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Structure and development of the saccular sensory epithelium in relation to otolith growth in the perch *Perca fluviatilis* (Teleostei)

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ABSTRACT

The development of the saccular sensory epithelium in relation to otolith growth in the inner ear of the perch *Perca fluviatilis* was studied by light and transmission electron microscopy. Development is studied from the early embryo (6 days after fertilization) when the otocyst first forms, to the newly hatched larva when the development of the inner ear approximates that of the juvenile. Light microscopy revealed: 1) The auditory vesicle and the otolith primordia or otoconia first appeared 6 days after fertilization. The saccular macula overlain by a small round otolith is established by 7 days after fertilization and the stato-acoustic ganglion which contains the neuronal precursors of the macular epithelium is also seen at this stage. 2) The saccular macula began to differentiate 12 days after fertilization and became well differentiated and distinctive at one day after hatching. It is composed of two types of epithelia: sensory and transitional. The former is differentiated into lightly-stained sensory hair cells and dark-stained supporting cells. Electron microscopy indicated: 1) The apical surface of each hair cell is covered with a ciliary bundle which varies in length in different epithelial regions. Each bundle is formed from a long single kinocilium and a short bundle of stereocilia. The supporting cells are provided with microvilli on their apical surface and seem to be covered with small and large vesicles, suggesting that they have a secretory function beside the supporting one. 2) Secretory materials, such as vesicular dilations, cytoplasmic extrusions, various kinds of vesicles and electron dense granules seemed to be produced by different cells of the saccular sensory epithelium and scattered into the endolymphatic lumen, just over the saccular macula region. These secretory materials probably contribute to the formation of the otolith and/or otolithic membrane. The present findings suggest also that the fish otolithic membrane may function to some degree in the otolith formation, but the function zone appears to be the subcupular meshwork layer and not the gelatinous layer. Most of these results are discussed with special regard to the environmental factors on early development in teleost fishes.

KEYWORDS: inner ear, development, sacculus, otolith, *Perca fluviatilis* (Teleostei)

INTRODUCTION

One of the most exciting aspects of using teleosts in auditory studies is that they show an extensive interspecific variation in peripheral morphology (Platt & Popper 1981). In addition, by careful selection of species, fishes can serve as an ideal group for comparative studies that can have major implications for our understanding of the development and functional anatomy of the auditory system in all vertebrate animals.

The teleost ear consists of three semi-circular canals and their associated receptor regions, as well as of three otolithic organs, the sacculus, lagena and utriculus (Fig. A). The utriculus and semicircular canals are present in most species, considered vestibular organs and being involved with determination of angular acceleration and responses to positional change or to preserve equilibrium (Platt 1983), while the sacculus and lagena are associated with audition (Kawamura 1984). The saccular macula appears to be the major receptor of sound in most fishes.

Each of the otolithic organs contains a sensory epithelium or macula, overlaid by a calcareous otolith. Because the sagitta (saccular otolith) is typically the largest otolith and its shape varies greatly among different fish groups, it is mainly used in species identification and also in fish ageing (Pannella 1971). It has been agreed that the teleost otolith is composed of calcium

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carbonate (Morales-Nin 1987) deposited on a small amount of an organic matrix (Degens *et al.* 1969). These secretory materials are believed to be secreted by the macular epithelial cells of the otolithic organs (Dale 1976; Dunkelberger *et al.*1980).

A series of literatures has demonstrated that there is considerable inter-specific variation in the gross morphology of the vertebrate ear (Fay & Popper 1980), a fact that is especially evident in the auditory portions of the inner ear in the teleost fishes, the sacculus and lagena (Platt & Popper 1981; Salem & Omura 1998). Inter-specific differences in the sacculus and lagena are seen in a number of structural features and include the shape and size of the otoliths and the area of the sensory epithelium that lies directly in contact with the otolith. While ultrastructural variations include the lengths of the cilia extending from the hair cells on different regions of the sensory epithelium and the orientations of these cilia on the sensory cells at different regions of the epithelium (Sokolowski & Popper 1987; Platt 1993).

Most previous studies were concentrated on the structure of the adult fish labyrinth, which has also been described for many different species of teleosts (Platt 1983). However, there are few studies on the growth of the sensory organs in the labyrinth (Becerra & Anadon 1993). These studies have demonstrated the addition of new sensory cells in fish over a long period time. It was known that the sensory epithelial cells of each otolithic organ is associated with branches of eighth auditory nerve. The role of innervation in the differentiation of the sensory cells in vertebrate ear is controversial. Some authors claim that the influence of the nervous system is necessary for cellular differentiation (Chandler 1984). However, others believe that cells can differentiate without this influence (Raymond 1987).

The present study was conducted to characterize the development and ultrastructure of one of the most important auditory parts of the inner ear, the saccular macula, in an attempt to understand the cellular contribution of the saccular sensory epithelium of the macula to otolith growth and otolithic membrane formation in the perch *Perca fluviatilis*, by using light and transmission electron microscopy. We will also discuss the factors affecting formation or deposition of the otolith increments. Data on this species is of particular interest because it is a member of order Perciformes, the largest and one of the most diverse of the teleost groups and one for which no TEM data on the ear are currently available.

MATERIALS AND METHODS

The fertilized eggs of the perch *Perca fluviatilis* were obtained from Gifu Prefectural Institute of Fish Larvae for Aquaculture (Japan) and kept in laboratory tanks with water temperature ranging between 15 - 17˚C. The fertilized eggs were maintained in recirculating water under a 12 hours light- 12 hours dark photoperiod; light was supplied by cool fluorescence lamp (30-50 lux). The eggs were hatched at 12 days after fertilization with total body length approximately 3-4mm. After deep anaesthetizing, whole bodies of six different developmental ages (6, 7, 8, 10 and 12 days after fertilization, and 1 day after hatching) were respectively selected, immersed in fixative (2% paraformaldehyde - 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3) and left at room temperature (18˚C) for 2-4 hours. After post-fixation in 1% Osmium tetroxide in 0.1 M phosphate buffer at pH 7.3, specimens were dehydrated in graded series of ethanol and embedded in epoxy resin.

For light microscopy observations, and on a Porter-Blum MT-1 ultramicrotome, serial semithin sections (1-2 micron) at the inner ear region were cut with glass knives and stained with 1% toluidine blue. Ultra-thin sections were stained with 3% uranyl acetate and 1% lead citrate and examined under a JEOL 100 CX transmission electron microscope at 80 kV.

RESULTS

Six days after fertilization, the auditory vesicle, which has an oval shape, appears on either side of the posterior portion of the head at the level of the developing rhombencephalon.

Numerous heavily-stained spherules with various sizes and shapes, representing the primordia or otoconia of the otolith, are observed in the lumen of the auditory vesicle (Fig. 1).
By seven days after fertilization, the auditory vesicle rapidly enlarges by expanding its lumen and forms a pear-shaped structure (Fig. 2). The first indication of the future sensory patches is the appearance of an otolith over a thickened region in the ventro-medial wall of the vesicle, representing the establishment of the saccular macula that lies in an oblique position (Fig. 2). The stato-acoustic ganglion (VIIIth) which contains the neuronal precursors of the macular epithelium is also clearly seen at this stage. This ganglion appears as a single ventro-medial mass beneath the medial floor of the saccular macular epithelium (Fig. 2).
At eight days after fertilization, the saccular macular which begins to invaginate medially is formed into a flattened region of arranged columnar cells overlain by an oval otolith (Fig. 3). The connective tissue layer lining the macular epithelium is highly vascularized forming a capillary bed. Ten days after fertilization, the cells of the saccular macula, which have a vertical position, are arranged in a somewhat radial manner but they not yet differentiated (Fig. 4).

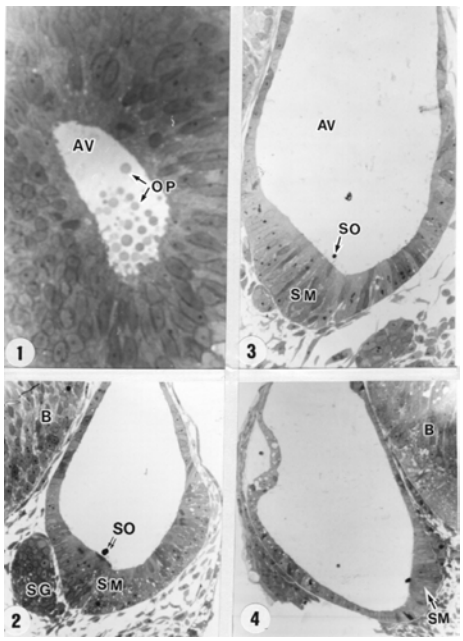
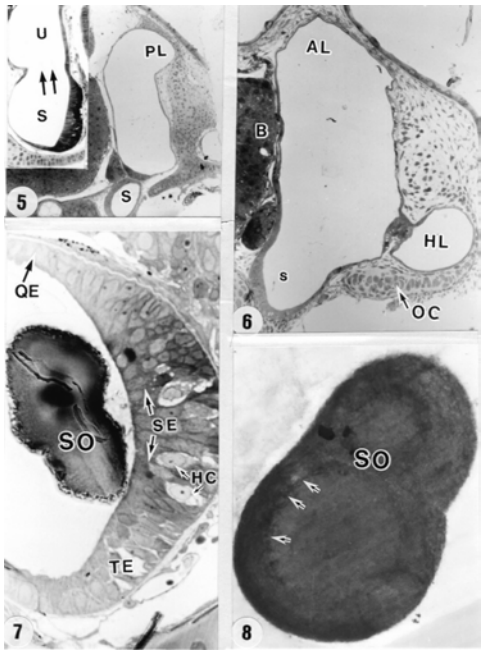


Fig. 1. *Perca fluviatilis*, 6 days after fertilization. LM micrograph of a transverse section across the inner ear region, showing the first appearance of the auditory vesicle (AV) containing the otolith primordia (OP). X 510.
Fig. 2. *Perca fluviatilis*, 7 days after fertilization. LM micrograph of a transverse section across the inner ear region, showing first appearance of the saccular otolith (SO) on an accumulation of polygonal cells representing the saccular macula (SM). B, brain. Note the stato-acoustic ganglion (SG) behind the saccular macula. X 400.
Fig. 3. *Perca fluviatilis*, 8 days after fertilization. LM micrograph of a transverse section across the inner ear region, showing enlargement of the auditory vesicle (AV) and arrangement of the saccular macular cells (SM) in an oblique position. X 360.
Fig. 4. *Perca fluviatilis*, 10 days after fertilization. LM micrograph of a transverse section across the inner ear region, showing the saccular macula (SM) in a vertical position and restricted to the medial wall. X 170.

The stage of twelve days after fertilization (hatching day) represented an advanced step in the development of the saccular macula and showed major differences compared with the previous stage, manifested mainly in the beginning of differentiation of the macular epithelial cells. The sacculus, which ends blindly, is more invaginated medially and its endolymph communicates with that of the utriculus via the utriculo-saccular canal (Fig. 5). The semicircular canals seemed to be well differentiated at this stage (Fig. 6). The sensory epithelium of the saccular macula, which is the thickest region in the saccular wall, is vertically oriented and essentially restricted to the medial wall. The peripheries of the sensory epithelium are transformed into a thinner transitional epithelia, which in turn gradually become thinner to form extremely thin squamous epithelium at the lateral sides of the macula (Fig. 7). The hair cells of the sensory epithelium, which begin gradually to differentiate, are concentrated in the middle part of the epithelium (Fig. 7). They are not numerous but very conspicuous. They are easily identified by their light staining and the large size of their nuclei. Situated in the saccular lumen is an elongate otolith, the sagitta, that lies in close association with the saccular macula (Fig. 7). The otolith seems to possess one incremental layer (growth ring), consisting of numerous spherules that are arranged in a wide ring (Fig. 8).



Figs. 5 & 6. *Perca fluviatilis*, 12 days after fertilization. LM micrographs of transverse sections through the saccule region, showing invagination of the saccule (S) towards the medial wall, and also the utriculo-saccular canal (double arrows). Note differentiation of three semicircular canals: PL, posterior canal; AL, anterior canal; HL, horizontal canal. U, utriculus; OC, otic capsule. Fig. 5: X 80 ; Fig. 6: X 140.

Fig. 7. *Perca fluviatilis*, 12 days after fertilization. LM micrograph of a transverse section through the sacculus, showing the sensory epithelium (SE), transitional epithelium (TE), squamous epithelium (QE) and saccular otolith (SO). Note the visible hair cells (HC). X 250.

Fig. 8. *Perca fluviatilis*, 12 days after fertilization. LM micrograph of a transverse section across the inner ear region, showing the saccular otolith (SO) and beginning formation of the first incremental layer (arrows). X 976.

One day after hatching, the saccular sensory epithelium which is overlain by an elongate otolith is well developed and differentiated. It consists of lightly-stained hair cells and dark-stained supporting cells (Figs. 9&10). Each hair cell exhibits a large, oval or elongate placed nucleus. The basal portion of these cells are approximately rounded and do not reach the basement membrane (Figs. 10 &17), while those of the supporting cells are seated up on the basement membrane, and their apical parts extending between the adjacent hair cells. Secrtory materials are observed in the cytoplasm of some hair cells (Fig. 10). Protrusion-like cilia are also seen on the apical surface of hair cells (Figs. 10&11). At the peripheries of the macula, the epithelium remains thickened but loses its sensory specialization before grading into the adjacent low cuboidal cells of the saccular wall. Between the sensory epithelium and the otolith is a thin layer of secretory materials or fibres, representing the otolithic membrane or the subcupular meshwork of the otolithic membrane (Figs. 11&19)

Because this stage represented an advanced step in the saccular sensory epithelium differentiation, it was important to examine it under the electron microscope. Two types of cells are clearly observed in the saccular sensory epithelium; hair (sensory) cells and supporting cells (Fig. 12). The hair cells are basically columnar but are slightly narrowed apically, possibly due to the expansion of the adjacent supporting cells at this level. Projecting from the luminal surface of each hair cell is a bundle of sensory hairs composed of numerous stereocilia and a single kinocilium (Figs. 12, 13, 14&15). Each hair bundle is planted in a cuticular plate which is a homogenous structure occupying the apical region of each hair cell (Figs. 12&16). Cellular organelles are abundant in the sensory hair cells; mitochondria are the most abundant, especially in the supra-nuclear part of the cells (Figs. 12&18). Junctional complexes, joining the hair cells with each other or with the adjacent supporting cells, are observed at the apical region of the sensory epithelial cells (Fig. 12).

A number of apocrine-like secretions seemed to be extruded from the apical surface of the sensory epithelium into the saccular lumen (Figs. 12&14). The extrusions appeared to have the same electron density as the subjacent apical cytoplasm, and seem to contain ribosome-like particles. Small empty vesicles, which appear to be liberated from multi-vesicular bodies and/or from the dilated microvilli of some supporting cells, are observed over the sensory epithelium (Figs. 14&15). These vesicles are restricted to the sensory region. Very few vesicles are also seen on the side of the transitional epithelium. Numerous spherules and electron dense granules associated with small vesicles seemed to be produced by the

sensory epithelial cells and scattered in the saccular lumen, especially at the subcupular meshwork zone of the otolithic membrane (Figs. 14&15).

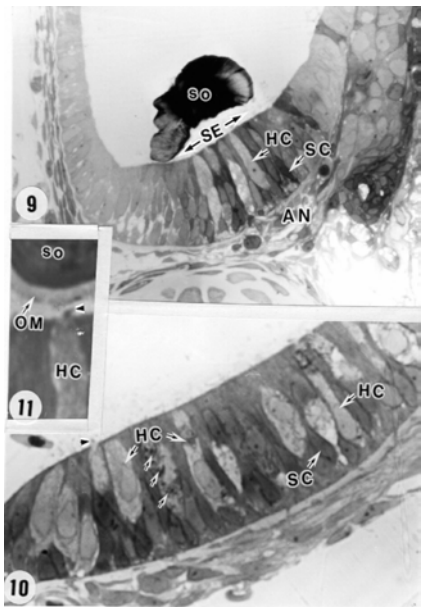


Fig. 9. *Perca fluviatilis*, one day after hatching. LM micrograph of a transverse section through the sacculus, showing differentiation of the saccular sensory epithelium (SE) into hair cells (HC) and supporting cells (SC). Note the saccular otolith (SO) covers the sensory epithelium region. AN, auditory nerve. X 220.

Fig. 10. *Perca fluviatilis*, one day after hatching. LM micrograph of a magnified part of the saccular sensory epithelium showing its differentiation into hair cells (HC) and supporting cells (SC). Note secretory materials (small arrows) in the cytoplasm of sensory hair cell. Arrowhead indicates protrusion-like cilia on the apical portion of a hair cell. X 700.

Fig. 11. *Perca fluviatilis*, one day after hatching. LM micrograph of a magnified part of a region between the saccular sensory epithelium and its otolith (SO), showing the otolithic membrane (OM). Arrowhead indicates protrusion-like cilia or a sensory hair bundle projecting from a hair cell (HC). X 1,320.

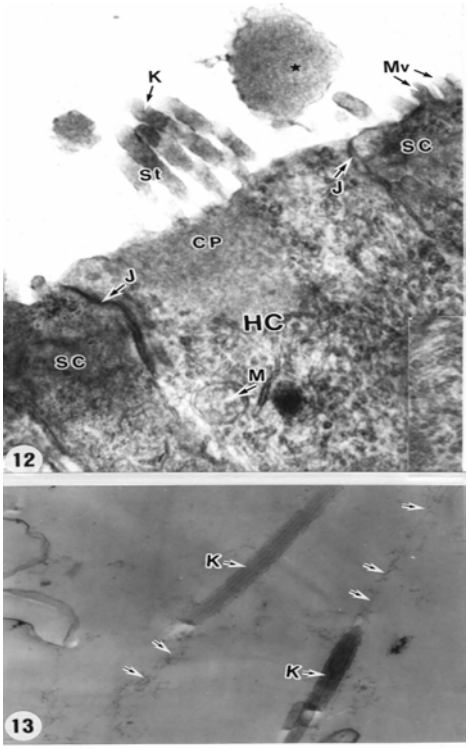
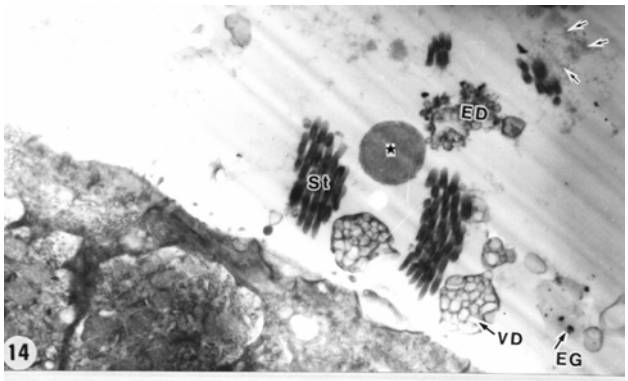
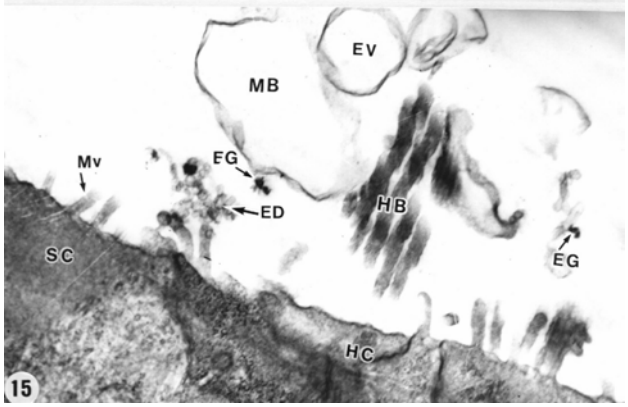


Fig. 12. *Perca fluviatilis*, one day after hatching. TEM micrograph of the saccular sensory epithelium, showing sensory hair bundle consisting of stereocilia (St) and single kinocilium (K), and cytoplasmic extrusion (star) over a hair cell and near the junctional complex (J). Microvilli (Mv) can be seen on the apical surface of a supporting cell (SC). M, mitochondria. X 28,000.

Fig. 13. *Perca fluviatilis*, one day after hatching. TEM micrograph of the subcupular meshwork zone, showing fine fibers or filaments (arrows) in contact with kinocilia (K). X 16,670.





Figs. 14 & 15. *Perca fluviatilis*, one day after hatching. TEM micrographs of the saccular sensory epithelium, showing an accumulation of various kinds of secretory materials over the saccular sensory epithelium; vesicular dilation (VD), cytoplasmic extrusion (star), empty vesicles (EV) released from multivesicular bodies (MB), numerous electron dense spherules (ED), and electron dense granules (EG). Arrows in figure 14. Indicate fibers or filaments near the otolith region. HB, sensory hair bundle. Fig. 14: X 10,000; Fig. 15: X 30,000.

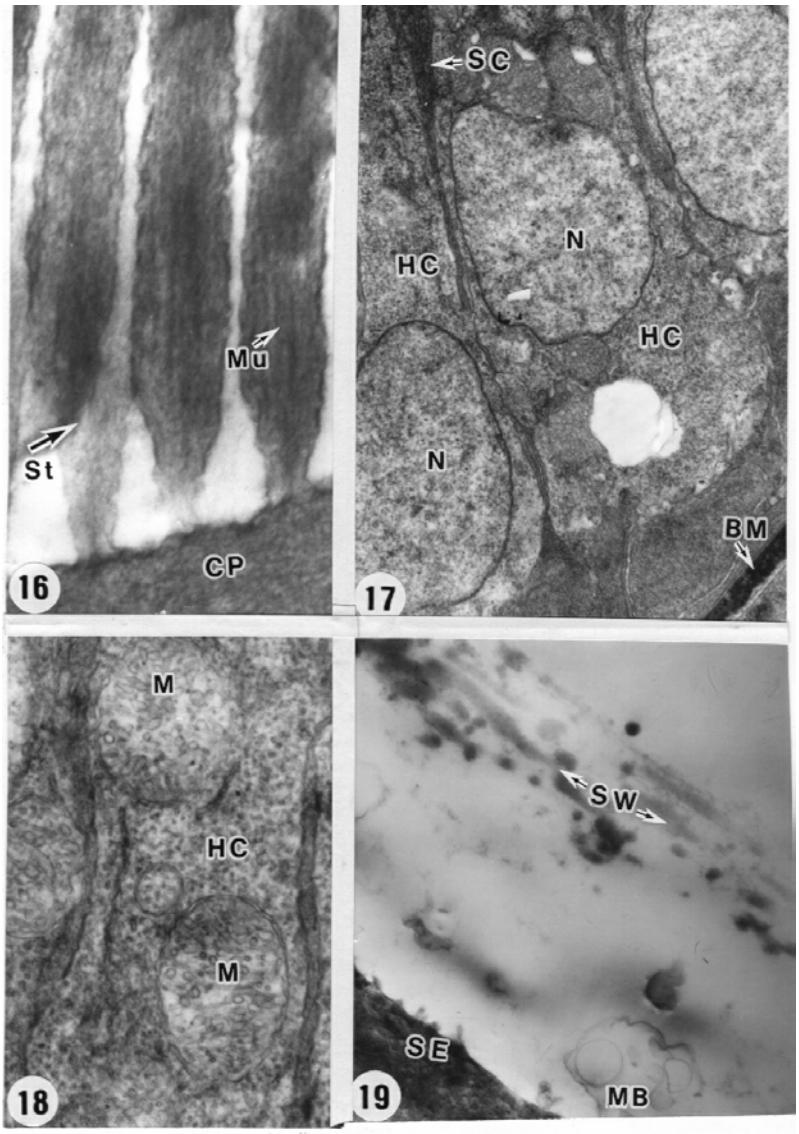


Fig. 16. *Perca fluviatilis*, one day after hatching. TEM micrograph of a sensory hair bundle, showing the stereocilia (St) which contain fine microtubules (Mu). Note the stereocilia are planted in the cuticular plate (CP) of the hair cell. X 50,000.

Fig. 17. *Perca fluviatilis*, one day after hatching. TEM micrograph of the saccular sensory epithelium, showing sensory hair cell (HC) and its nucleus (N). Note the hair cells do not reach the basement membrane (BM). X 7,500.

Fig. 18. *Perca fluviatilis*, one day after hatching. TEM micrograph of the saccular sensory epithelium, showing numerous mitochondria (M) in the supranuclear part of the hair cell (HC). X 18,330.

Fig. 19. *Perca fluviatilis*, one day after hatching. TEM micrograph of an accumulation of secretory materials forming the subcupular meshwork (SW) of the otolithic membrane. X 9,750.

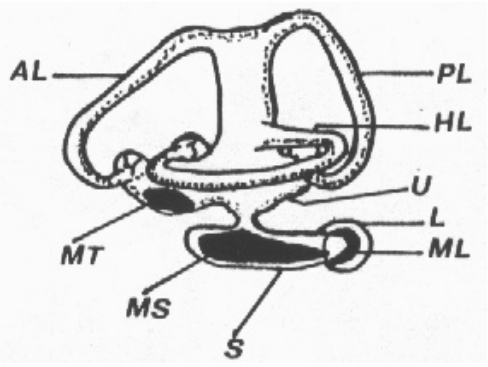


Fig. A: Diagram illustrating the membranous labyrinth in the teleost. AL, anterior canal; HL, horizontal canal; L, lagena; ML, macula lagena; MS, macula sacculus; MT, macula utriculus; PL, posterior canal; S, sacculus; U, utriculus.

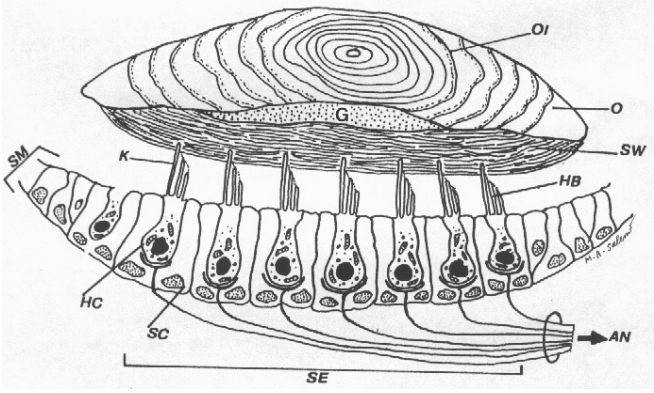


Fig. B: Artist's conception of relationship between otolith, otolithic membrane, and sensory epithelium. The figure represents a cross section through the middle of the otolith and the sensory epithelium. The otolithic membrane lies between the otolith (O) and the sensory epithelium (SE), consists of upper gelatinous layer (G) and the lower subcupular meshwork (SW). The former covers only the sensory region, while the subsupular meshwork covers the entire surface of the otolith and also most of the saccular macula (SM). The hair bundles (HB) seem to embed into the subsupular meshwork. AN, auditory nerve; HC, hair cell; K, kinocilium; OI, otolith increment; SC, supporting cell.

DISCUSSION

Although the overall pattern of development is similar amongst the vertebrates, the initial development of the auditory vesicle of teleosts begins at different times depending on the species and/or environmental parameters such as water temperature. In the present study, the auditory vesicle is formed at 6 days after fertilization in embryos kept at 15-17°C, whereas the vesicle appeared 7 days after fertilization at 18-20°C in the *Opsanus tau* (Tracy 1959). In comparison, the auditory vesicles formed at 48 hours after fertilization in the herring embryos (kept at 7°C) (Von Noorden 1883) and at 15 hours after fertilization in the zebrafish embryos (kept at 28°C) (Waterman & Bell 1984).

Development of the vertebrate inner ear begins with the formation of the sacculus and utriculus, followed by formation of the vertical and horizontal semicircular canals (Sokolowski & Popper 1987). The development of the inner ear in the perch follows this general sequence with the sacculus forming at 7 days after fertilization and the utriculus at 10 days after fertilization, followed by the semicircular canals at 12 days after fertilization. A similar result was found in *Kryptopterus bicirrhis* (Jenkins 1977), *Opsanus tau* (Sokolowski & Popper 1987) and in *Plecoglossus altivelis* (Salem & Omura 1998). This pattern of development is also the same in frogs (Villy 1890) and lizards (Landmann 1972). The early appearance of the sacculus before the utriculus and semicircular canals in some fishes and in the present study, suggests that the auditory sense is established earlier than the sense of equilibrium. However, in other teleost species, such as *Paralichthys olivaceus* (Kawamura & Ishida 1985), the equilibrium sense is established earlier than the auditory sense; this developmental differences probably depend on the behavioral and habitual requirements of the fish.

Numerous heavily-stained spherules with various sizes and shapes, representing the primordia or otoconia of the saccular otolith, were observed in the auditory vesicle lumen at 6 days after fertilization. Similar results have been recorded in the embryos of *Fundulus heteroclitus* (Radtke & Dean 1982), *Opsanus tau* (Sokolowski & Popper 1987), *Oreochromis niloticus* (Zhang & Runham 1989) and *Plecoglossus altivelis* (Salem & Omura 1998). These primordia or octoconia in different species of fishes are either separated or fused during the embryonic development (Brothers 1984). The otolith primordia (otoconia) in the perch, as seen with the light microscope, are first appeared as several separate particles or spherules. Brothers (1984) suggested that the type of fusion or separation, shape and size, in addition to the number of the primordia are a taxonomically useful characteristic. For example, in the Salmoniformes the otoconia are separate, while in the Atheriniformes the otoconia are grouped tightly. However, the otoliths initially develop at different times in the inner ear of different species. The saccular otolith in the perch appeared in developing in embryos 7 days post-fertilization. In comparison, in the herring (Von Noorden 1883) and killifish (Radtke & Dean 1982) the saccular otolith appeared at 48 hours and 72 hours after fertilization respectively, while it appeared in the embryonic zebra fish at 19 hours after fertilization (Waterman & Bell 1984). This disparity in the appearance time of the otoliths is presumed to be a species-specific feature.

In the perch of the present investigation, the stato-acoustic (VIII th) ganglion appeared before the differentiation of the saccular sensory epithelium. This result is similar to that found in the brown trout *Salmo trutta fario* (Beccera & Anadon 1993) and zebrafish *Danio rario* (Haddon & Lewis 1996). It is possible to suppose that the stato-acoustic ganglion is necessary to start differentiation of the saccular sensory cells. Studies of denervated otocysts in chick embryos, however, have shown that in the cochlea development of stereocilia is not directed by neurons (Corwin & Cotanche 1989). The pattern of innervation in mammals may be also dependent on attractant fields produced by sensory cells (Van De Water *et al.* 1989).

Accordingly, it is possible that in fish, sensory cell differentiation can occur without nerve influences, although the present observations are compatible with the opposite hypothesis. Further experimental applications are necessary to show whether or not the stato-acoustic ganglion (nerve fibres) is required for sensory cell differentiation in fishes.

It is known that the otoliths attach to the sensory epithelium by the otolithic membrane. Although the latter in bony fishes has been treated as a relatively familiar structure throughout the literature, its fine structural features are still limited. This probably due to the discreteness of its organic phase as well as the technical difficulties involved in preparing sections of the mineralized otolith. In general, the otolithic membrane in teleosts, including the present study, consists of an upper gelatinous layer and lower subcupular meshwork (Fig. B). Dunkelberger *et al.* (1980) in *Fundulus heteroclitus* and Zhang (1992) in *Oreochromis niloticus* have been observed that the gelatinous layer covers only the sensory epithelium region of the saccular macula while the subcupular meshwork covers both sensory and non-sensory regions. The otolithic membrane has been considered to be an apparatus for mechanoreception of gravitational forces, i.e. it transfers the mechanical energy from the otolith to the sensory hairs of the macular cells. Changes in gravity and linear acceleration produce a sliding motion of the otolith which stimulates the sensory macular cells (Dale 1976). On the other hand, structural observations suggest that the gelatinous layer of fish may act as a buffering zone between the otolith and the sensory hairs (Dunkelberger *et al.* 1980).

In vertebrates other than fishes, a few studies have suggested that the otolithic membrane may participate in otolith formation. Marco *et al.* (1971) indicated that in the guinea pig, the central parts of the otoliths and the two zones of the otolithic membrane were similar in morphology and that otolithic membrane material penetrated through fissures in the otolith. These findings led the authors to believe that otoliths were generated within the otolithic membrane.

Electron microscopic observations showed that, fine fibres or filaments from the subcupular zone are projected into the otolith, and some of these fibres or filaments made a connection or attachment with the distal end of the kinocilia of the sensory hair cells (see Figs. 13&14). As suggested by Dunkelberger *et al.* (1980), these fibres of the subcupular layer are incorporated into the otolith of juvenile *Fundulus heteroclitus* and probably contribute to the formation of the organic matrix of the otolith. This is probably the case of the perch. The attachment of these fibres to the kinocilia and stereocilia provides a mechanical link between the otolithic membrane and hair cell bundles of the sensory cells. This, coupled with attachment of the otolithic membrane to the otolith, establishes an arrangement whereby rotational movements of the otolith during vibratory stimulation could be transmitted as shearing forces to the ciliary bundles of the sensory cells (Jenkins 1979). Similar attachments, at least with the kinocilia, have been also reported in reptiles and birds (Dohlman 1971).

The present observations showed various kinds of secretory materials over the saccular sensory epithelium. These secretions, which seemed to be produced by the sensory epithelial cells of the saccular macula, include; vesicular dilations, cytoplasmic extrusions, multivesicular bodies associated with empty vesicles, numerous spherules and electron dense granules. The vesicular dilations, containing small vesicles, appeared to be detached and free in the endolymphatic lumen. Such dilations containing vesicles are probably identical to the veils that observed in the otolithic organs of pigeons (Dohlman 1971) and clinchillas (Lindmann 1973). According to Dohlman (1971), veils seemed to be secreted from microvilli of the supporting cells and it gradually packed together into the gelatinous layer of the otolithic membrane. If this is also the case of the perch, the gelatinous layer might be derived from the vesicular dilation of microvilli of the supporting cells.

Multivesicular bodies associated with numerous empty vesicles were observed within the subcupular meshwork zone, suggesting that they have an important role in the formation

of the otolithic membrane and/or the otolith. Such a result agrees with that found in *Oreochromis niloticus* (Zhang 1992). It has also been reported by Dale (1976) that the subcupular meshwork of the otolithic membrane of *Gradus morhua* seemed to be consisted of the same sort of the empty vesicles. Saitoh & Yamada (1989) also suggested that the vesicles in the sacculus of *Oreochromis niloticus* could be incorporated into the subcupular meshwork, which is responsible for otolith protein matrix formation (Dunkelberger *et al.* 1980). Similar structures were also observed and considered to contribute to the organic matrix of otoconia in frogs (Harda 1972) and rats (Salamat *et al.* 1980).

In the perch, numerous spherules and electron dense granules associated with small vesicles are found over the saccular macula and also scattered in the otolithic membrane region. The chemical nature of these spherules remains unknown. Saitoh & Yamada (1989) presumed that such spherules and electron dense granules contain the precursor materials of the organic matrix of the otolith, and also suggested that these materials are incorporated into the subcupular meshwork, which later becomes an organic constituent of the otolith matrix. Abundance of mitochondria, particularly at the apical regions, is observed in the saccular sensory epithelial cells of the perch. Such abundance of mitochondria was believed to be responsible for supplying calcium for otolith growth (Mugiya 1974). Previous studies on the otolith growth have reported that otolith increments (growth rings) are formed daily in various marine and freshwater fishes (Pannella 1971). In contrast, the daily otolith increments are not always produced in some species (Wild & Foreman 1980) and at some ages (Pannella 1971). The present investigation showed that the saccular otolith of the perch possessed one incremental layer at 12 days after fertilization (hatching day) (under photoperiod: 12 h light - 12 h dark). Meanwhile, in the sockeye salmon, the first daily increment appeared after hatching (Marshall & Parker 1982). Moreover, the initial formation or deposition of the otolith increments were appeared at 11 days after fertilization in the killifish (Radtke & Dean 1982), and at 6 days after hatching in the anchovy (Methot & Kramer 1979). Accordingly, the development of the daily otolith increments either in the embryos or larvae is presumed to be species-specific (Sokolowski 1986), although it may be affected by environmental factors such as photoperiod, temperature and feeding (Campana & Neilson 1982). Mugiya (1987) indicated that photoperiod was the major factor controlling the periodicity of otolith increment formation in young tilapia and rainbow trout embryos, respectively. However, Radtke & Dean (1982) showed that mummichog embryos incubated under constant light (24 h) had the same number of otolith increments as under 12 h light-12 h dark, and that those under constant dark (24 h) had a lower number of otolith increments. Neilson & Geen (1982) have reared chinook salmon (*Oncorhynchus tshawytscha*), free-swimming fry and alevins, in constant light and total darkness, respectively and noticed clear daily otolith increment production in all cases. Brothers (1978) regarded temperature fluctuation as the key factor to the timing of otolith increment deposition in temperate stream fishes. While, Neilson & Geen (1982) found a positive influence of feeding frequency on the number of otolith increments in juvenile chinook salmon. It seems finally that, effect of photoperiod, temperature and feeding frequency on the formation of otolith daily increments are still conflicting and controversial in many teleost species.

It has been reported that the stereocilia are essential to the process of sensory transduction (Hudspeth 1983). The present electron microscopic observations showed differences in length of the stereocilia and also in length of the kinocilia in the ciliary bundles of sensory hair cells. Saunders & Dear (1983) mentioned that the bundles with long stereocili may be tuned to low frequencies, while bundles with shorter stereocilia may be tuned to higher frequencies. However, Platt & Popper (1984) proposed that the differences in the length of the ciliary bundles may be related to the nature of the stimulus applied to the ear fluid and tissues.

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الملخص العربى

تركيب وتطور الطلائية الحسية للكُييس وعلاقتها بنمو الحُصّية الأذنية فى سمكة القَرْخ (*Perca fluviatilis*)

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يتناول هذا البحث دراسة تركيب وتطور الطلائيه الحسية للكُييس فى الأذن الداخلية لسمكة القَرْخ وكذلك علاقة هذا التركيب الجنينى بنمو الحُصّية الأذنيه للكييس، وقد اعتمدت الدراسة على أخذ عينات مختلفة الأعمار فى المراحل الجنينيه، وتم تحضير سلسلة من القطاعات العرضية السميكة والرقيقة فى منطقة الأذن الداخلية وبالتحديد فى منطقة الكُييس sacculus وتم صباغتها وفحصها بالميكروسكوب الضوئى والإلكترونى، وقد أوضحت الدراسة ما يلى:

- عند 6 أيام بعد الإخصاب، بدأ ظهور الحويصلة السمعية وقد احتوت على كُرّيات صغيرة يعتقد أنها طلائع الحُصّيه الأذنيه otolith primordia .

- بدأ ظهورالبقعة الحسية للكُييس saccular macula بعد 7 أيام من الإخصاب، وكذلك الحُصّيه الأذنيه . saccular otolith وأيضا تكوين العقدة العصبيةstato-acoustic ganglion والتى أشارت إلى بداية تأسيس العصب السمعى، وظهور هذه العقدة فى مرحلة مبكرة قد يكون ضرورى لبداية تميز المناطق الحسية الموجودة بالبقعة الحسية للكُييس.

- حدث انغماد للبقعة الحسية إلى الداخل وكذلك ترتيب خلاياها فى وضع رأسى بعد 8 و 10 أيام من الإخصاب، أما بعد 12 يوم من الإخصاب لوحظ تميز تدريجى لخلايا الطلائية الحسية كما لوحظ أيضا نمو وكبر حجم الحُصّيه الأذنيه وتكوين حلقة واحدة من حلقات النمو otolith increment.

- عند عمر يوم بعد الفقس فقد تميزت بوضوح الطلائيه الحسية للكُييس إلى خلايا شُعيرية حسية وخلايا دعامية، وتحمل كل خلية شُعيرية حزمة شعيرية حسية على سطحها العلوى. ولوحظ أيضاً وجود كمية كبيرة من المواد الإفرازية فوق هذه الطلائية والتى يعتقد ان تكون لها علاقة وثيقة ودور فعال فى تكون الحُصّيه الأذنيه والغشاء الحُصّي otolithic membrane وكذلك طبقات النموالموجوده بالحُصّيه incremental layers.

- فى ضوء النتائج التى تم التوصل اليها يمكن القول بأن هناك خلايا متخصصة داخل الطلائيه الحسية للكُييس تقوم بافراز مواد مختلفة فى الشكل والنوع ، وهذه المواد الافرازية تكون المصدر الرئيسى لتكوين الحُصّيه الأذنيه والغشاء الحُصّي وكذلك طبقات النمو الموجوده بالحُصّيه أثناء المراحل الجنينية.