenetic series, the formation of the foramen olfactorium evehens was hard to follow because the interorbital cartilage was not stained by alcian blue in all samples from all the investigated stages. The presence of the interorbital cartilage was first noticed in histological sections of 25 dph old Ohrid trout, and a completely developed foramen olfactorium evehens was observed at 92 dph, when the presence of the os orbitosphenoid was noted. This bone, which contributes to the bordering of the foramen olfactorium evehens, develops as a perichondral lamella of the interorbital septum and interorbital cartilage. The formation of the interorbital cartilage has not been observed in *Onchorynchus kisutch* (Walbaum) (Jollie, 1983) and *S. salar* (Sanford, 2000). As the interorbital cartilage did not stain well with alcian blue and could only be discerned in the histological sections, it cannot be excluded that it may have been overlooked in those two studies (which did not use histological sections). According to those two authors, a cartilaginous precursor for the orbitosphenoid is also lacking. The formation of the interorbital cartilage has been noticed in other salmonids like *O. mykiss* and *S. trutta*.

The commissura lateralis, which represents the border between the orbito-temporal region and the floor of the otic capsule, is present in *S. letnica* at 1 dph. It forms the lateral wall of the trigeminofacial chamber, where a vein (presumably the vena mandibularis) and the orbital artery enter between the commissura basicapsularis anterior and the lateral commissure, whereas the hyomandibular branch of the facial nerve exits the neurocranium through the trigemino-facial chamber. During ontogeny, an extra strut of cartilage develops in the foramen facialis of *S. letnica*. According to de Beer (1927, 1937) this cartilage is present in *S. salar* and *Salvelinus fontinalis* (Mitchill) but has not been observed either in *S. trutta* (de Beer, 1937) or in *O. mykiss* (Verraes, 1973).

The trabecular bars do not lie in the same position as the rest of the neurocranial floor, at the level of the fusion with the parachordal cartilage. This inclination may be related to the necessity of extra space for the rectus muscles of the eye, thus forming the posterior myodome. At 92 dph (28.5–31.2 mm L_T) the caudal part of the trabecular bars starts to become resorbed, which could only be observed in the histological sections. According to Daget (1964), the cartilage resorption of the caudal trabecular bars is typical of teleosts and is not

accompanied by a process of perichondral or endochondral ossification. In other salmonids this resorption occurs in the same developmental period, as for example observed in *S. salar* (de Beer, 1937) and *O. mykiss* (Verraes, 1974d). Compared to some non-salmonid teleosts, such as *Haplochromis elegans* Trewavas (Cichlidae) (11 dph) (Ismail, 1979), *H. longifilis* (10 dph) (Vandewalle *et al.*, 1997) and *B. barbatus* (16 dph) (Vandewalle *et al.*, 1992), this resorption takes place later. As suggested by Verraes (1974d), the timing of this cartilage resorption is functionally related to the developmental speed of the os parasphenoideum.

The major part of the floor of the otic capsules is present in *S. letnica* at 1 dph. These capsules are connected to the parachordal plates at the levels of the commissura basicapsularis anterior and the commissura basicapsularis posterior. The fenestra between these two commissures is the fenestra basicapsularis. The histological sections show that a nerve exits the neurocranium at the caudal part of this fenestra. Judging from its location, this nerve must correspond to the n. glossopharyngeus. The fenestra basicapsularis closes up during later development due to further chondrification, leaving only a small foramen for the n. glossopharyngeus. At some point during ontogeny in *S. trutta*, the fenestra basicapsularis becomes subdivided into two parts, consequently forming the fenestra basicapsularis anterior and posterior. This subdivision could not be observed in *S. letnica*, nor has it been observed in *O. mykiss* (Verraes, 1973).

The tectum synoticum, together with the tectum posterius, constitutes the posterior roof of the cartilaginous neurocranium. This pattern of formation of the posterior part of the chondrocranial roof is typical for many salmonids (de Beer, 1927, 1937; Verraes, 1974d; Balon, 1980), but also for many non-salmonid teleosts *e.g.* Cyprinidae (Cubbage & Mabee, 1996) or Percomorpha (Mabee & Trendler, 1996).

Generally, during the early development of the fish skull, the cartilaginous roof comprises the taeniae marginales (usually referred to as orbital cartilages). In some fishes the taeniae marginales arise as a rostral extension of the anterior otic cartilage (Srinivasachar, 1958b, 1959; Vandewalle *et al.*, 1992; Cubbage & Mabee, 1996; Mabee & Trendler, 1996; Adriaens & Verraes, 1997a) whereas in other fishes they develop as isolated cartilages (de Beer, 1937; Daget, 1964; Verraes, 1974d). In *S. letnica* they also arise as a separate element on the first day after hatching, a condition also observed in *S. trutta* (de Beer, 1927) and *O. mykiss* (Verraes, 1974d). Compared to *S. trutta*, this cartilage is more pronounced in *S. letnica*. The initiation of the formation of the future epiphyseal bridge was also noted in 1 dph old *S. letnica* whereas in *S. trutta* the initiation of the formation of this bridge was only noticed at 7 dph. The chondrification of the anterior part of the skull roof in *S. letnica* takes place at the same time as that in *O. mykiss* and *S. trutta*. In *O. mykiss* two small foramina have been observed in the anterior part of the skull roof, which could not be observed in *S. letnica*. Based on the study by Verraes (1974d), the position of these openings suggests that the closing of the fontanella praepinealis is first realized by the formation of a medial, longitudinal rod, the taenia tecti medialis anterior, which further expands in all directions. With the samples and the methods used in this work, it was not possible to observe the formation of the taenia tecti medialis anterior in *S. letnica*. The onset of the formation of the taenia tecti medialis posterior was

observed in *S. letnica* at 39 dph and was fully formed at 92 dph, which is in accordance with that in *O. mykiss*.

The processus pterygoideus in *S. letnica* is already present at 1 dph (13.4–14.4 mm L_T), with an independent pars palatina at its rostral part. This corresponds to the observations in *O. mykiss* (12.7 mm L_T , 1 dph) (Verraes, 1973). According to de Beer (1927), the first signs of the pars palatina in *S. trutta* are observed only at 8 dph (16 mm L_T) (Table I). In Ohrid trout the palatine later on fuses with the pterygoid, at 5 dph. The formation of an independent pars palatina has also been observed in some other teleosts apart from salmonids. An isolated palatine is considered a synapomorphy of the Siluriformes, where it plays a crucial role in the palatine-maxillary mechanism for the controlled movements of the maxillary barbels (Gosline, 1975; Fink & Fink, 1981, 1996; Ghiot *et al.*, 1984; Arratia & Schultze, 1990; Arratia, 1992; Adriaens & Verraes, 1997b). In most teleosts, however, the pars palatina is fused to the pterygoquadrate from the moment it is formed. Until now, no functional evolutionary significance has been suggested for this pattern in salmonids.

During further development, the rostral part of palatoquadratum in *S. letnica* forms a hook into which the processus anterior of the maxilla fits (Fig. 9), which is typical for species of the genus *Salmo* (Sanford, 2000). Two cartilaginous processes are also formed in the dorso-caudal part of the palatoquadratum, known as the processus basalis and the processus metapterygoideus. Only the latter articulates with the hyoid arch. The present investigations do not show any direct evidence for a specific role of the processus basalis. It may play a crucial role, however, for the early support of the dermal entopterygoid bone which initially develops against the processus basalis.

In teleosts, the left and right Meckel's cartilages can be fused rostrally at some stage during development (*e.g.* Ariidae, Ictaluridae, Heteropneustidae and Clariidae) (Srinivasachar, 1958a, b, 1959; Adriaens, 1998) or remain separate (*e.g.* Cyprinidae and Percomorpha) (Ismail, 1979; Vandewalle *et al.*, 1992; Cabbage & Mabee, 1996). The latter seems to be the case in salmonids, where in *S. letnica*, the anterior parts of Meckel's cartilage only touch from the first day until 92 dph, but never fuse. Even though in some teleosts the palatoquadrate is initially fused to the Meckel's cartilage, the interhyal, and even the neurocranium (de Beer, 1937; Srinivasachar, 1958a; Arratia, 1990; Adriaens & Verraes, 1997a), such a fusion could not be discerned in *S. letnica* at hatching. The fusion between the lower jaw and the hyosymplectic in catfishes has been suggested to be crucial in mouth opening during early ontogeny (Surlmont *et al.*, 1989; Adriaens *et al.*, 2001).

At the moment of hatching, most of the cartilaginous elements of the branchial basket are present in *S. letnica*, which is also the case in *O. mykiss* (Claeys & Verraes, 1984). In *S. trutta*, however, the presence of these elements has only been observed until a few days later (de Beer, 1927). In *S. letnica* a fully developed branchial basket was seen at 25 dph, when the last cartilage component, the 'interbranchial IV,' was also formed (according to the nomenclature of Claeys & Verraes, 1984; this corresponds to the 'epibranchial V' of Sanford, 2000, and postepibranchial of Vandewalle *et al.*, 1992). Such a cartilage was not observed in *S. trutta* (de Beer, 1927, 1937).

Even though some subtle differences in timing presumably exist in the ontogeny of different salmonid species (which could not be discerned based on the

literature and present results), it does seem that the ontogeny of the chondrocranium is similar in several salmonid species. The general salmonid pattern does seem to involve the presence of most cartilaginous elements at the moment of hatching. In *S. letnica* a fully formed chondrocranium, with the first signs of cartilage resorption is present at 92 dph.

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