

Calcified structures have different ontogenic origins and properties. Therefore, in order to appreciate the potential uses and limitations of otoliths, scales and skeletal tissue for sclerochronological studies it is important to understand the nature and formation of increments within these different CS. In this chapter, we describe the function, morphology and structure for the different CS. Further, we review the current understanding of accretion processes and how these are influenced by internal and external factors.

A. Otoliths

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1. Description and function

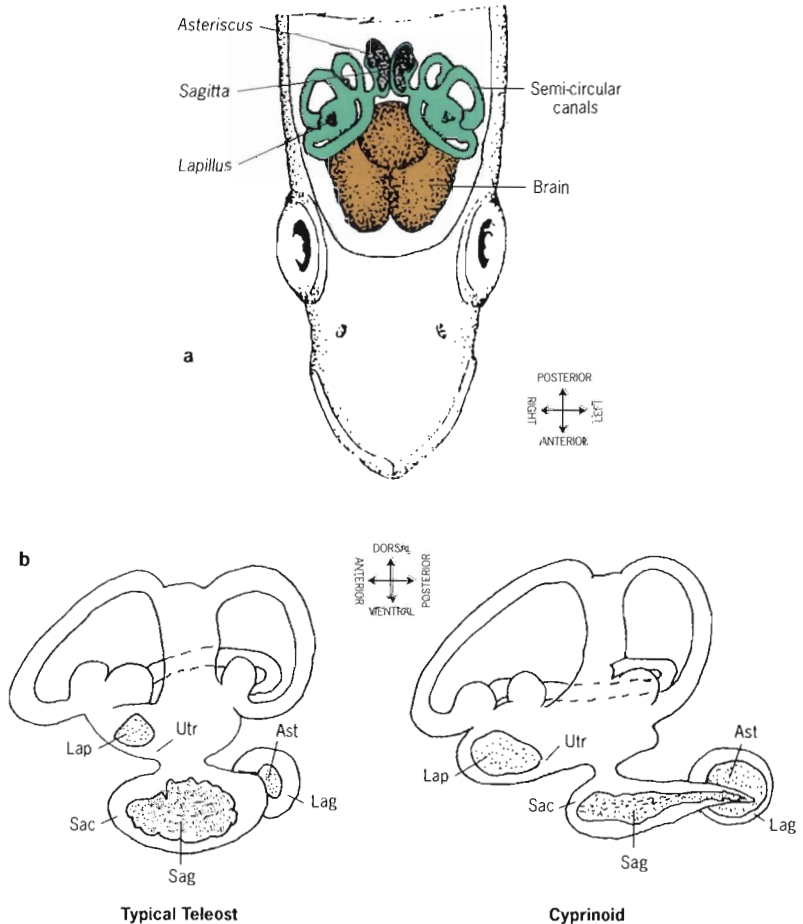
The inner ear, which is found in all jawed Vertebrates, functions both as an auditory system that detects sound waves and a vestibular system which detects linear and angular accelerations, enabling the organisms to maintain balance. In fish, the inner ear is a paired structure embedded in the cranium on either side of the head close to the midbrain. Each ear is a complicated structure of canals, sacs and ducts filled with endolymph, a fluid with special viscous properties (fig. II.A.1). The gross anatomy of these labyrinths and the structure of the labyrinthine mechanoreceptor organs are known from many fish species (Lowenstein, 1971). Teleosts have three semi-circular canals arranged orthogonally to each other, which detect angular accelerations. The canals open into a series of expanded interconnected chambers or otic sacs that contain a sensory tissue, the *macula*, that detects both linear accelerations and sound.

In Osteichthyan species there are three such otic sacs, each containing a calcareous structure, an otolith, that acts as a mechanoreceptor stimulating the kinocilia ("hair" cells) of the *macula*. The three otic sacs are the *sacculus*, *utricle* and *lagena*, which contain the *sagitta*, *lapillus* and *asteriscus* otoliths, respectively (fig. II.A.1). Each otolith is fixed over the *macula* by an otolithic membrane, into which sensory cilia project. According to Dunkelberger *et al.* (1980), the otolithic membrane consists of two zones: a structured gelatinous zone that covers the sensory region of the *macula*, which usually exhibits a reticulated or honeycomb architecture, and the sub-cupular zone, which consists of very loose networks of fibres covering sensory and non-sensory regions of the *macula*. The gelatinous zone extends from the otolith surface to the tips of the sensory hairs and its primary function is probably that of

mechanoreception. The lumen of the entire system is filled with endolymph. In species of Ostariophysii the swimbladder is used to enhance auditory stimulation of the inner ear (Popper & Fay, 1993).

Figure II.A.1
Position of the otoliths within the inner ear of Teleost fish (modified from Secor *et al.*, 1992).
a) Dorsal view of the vestibular apparatus in a typical Teleost species. The top of the cranium is cut away (frontal section).
b) Otoliths within the labyrinth systems of typical Teleost and Ostariophysian fishes.

Ast = asteriscus;
Lag = lagena (vestibule);
Lap = lapillus;
Sac = sacculus (vestibule);
Sag = sagitta;
Utr = utriculus (vestibule).



1.1. Description

The otoliths of the three otic sacs differ in size and shape (fig. II.A.2). Differences in otolith shape tend to reflect phylogeny and development, although there is considerable inter- and intra-specific variation (fig. II.A.3). Inter-specific differences in shape appear to be due to both genetic and environmental influences (Lombarte & Leonart, 1993; Nolf, 1995; Totres *et al.*, 2000). Due to their inter-specific variation in shape, otoliths have been found to be useful in taxonomy (Hechr, 1979), as well as permitting the study of food webs from partially digested remains (Surer & Morel, 1996; Olsson & North, 1997; Watanabe & Saito, 1998; Alonso *et al.*, 1999, *inter alia*). Similarly, otoliths from archaeological and paleontological finds have also been

used in the reconstruction of paleoenvironments and paleofauna (Nolf, 1995). Otolith morphometrics have also been used in species identification and to study geographical variations in populations and stocks of fish (Messieh *et al.*, 1989; Castonguay *et al.*, 1991; Campana & Caselman, 1993; Friedland & Reddin, 1994).

Figure II.A.2
The three otoliths
of *Vinciguerria nimbaria*
(Photichthyidae). S, sagitta;
L, lapillus; A, asteriscus.
Scale bar = 300 μ m (from
Tomás & Panfili, 2000).

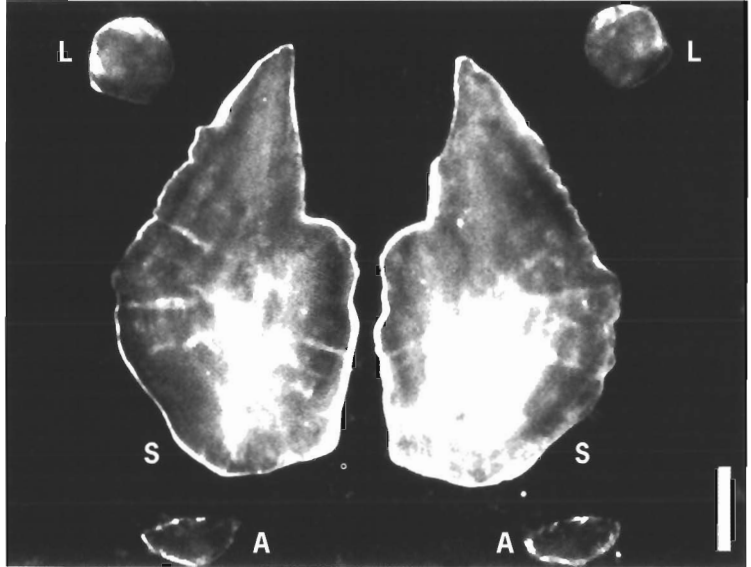
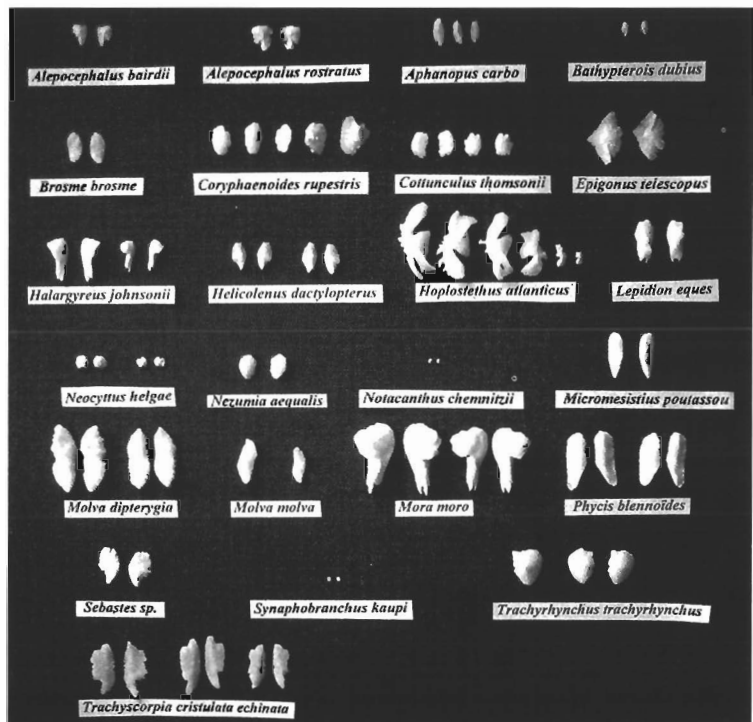


Figure II.A.3
Examples of various otolith
shapes from deep water
species (photo V. Allain).
Scale bar = 10 mm.

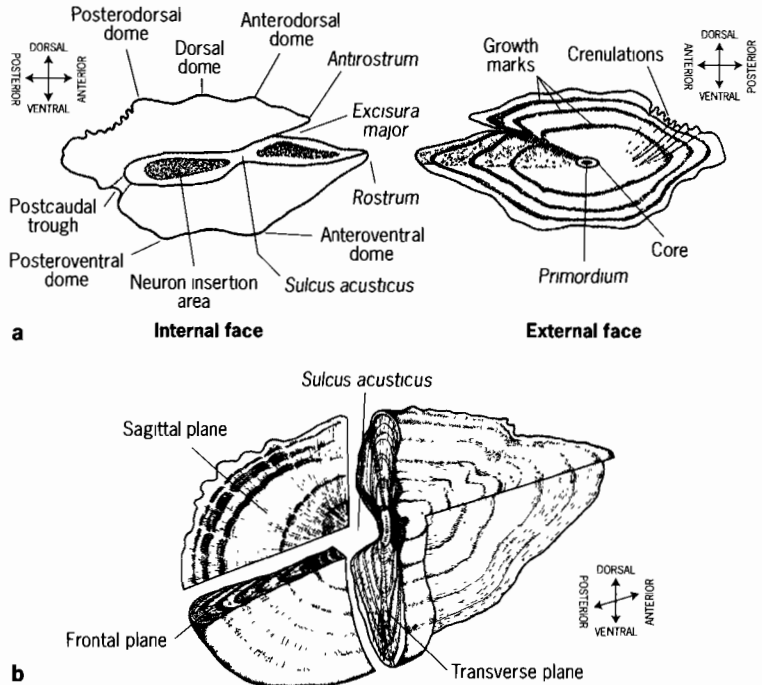


In most species the *sagitta* is the largest otolith and is most often used in age estimation. However, the *asteriscus* is the largest otolith in Ostariophysean species (Adams, 1940). Most studies of otolith formation have focused on the *sagitta* and *sacculus*. In the literature, the term “otolith” is often used to describe any one of the three pairs, generally the *sagitta*, but it is important to define this in any study.

Otoliths are generally laterally compressed and left-right symmetrical, except in flatfish and catfish. Details of the terminology utilised in describing otolith morphology are given in figure II.A.4a. An otolith has three planes of orientation, following those of the fish; sagittal, frontal and transverse (fig. II.A.4b). This orientation must be defined carefully in describing any otolith preparation, and reference should always be made to the standard terminology (e.g. transverse, sagittal, frontal sections). The proximal face of the *sagitta* has a groove, the *sulcus acusticus* (fig. II.A.4 a, b), which allows contact with a sensory epithelium (*macula*) of the *sacculus* (Dunkelberger *et al.*, 1980; Fay, 1980; Platt & Popper, 1981). A typical *sagitta* is elliptical on its sagittal plane, is compressed in its internal-external axis, with a convex proximal face and a concave distal face, and a main axis of growth oriented in the antero-posterior direction (fig. II.A.4). However, in several epipelagic and pelagic fish such as tunas, Istioforids, dolphin-fish, Cyprinidae and deep water species dorsal and ventral sides of the otoliths are asymmetrical, displaying a butterfly shape.

Figure II.A.4
A cross section through a typical *sagitta* illustrating the component parts (modified from Pannella, 1980).

a) Internal and external faces of a typical *sagitta*.
b) The three planes of orientation of a typical *sagitta*.



Otoliths are formed extracellularly from the crystallisation of the aragonite form of calcium carbonate onto an organic matrix template composed largely of a keratin-like protein, otolin, which is rich in aspartate and glutamate residues (Degens *et al.*, 1969; Watabe *et al.*, 1982; Morales-Nin, 1987a). The otolith grows or accretes by the addition of concentric layers of proteins and calcium carbonate, resulting in a structure somewhat comparable to that of an onion (chap. III.C).

1.2. Function

Fish labyrinths are involved in the maintenance of equilibrium and have nervous cells that are sensitive to pressure, gravity, angular movement and sound vibration (Grassé, 1958; Lowenstein, 1971; Blacker, 1974). The *pars superior* of the labyrinth (semi-circular canals and *utricle*) deals with postural information whilst the *pars inferior* (*sacculus* and *lagena*) is the sound receptor (fig. II.A.1). Teleost otoliths are similar to, but larger than, the otoconia of other Vertebrates. The otoliths are involved in mechanoreception, acting as electromechanical sound and displacement transducers that convert shear forces into electrical impulses by distorting the kinocilia of the nervous end-organ or *macula* in the fish inner ear (Popper & Hoxter, 1981). Relative motion between the sensory epithelium and the otolith bends the ciliary bundles and stimulates the eighth cranial nerve. The otoliths add mass to the gelatinous layer of the three otic sacs, increasing their sensitivity to gravitational and other linear acceleration forces (Ross & Pote, 1984). The *sulcus acusticus* of the otolith has a direct relationship with the *macula* of the vestibular epithelium, which is directly connected with the auditory nerve (Grassé, 1958). The receptor systems are rather different for Ostariophysean species, in which the internal ear is in contact with the swimbladder through a complex of bones known as the Weber complex (Grassé, 1958).

It has been hypothesised that sound reaches the fish ear via two different pathways. Because the fish's body is approximately the same density of water, it moves with the water in response to an impinging sound field. The otolith, however, is denser than the rest of the body, and so moves with a different amplitude and phase from the sensory *macula* and the body. Thus, the sound source directly stimulates the inner ear. In addition, because the swimbladder contains gas less dense than the body, the walls of the swimbladder vibrate. This produces indirect stimulation through otolith displacement (Popper & Lu, 2000).

2. Periodic increments

Otoliths exhibit a range of incremental structures that are often formed regularly over time scales ranging from sub-diurnal to annual. Unlike skeletal calcium, which may be mobilized for homeostasis

(Simkiss, 1974), otoliths do not appear to be subject to mineral resorption except under extreme stress (Mugiya & Uchimura, 1989). Consequently, otoliths appear to be highly suitable for age estimation. Fish age estimation depends on visible changes in otolith growth. The growth patterns of most interest are at four levels of resolution:

- primary increments, permitting a resolution of days;
- seasonal zones, permitting a resolution of several months or a growth season;
- annual increments, permitting a resolution of years;
- discontinuities in the otolith (ultra)structure, which correspond to various stresses that were not necessarily regular during the life history of the individual.

The mechanisms which produce these visible patterns are slightly different, although at the operational level they are the result of variations in the relative calcium and protein content of the increments or zones (Dannevig, 1956; Morales-Nin, 1987a).

2.1. Primary increments

Primary increments are formed from the successive deposition of a mineral-rich and a matrix-rich, mineral-deficient layer around a core (Watabe *et al.*, 1982; Morales-Nin, 1987a; Mugiya, 1987; Zhang & Runham, 1992a). Various names have been given to the two layers that form these primary increments. A review of otolith terminology presented at the first international symposium on otolith research proposed the terms L- and D-zones (Kalish *et al.*, 1995), for the mineral- and matrix-rich layers respectively. These terms refer to the bipartite appearance of the increments, L- and D-zones appearing light and dark respectively when viewed under transmitted light (fig. II.A.5a, b). The difference in the chemical composition of the two zones also leads to their different appearance under scanning electron microscopy following acid etching. The L-zone is rich in calcium carbonate and appears elevated in SEM whereas the D-zone is richer in protein and poorer in calcium and appears like a ridge in SEM (fig. II.A.5c, d, e). This terminology will therefore be used in the following review.

Pannella (1971; 1974) first discovered primary increments in otoliths and suggested that they were deposited daily. The large literature on daily increments has led many researchers to infer that primary increments can be assumed to be formed daily. However, such an assumption is invalid for a number of reasons. Otolith increment deposition may not be daily or be easily discernible in all species (Geffen, 1982; McGurk, 1984; Al-Hossaini & Pitcher, 1988; Morales-Nin, 1992). Inter-observer comparisons have shown that otolith structures are often interpreted differently by different readers (Campana & Mokness, 1991). Primary increments may not be deposited daily until some time after hatching (Geffen, 1987).

Sub-daily increments and discontinuities in the increment record may also occur (Campana & Neilson, 1985). The daily deposition of increments generally appears to cease in the adult and/or juvenile life history stages of long-lived fish (Pannella, 1971, 1980). In some cases this apparent cessation in the daily periodicity might be related to the formation of very narrow growth increments below the detection limit of the light microscope (Morales-Nin, 1988; Morales-Nin & Ralston, 1990). However, ultrastructural investigations have also demonstrated that primary increments are not deposited daily in some species (Volk *et al.*, 1995). Clearly then, the interpretation of microstructural growth patterns in wild fish requires an understanding of the physiological process and regulation of otolith accretion and of the environmental factors that influence them (Campana & Neilson, 1985). For otolith primary increments to be of use in age estimation, the processes involved in their regulation must either be synchronized to cyclical environmental events or possess an endogenous circadian rhythm, entrained to a diel environmental cycle (Geffen, 1987). In addition, increment formation must be independent of

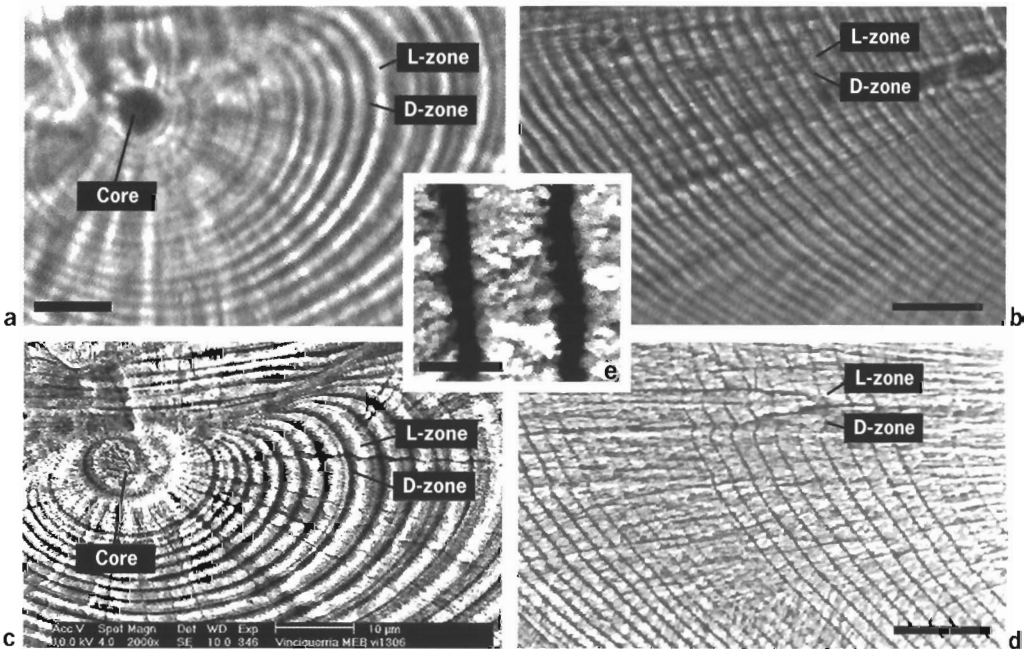


Figure II.A.5 - A transverse thin section of the otolith of *Vinciguerria nimbaria* (Photichthyidae). The primary increments composed of L- and D-zones are clearly visible.

a) Detail of the core area under transmission light microscopy. Scale bar = 10 μm (photo J. Panfili).

b) Detail of the adult growth area under transmission light microscopy. Scale bar = 10 μm (photo J. Tomás).

c) Detail of the core area after acid etching under SEM. Scale bar = 10 μm (photo L. Marec & E. Dabas).

d) Detail of the adult growth area after acid etching under SEM. Scale bar = 10 μm (photo J. Tomás).

e) Detail of the primary increments after acid etching, under SEM. The L-zone is rich in aragonite crystals whereas the D-zone corresponds to deep grooves. One primary increment is equal to 1 L-zone + 1 D-zone. The figure shows one complete L-zone and 2 complete D-zones. Scale bar = 1 μm (photo L. Marec & E. Dabas).

somatic growth. Experiments have shown that otoliths continue to accrete even when somatic growth has naturally ceased (Brothers, 1981; Wright *et al.*, 1990; Mugiya & Tanaka, 1992) or has been artificially restricted (Mosegaard *et al.*, 1988). This continuity may be related to differences between the growth of sensory systems such as the inner ear and other parts of the body.

Primary increments are only visible at high magnification (light microscopy or electron microscopy). They vary in size from less than 1 μm to 12 μm (Pannella, 1974). The width of the D-zone is always less than 1 μm (around 200 to 500 nm) whereas the width of the L-zone is more variable (from around 0.4 μm to 10 μm). However, because otoliths do not grow uniformly the increment width will also depend on the *radius* along which it is measured and how the otolith is sectioned (chap. III.C). Sub-daily increments may be laid down particularly during periods of fast growth. These structures can generally be differentiated from daily increments because they tend to be less well-defined and distinct than daily increments (Campana, 1992).

2.1.1. *Primordium*

The otolith develops from one or more partially calcified *primordia* exocytosed by epithelium cells in the inner ear (Mann *et al.*, 1983). These cores have been termed primordial granules and they are the primary or initial components of the *primordium*. In *sagittae* the granules may be composed of vaterite, whereas the rest of the *primordium* is typically aragonite. Examples of these different types of *primordium* are shown in figure II.A.6. The *primordium* can be either circular, elongated or multiple, depending on the species. In the case of multiple *primordia* these coalesce to form the core of the otolith (fig. II.A.6). The term "*nucleus*" has also been used to describe the core region of the otolith, although this term is not recommended since it has also been used to describe a much larger central area of the otolith (see glossary).

2.1.2. First ring

Otoliths develop in the later part of the egg stage in fish. For some time after formation, the otoliths grow continuously and mostly without obvious incremental accretion. The time at which incremental deposition begins differs from species to species. This point in time is often (conveniently) marked by a distinctive feature, usually a prominent check (fig. II.A.6d, e). There is considerable confusion about the terminology used to name this feature, as well as about its biological significance and timing. This first increment may not have the same physiological basis in all species. However, there are practical advantages in standardising counting procedures using this as a reference point (Neilson & Geen, 1982).

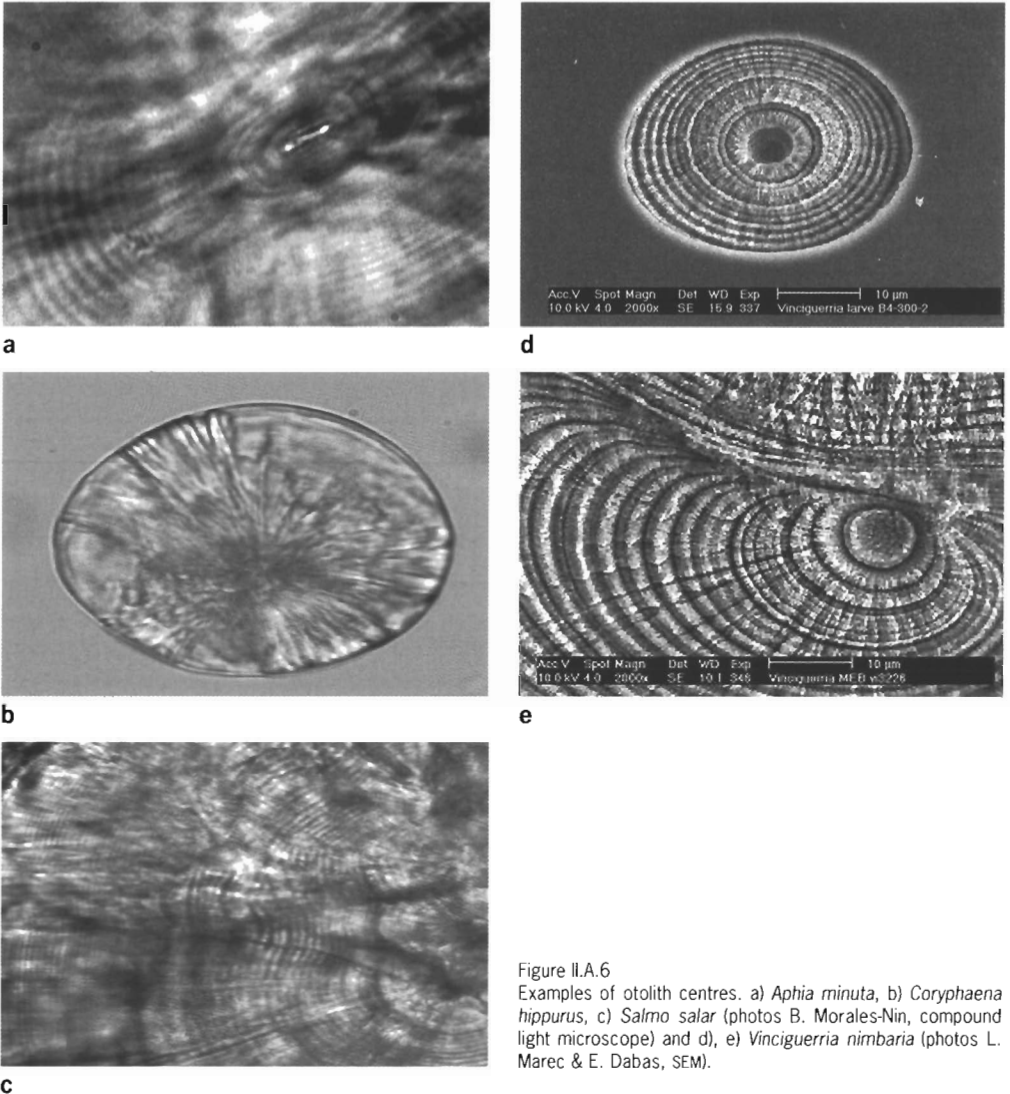


Figure II.A.6
Examples of otolith centres. a) *Aphia minuta*, b) *Coryphaena hippurus*, c) *Salmo salar* (photos B. Morales-Nin, compound light microscope) and d), e) *Vinciguerria nimbaria* (photos L. Marec & E. Dabas, SEM).

In several species increments have been noted which are most likely to have been formed before hatching (fig. II.A.7). It has not been demonstrated conclusively whether or not these increments are true structures or merely optical artefacts. The opacity of pre-hatch increments is rather different from post-hatch primary increments (fig. II.A.7). These structures are most apparent in young larvae with small otoliths. Hatching is not really a developmental stage in fish larvae, since a single batch of siblings may hatch at different stages of morphological development. In many species the first increment or otolith check may

be formed on the day of hatching, and may be properly termed a hatch ring. However, in other species, the first increment may be formed in association with a particular developmental stage, irrespective of whether the embryo has hatched or not. For example, in *Solea solea* the increment that is formed when the mouth opens is more prominent than any preceding increments, and this increment is used as the reference point for counting (Lagardère, 1989). In *Clupea harengus*, the first prominent increment is formed well after hatching and towards the end of the yolk-sac stage (Geffen, 1982). In both species the timing of this increment varies, and depends on developmental rates. In juvenile otoliths of the Siluriforme species *Hoplosternum littorale* and *Megalechis thoracata*, the hatch check is well differentiated (Ponton *et al.*, 2001) (fig. II.A.7). In these species the hatched larvae are well developed and the otoliths appear to exhibit some increments before the hatch check (fig. II.A.7). In a number of other species, the transition to exogenous feeding is represented by a prominent increment. Examples of first increments cited in the literature are presented in table II.A.1.

Figure II.A.7
Examples of otolith hatch check in *Megalechis thoracata*. The black arrows indicate the hatch check and the white arrow the multiple *primordia* cores. The hatch check has been localised after a validation experiment. Some increments are also present inside the check and then before birth. Scale bar = 50 μ m (photo D. Ponton).

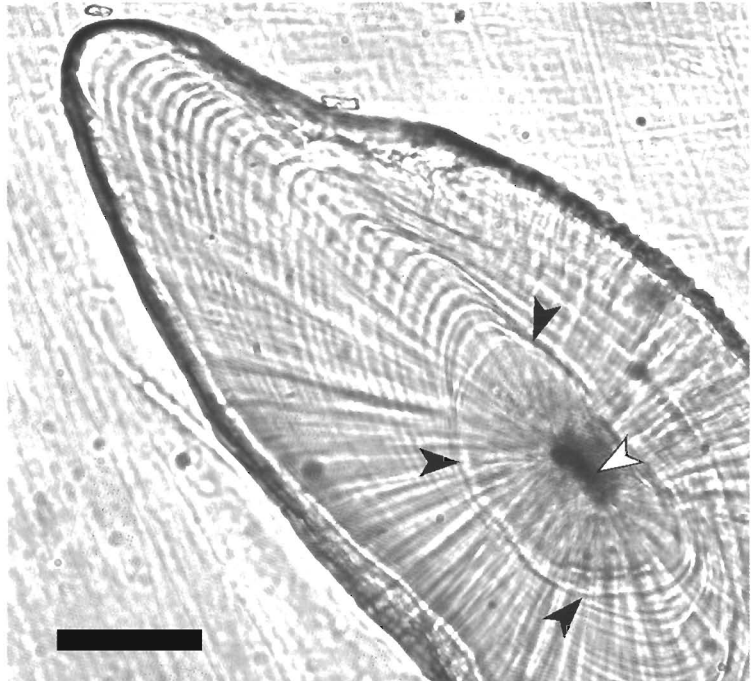


Table II.A.1. - References on otolith structures associated with life history events. Otolith structures refer to those found in *sagittae* except those denoted with an asterisk, which were *lapilli* (from Wright *et al.*, 1998).

| Species | Term used by author | Alternative terminology | Related life history event (timing) | Reference |
|----------------------------------|----------------------------|----------------------------|---|------------------------------------|
| <i>Anmodytes marinus</i> | Yolk-sac absorption check | | Yolk-sac absorption | (Wright, 1993) |
| | Secondary growth centre | Accessory <i>primordia</i> | Metamorphosis & settlement | (Wright, 1993) |
| <i>Anguilla anguilla</i> | First ring | First check | End of yolk-sac phase | (Lecomte-Finiger, 1992) |
| | Deep groove | | First ingestion | (Lecomte-Finiger, 1992) |
| | Transition ring | Transition ring | End of metamorphosis | (Lecomte-Finiger, 1992) |
| <i>Chromis atripectoralis*</i> | Check | Settlement check | Time of settlement | (Thorrold & Milcich, 1990) |
| <i>Clupea harengus</i> | First heavy ring | First check | End of yolk-sac phase | (Geffen, 1982) |
| | Hatch check | First check | End of yolk-sac phase | (Moksness, 1992) |
| | Hatch check | First check | Between hatch and yolk-sac absorption | (Hoei, 1997) |
| <i>Engraulis encrasicolus</i> | Check ring | First check | End of yolk-sac phase | (Palomera <i>et al.</i> , 1988) |
| <i>Gadus morhua</i> | Nuclear check | First check | Hatching | (Bolz & Lough, 1983) |
| | Yolk-sac check | | End of yolk-sac | (Bolz & Lough, 1983) |
| | Hatch check | First check | Time of hatching | (Campana, 1989) |
| | Check | First check | Hatching | (Geffen & Nash, 1995) |
| <i>Halichoeres tenuispinis</i> | Hatch check | First check | Hatching | (Kishiro & Nakazono, 1991) |
| <i>Hoplosternum littorale*</i> | Hatch check | First check | Hatching | (Ponton <i>et al.</i> , 2001) |
| <i>Melanogrammus aeglefinus*</i> | Nuclear check | First check | Hatching | (Bolz & Lough, 1983) |
| | Yolk-sac check | | End of yolk-sac phase | (Bolz & Lough, 1983) |
| | Hatch check (two) | | Hatching | (Campana, 1989) |
| <i>Merluccius merluccius</i> | Accessory <i>primordia</i> | Accessory <i>primordia</i> | Metamorphosis & settlement | (Morales-Nin & Aldebert, 1997) |
| <i>Microstomus pacificus</i> | Accessory <i>primordia</i> | Accessory <i>primordia</i> | Metamorphosis (eye migration to settlement) | (Toole <i>et al.</i> , 1993) |
| Myctophidae (55 species) | Accessory <i>primordia</i> | Accessory <i>primordia</i> | Transforming larvae | (Linkowski, 1991) |
| <i>Oncorhynchus keta</i> | Hatching check | First check | Hatching | (Volk <i>et al.</i> , 1984) |
| | Seawater transfer ring | Transition ring | Seawater transfer | (Volk <i>et al.</i> , 1984) |
| <i>Oncorhynchus gorbuscha</i> | Transition zone | Transition zone | Seawater transfer | (Volk <i>et al.</i> , 1995) |
| | Emergence check | Emergence check | Emergence from bottom | (Mortensen & Carls, 1995) |
| <i>Oncorhynchus nerka</i> | Hatch ring | First check | Hatching | (Marshall & Parker, 1982) |
| | First feeding check | | Critical period | (Marshall & Parker, 1982) |
| <i>Oreochromis niloticus</i> | Hatching check | First check | One day after hatching | (Zhang & Runham, 1992a) |
| <i>Pleuronectes americanus</i> | Accessory growth centres | Accessory <i>primordia</i> | During and after metamorphosis | (Jearld <i>et al.</i> , 1993) |
| | Secondary growth centre | Accessory <i>primordia</i> | Changing habitat Metamorphosis | (Sogard, 1991) |
| <i>Pleuronectes platessa</i> | Accessory <i>primordia</i> | Accessory <i>primordia</i> | Metamorphosis | (Al-Hossaini <i>et al.</i> , 1989) |
| | Accessory <i>primordia</i> | Accessory <i>primordia</i> | Settlement | (Karakiri & Westernhagen, 1989) |

| | | | | |
|--------------------------------------|----------------------------|----------------------------|-----------------------|--------------------------------|
| <i>Pollachius virens</i> | Hatch check | First check | After hatching | (Campana, 1989) |
| <i>Pomacentrus coelestis</i> * | Check | Settlement check | Time of settlement | (Thorrold & Milicich, 1990) |
| <i>Scophthalmus maximus</i> | First heavy ring | First check | End of yolk-sac phase | (Geffen, 1982) |
| <i>Sebastes jordani</i> | Extrusion check | First check | Parturition | (Ralston <i>et al.</i> , 1996) |
| <i>Solea solea</i> | Hatch check | First check | Hatching | (Lagardère, 1989) |
| | Mouth opening check | | First feeding | (Lagardère, 1989) |
| | Yolk-sac exhaustion check | | Starved larvae | (Lagardère, 1989) |
| <i>Theragra chalcogramma</i> | Check | Check | Physiological changes | (Nishimura, 1993) |
| <i>Trachyrhynchus trachyrhynchus</i> | Accessory <i>primordia</i> | Accessory <i>primordia</i> | Migration | (Massuti <i>et al.</i> , 1995) |
| <i>Vinciguerria nimbata</i> | Hatch check | First check | Hatching | (Tomás & Panfili, 2000) |

Salmonid otoliths display a number of prominent increments, each related to a different developmental event. The earliest increments which surround the entire set of *primordia* coincide with vascularisation of the yolk sac and the development of red blood cells. There is a prominent increment which marks hatching, and another prominent increment which marks emergence from the substratum. Under hatchery conditions, checks which correspond to first feeding have also been observed. For ecological studies, age estimations are based on counts from the emergence mark.

2.2. Accessory growth centres

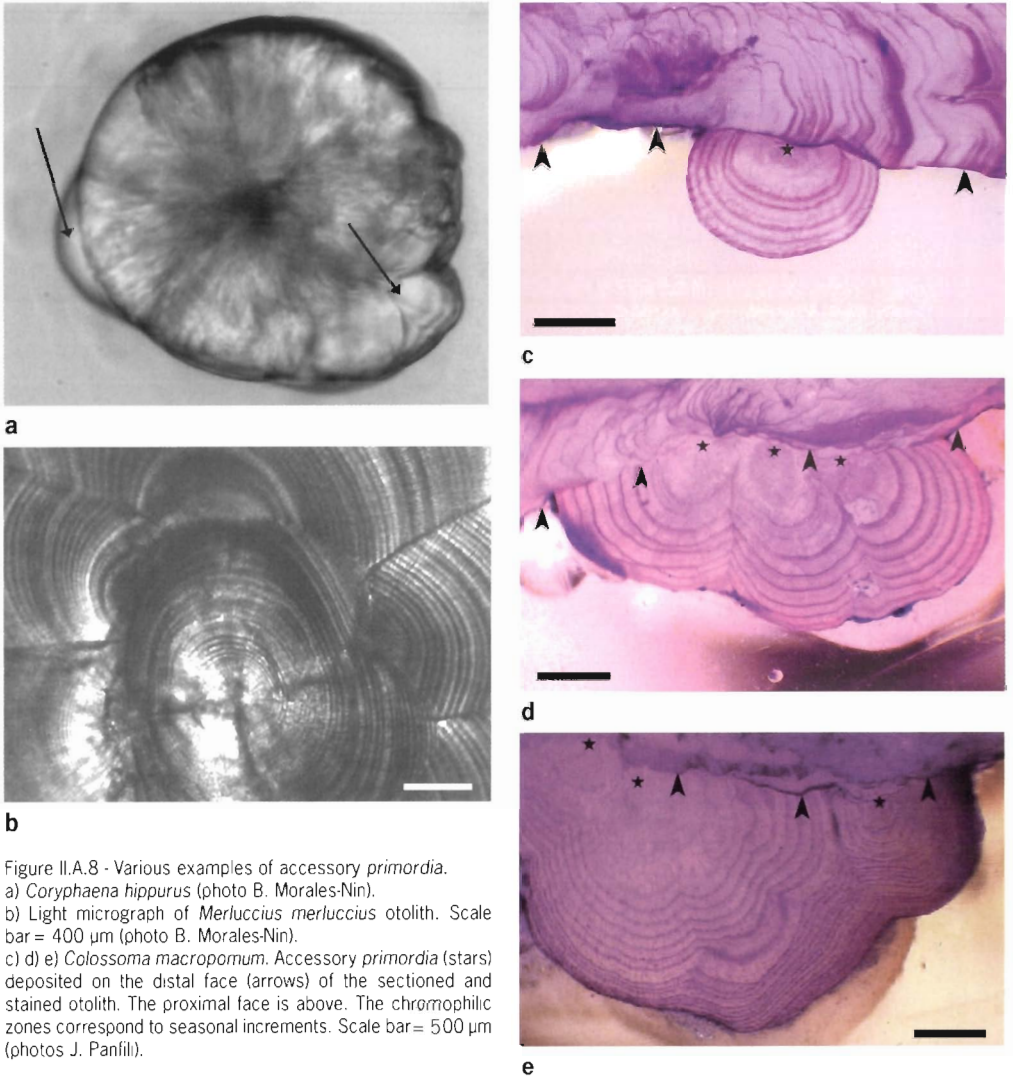
During the larval phase most otoliths continue to accrete around the *primordium*. However, in the otoliths of many species additional planes of growth are formed at later developmental stages and from these new series of increments emanate. These new planes in otolith growth result from the development of accessory growth centres. Accessory growth centres are particularly common in the largest otolith (*sagitta*) of most species and the *lapillus* of Cyprinids or *asteriscus* of Ostariophyseans. Accessory growth centres are often referred to as accessory *primordia*. However, the term accessory growth centre is preferred, in order to avoid confusion with *primordia* which contain multiple primordial granules. Figure II.A.8a, b shows examples of accessory *primordia* in two marine species.

Since otolith shape influences sensitivity to sound frequencies (Popper & Hoxter, 1981), the formation of accessory growth centres may be related to a transition in physiology, habitat or behaviour. These structures are found in many species that undergo a marked habitat change at the transition from the larval to the juvenile stage. Examples of these structures in juvenile fish are shown in table II.A.1. Accessory

primordia can also be found on the otoliths of adults. For example, they are very common on the distal face of the *asteriscus* of *Colossoma macropomum*, where they appear as autonomous structures which grow with the otolith, also showing seasonal increments (fig. II.A.8c, d, e).

2.3. Seasonal and annual increments

Seasonal increments, also termed seasonal zones, marks, rings or *annuli* (see glossary), are often distinguishable on otoliths. They are often visible in tropical as well as temperate species. These zones can be visible in both whole (untreated) otoliths and/or after some form of preparation (chap. VIII). The two main types of seasonal zones have



different opacities. Under transmitted light the opaque zone is dark and the translucent zone is bright, and under reflected light the opaque zone is bright and the translucent zone is dark (fig. II.A.9). In addition to their macroscopic appearance the two seasonal zones differ with respect to the width of primary increments, the thickness and size of aragonite crystals (Morales-Nin, 1987a), the frequency of growth discontinuities and organic layers (Mugiya *et al.*, 1985), the ratio of calcium carbonate to protein matrix (Casselman, 1974, 1982, 1987; Mugiya, 1984), and elemental ratios (Casselman, 1982, 1983; Kalish, 1989, 1991a). It is the combination of these factors that leads to differences in the optical densities of the two zones. Seasonal zones can reach a few hundred microns in width and are therefore visible to the naked eye or at low magnifications (10x – 40x). The difference in the organic matrix content of the two zones can be underlined after burning which turns the organic matrix a rich opaque brown, or after staining, which colours the chromophilic organic zones (fig. II.A.10 and chap. VIII).

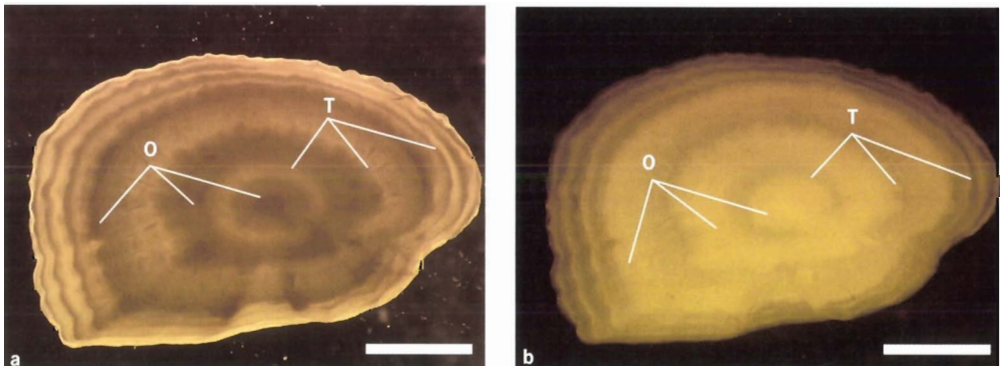
Annual increments, also termed annual marks, rings or *annuli*, are often interpreted when taking into account the succession of several seasonal increments. Most temperate and many tropical species exhibit annual increments, usually comprising opaque and translucent zones. However, in some tropical species biannual growth increments have been reported, probably related to multi-annual changes in environmental and hydrological factors (Yosef & Casselman, 1995).

Figure II.A.9

Whole otolith of a plaice, *Pleuronectes platessa* (47 cm TL), showing opaque (O) and translucent (T) zones viewed under transmitted light (a) and under reflected light against dark background (b). The opaque zones are dark in transmitted light and bright in reflected light, and inverse for translucent zones. Scale bar = 2 mm (photos J. Panfili).

2.4. Lunar-related structures

A common feature of the otoliths of juvenile and adult fish is a pattern of thick increments separated by numerous less prominent increments. This pattern is usually repeating and has been hypothesised to reflect lunar cycles. The best examples of lunar patterns are seen in the otoliths of juvenile flatfish, but they have been described in a wide range of species. Presumed lunar patterns have been described in



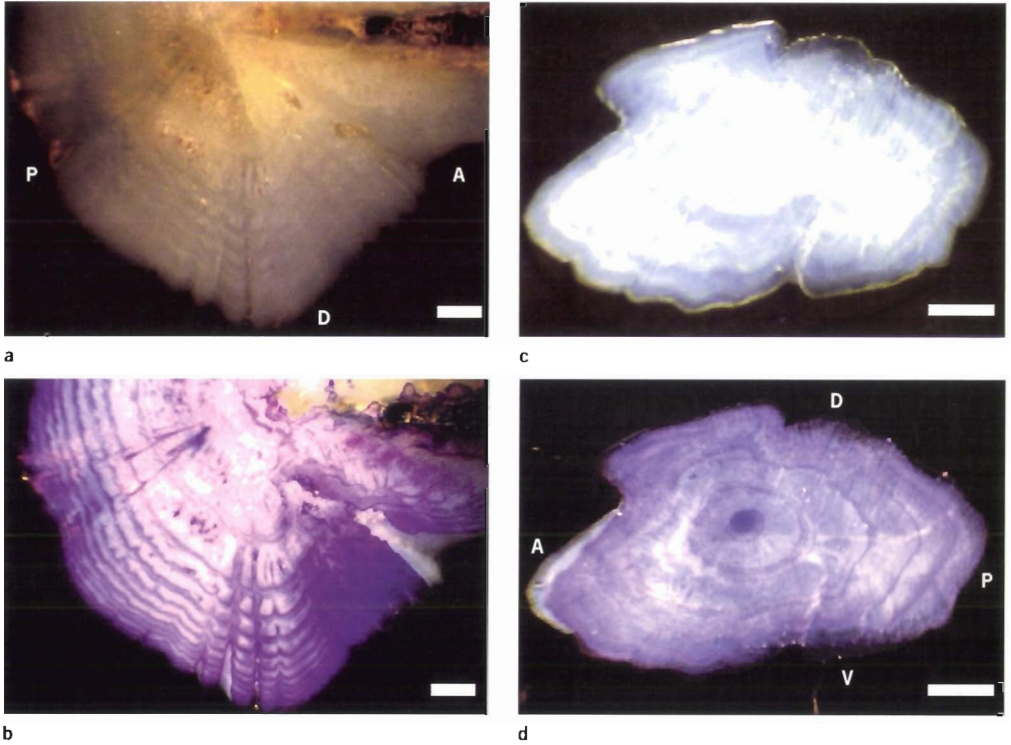


Figure II.A.10 - Enhancement of the otolith zones after staining. A, anterior; D, dorsal; P, posterior; V, ventral (photos J. Panfili). a) Sagittal section of an otolith (asteriscus) of *Colossoma macropomum* (Serrasalimidae, 63 cm SL). Central and dorsal areas observed in reflected light against a dark background. Scale bar = 1 mm. b) Sagittal section of the *Colossoma macropomum* otolith (a) after acid etching (EDTA) and staining with toluidine blue. Central and dorsal areas observed in reflected light against a dark background. The growth zones are enhanced. Scale bar = 1 mm. c) Sagittal section of an otolith of *Anguilla anguilla* (Anguillidae, 55 cm TL) observed in reflected light against a dark background. Scale bar = 500 μ m. d) Sagittal section of the *Anguilla anguilla* otolith (c) observed in reflected light against a dark background, after acid etching and staining with toluidine blue. Scale bar = 500 μ m.

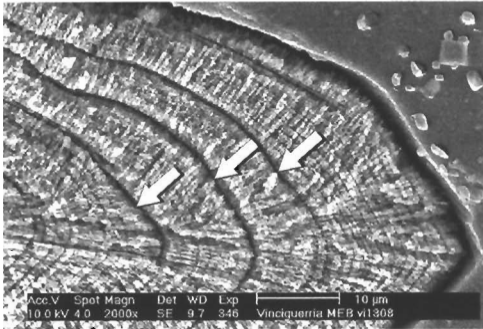
bathypelagic fish otoliths as well, bringing into question the actual cause of the patterns and what cyclical (physiological or behavioural) processes they may reflect.

The terminology used to refer to the presumed lunar pattern varies (Pannella, 1980). "Lunar pattern" usually refers to sets or sequences of increments, each beginning with a prominent, high-contrast, increment which is most often termed a "check" (discontinuity; see 2.5) regardless of the cause of formation. The check is followed by a set of lower-contrast increments. The widths of the L-zone of the check and subsequent increments are usually uniform. Several authors have made use of these repeating features for age determination, on the assumption that each sequence represents a 14-day lunar cycle. Fewer have attempted to discover the source of the environmental signal that

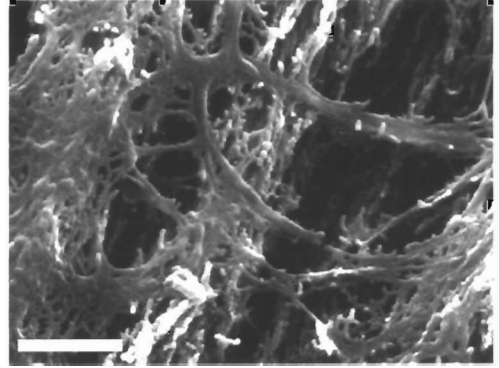
imprints the distinctive pattern. Campana (1987) described alternating patterns of high and low contrast increments, each containing either approximately seven or 14 increments. The formation of these patterns corresponded well to variations in tidal height and the lunar cycle. Geffen & Nash (1995) showed that the pattern in *Pleuronectes platessa* contained seven increments, and that the discontinuity which separated the normal increments coincided with dates half-way between spring and neap tides. Linkowski (1996) has also described a clear lunar pattern in the primary increment growth of four species of the genus *Hygophum* in the North Atlantic.

2.5. Structural discontinuities

Structural discontinuities, also known as checks, are breaks within the regular arrangement of the primary increments (Pannella, 1980; Campana & Neilson, 1985; Gauldie, 1987; Morales-Nin, 1987a; Gauldie & Nelson, 1988). These may interrupt the succession of seasonal or daily increments in a cyclic or acyclic way. They can be distinguished under high or sometimes low magnification, usually after some preparation. The discontinuity affects the growth pattern or the direction of growth. They generally appear after acid etching as deep grooves in the otolith surface and under the microscope they appear as wide D-zones (fig. II.A.11a). The organic matrix is usually abundant in these discontinuities (fig. II.A.11b) (Morales-Nin, 1986b), which may be why they are often stainable after acid etching by certain histological dyes (Pannella, 1980). A discontinuity preceded by increments of decreasing width might correspond to a seasonal ring (fig. II.A.11c). Discontinuities are typical of all species and are probably induced by disturbances or stresses suffered by individuals in their biotope. Pannella (1980) proposed a classification for discontinuities (checks) according to their structures and presumed causes, although without much justification. However, research has demonstrated that certain discontinuities are related to developmental events, such as the change from pelagic to demersal life, or settlement in coral reef species. For example, rhythmic growth patterns and checks in *Merluccius capensis*, *M. paradoxus* and *Genypterus capensis* were found to be related to activity patterns and different life strategies (Morales-Nin, 1987b). As a discontinuity represents an interruption in growth of unknown duration, its interpretation can be ambiguous and may pose problems in making estimates of daily age (Campana & Neilson, 1985). When the duration of formation of a discontinuity is known, for example with a winter stress check, such structures can be useful for estimating annual age.



a



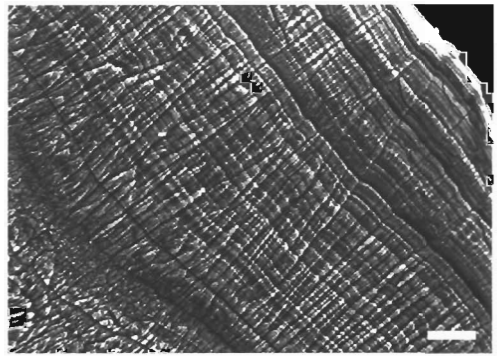
b

Figure II.A.11 - Discontinuities found within otoliths.

a) Discontinuities (arrows) in the regular arrangement of otolith primary increments of *Vinciguerria nimbaria* (Photichthyidae) (photo J. Panfili).

b) Scanning electron micrograph of a demineralised otolith of *Dicentrarchus labrax* showing the organic matrix and the transversal groups of fibres corresponding to discontinuities. Scale bar = 1 µm (photo B. Morales-Nin).

c) Scanning electron micrograph of a *Dicentrarchus labrax* demineralised otolith showing the decreasing increment width and the structural discontinuity corresponding to a seasonal increment. Scale bar = 10 µm (photo B. Morales-Nin).



c

2.6. Secondary growth zones

In many species the estimation of annual increments is made difficult by the presence of non-periodic “secondary” zones. This term applies to a range of non-seasonal zones characterised by different opacities and thickness. The two major types of secondary zones are false and split rings. False rings appear as translucent zones within an opaque zone. They are particularly common in the first year of otolith growth and in many cases are easily confused with the first annual increment (fig. II.A.12). For instance, depending on the spawning period, age-0 *Trachurus mediterraneus* presents four types of otoliths that differ in the presence, number or appearance of false rings (Karlou Riga, 2000). Split rings appear as double structures, almost as though they were composed of two unusually thin translucent bands separated by a very thin opaque band. In some species the annual increment is composed of multiple rings, with a narrow well-defined translucent zone followed by some very opaque material (fig. II.A.13).

Problems in distinguishing between secondary and true seasonal zonations is a major cause of age-reading errors. There are currently no objective criteria for identifying secondary structures, despite extensive reviews of this problem. Little is known about the causes of these secondary structures, although a number of factors including temperature, food intake and developmental transitions have been implicated in their formation.

Secondary growth structures are also present at the primary increment level in species with peculiar life histories, such as Myctophids and related species which start their life in the upper layers of the water column and later make diel migrations between deep water during the day and the surface at night. Secondary related daily increments have been described in three species of tropical Myctophids (Gartner, 1991) and in *Vinciguerria nimbaria* (Tomás & Panfili, 2000). These secondary increments appear to be sub-daily increments. Due to the possible variation in incremental structures discussed above, it is important to describe all structures recorded carefully according to the standard terminology (see glossary).

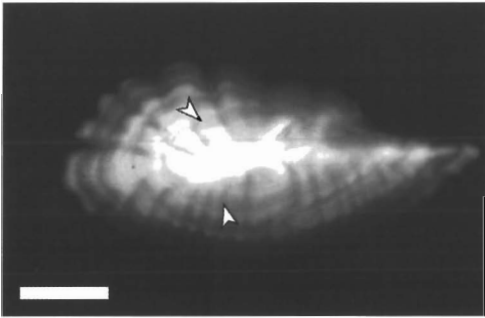
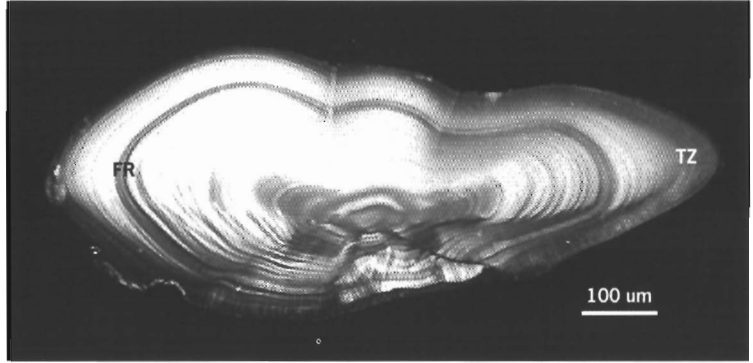
3. Regulation of incremental deposition

3.1. Exogenous influences on primary increment periodicity

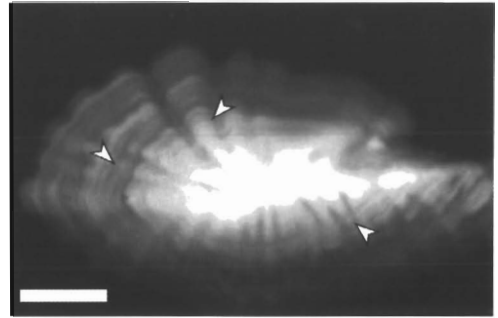
Several studies have examined the relationship between increment formation and specific environmental factors and a number of possible synchronising factors have been proposed. Pannella (1980) suggested that increment periodicity may be related to the number of peaks in feeding activity. Feeding frequency has been reported as influencing increment periodicity in some species, for example, *Oncorhynchus tshawytscha* (Neilson & Geen, 1982) and *Pleuronectes platessa* (Al-Hossaini & Pitcher, 1988), but not in others such as *Lepomis macrochirus* (Taubert & Coble, 1977), *Oncorhynchus nerka* (Marshall & Parker, 1982), *Platichthys stellatus* (Campana, 1983) and *Salmo salar* (Wright *et al.*, 1991). Moreover, starved fish often continue to deposit daily increments (Taubert & Coble, 1977; Marshall & Parker, 1982; Campana, 1983; Wright *et al.*, 1990). There thus appears to be little evidence to support Pannella's hypothesis of a relationship between increment periodicity and peaks in feeding activity.

Otolith growth is sensitive to temperature in a number of species (Brothers, 1981; Mosegaard *et al.*, 1988) and Brothers suggested that temperature fluctuations are a major influence on increment formation in temperate stream fish. Thermally-induced marks on otoliths demonstrate how strong and sudden temperature variations may disrupt otolith growth (Volk *et al.*, 1994). Gauldie & Nelson (1990a) proposed a carbonic anhydrase-regulated system for otolith formation.

Figure II.A.12
False ring (FR) in the sagitta
of a 1-year old whiting,
Merlangius merlangus.
A translucent zone (TZ)
of an annulus is also shown
for comparison.
Scale bar = 0.1 mm
(photo P.J. Wright).



a



b

Figure II.A.13
False rings.
Scale bar = 1 mm
(photos B. Morales-Nin).
a) Sagittal otolith
of *Trachurus trachurus*
capensis showing
the multiple zones
in the nuclear area.
b) Multiple growth zones
in a *Trachurus trachurus*
capensis otolith.

Such a chemical system would have temperature as its main external controlling factor. However, the role of the organic matrix in otolith formation is not well understood, and this might also be related to various external cues (chap. II.3.4).

Light-dark cycles appear to be necessary for daily increment formation in larval *Lepomis macrochir* (Taubert & Coble, 1977) and *Fundulus heteroclitus* (Radtke & Dean, 1982). Campana & Neilson (1985) suggested that such dependence on light-dark transitions may be mediated by age, as light-dark cycles appear to be essential for daily increment deposition in the larval but not the juvenile stages of *Porichthys notatus* (Campana, 1984). Many deep-sea fish exhibit microscopic increments with rhythmic groupings and a similar structure and thickness to those found in shallow-water fish, despite the absence of light and feeding daily rhythms (Gauldie, 1987; Lombarte & Morales-Nin, 1989; Gauldie, 1990; Morales-Nin *et al.*, 1996b). In these species, small variations in tidal currents along the slope or vertical migrations of planktophagous prey may act as a daily *Zeitgeber*.

In a review of environmental manipulation experiments Campana & Neilson (1985) suggested that the endogenous circadian rhythm controlling otolith accretion was entrained to photoperiod, but could be masked by sub-daily temperature cycles or feeding patterns. If increment periodicity is controlled by an endogenous circadian rhythm then increment deposition would be expected to continue in the absence of entraining stimuli, although the absence of an entraining stimulus would be expected to eventually lead to a divergence from a daily deposition rate. Several studies have shown a continued daily increment deposition rate in the absence of one potential entraining stimulus such as light-dark transition. Constant daily rates of increment formation have been reported in juvenile fish held under constant light (Campana, 1984), darkness (Radtke & Dean, 1982) and in the absence of cyclical variations in light, temperature or feeding frequency (Wright *et al.*, 1991). However, environmental manipulation experiments do not provide unambiguous experimental evidence of an endogenously regulated cycle of increment formation, since fish may have an endogenous feeding rhythm. Moreover, no study has demonstrated a divergence from a single increment per day, as might be predicted when there is no entraining stimulus, although this may reflect the short (<30 days) duration of these experiments.

Support for light-dark transitions as a sign for entrainment has come from ultrastructural and radio-labelling experiments. Tanaka *et al.* (1981) demonstrated that in *Tilapia nilotica*, the order of formation of the L- and D-zones was dependent on photoperiod, as a reversal of the light-dark cycle was found to induce a reversal in the order of the two layers. Using radiolabelled calcium (^{45}Ca) to study *in vivo* otolith calcification in *Carassius auratus*, Mugiya and coworkers (1981) found an apparent diel cycle in calcification that was associated with photoperiod. However, these experiments were flawed because no consideration was given to the possible effects of isotopic equilibration on ^{45}Ca incorporation. Nevertheless, later *in vivo* experiments, involving juvenile *Salmo salar* subjected to an isotopic equilibration period, did demonstrate that otolith calcification was entrained to dark-light transitions (Wright *et al.*, 1992). Radiolabelling experiments have also demonstrated diel cycles of both calcification and organic matrix formation, associated with photoperiod, within isolated *sacullae* (Mugiya, 1987).

3.2. Exogenous regulation of annual increment periodicity

At present the regulation of annual increment formation in otoliths is not well understood, although it is commonly assumed that seasonal zones are related to seasonality in somatic growth and environmental factors. One view is that seasonal variation in otolith formation is related to cyclical physiological changes in the fish such as the onset

of reproductive activity or the accelerated somatic growth that occurs in spring (Johnson, 1983; Fowler, 1990). An alternative suggestion is that the physiology of otolith formation is independent of other somatic and reproductive processes taking place within the fish, and is an independent physiological response to environmental variation (Loubens, 1978; Fowler & Doherty, 1992). Evidence for and against these hypotheses generally takes the form of correlations in the timing of the different processes and is usually too weak and insufficient to allow either hypothesis to be rejected. The formation of the zones in relation to reproductive activity is controversial, given that in several species the opaque zone coincides with the time of year when fish are reproductively active, while in others the formation of regular translucent zones has been related to maturity and spawning. However, zone formation is frequently seen in the juvenile stages of many fish species (Johnson, 1983; Fowler, 1990). Furthermore, it is difficult to envisage how reproductive activity could directly affect otolith composition since, although reproducing females have elevated plasma calcium concentrations, this takes the form of protein-bound calcium which will not affect calcium ion levels in the endolymph (Kalish, 1991a). As yet no experimental studies have been performed in order to distinguish between calcium and organic components in plasma and otolith formation.

The season of formation of opaque and translucent zones may change during development and in relation to geographical distribution. In *Gadus morhua* from the North Sea, for example, the opaque zone forms earlier towards the southern extremes of this species' range and becomes progressively later further north. Within each stock younger fish begin to lay down the opaque zone up to four months before older fish. Spawning occurs when the translucent zone is well into the process of formation. The temporal delay in opaque-zone formation increases with age (Williams & Bedford, 1974). Vianet *et al.* (1989) provide other examples of geographical differences in four Pleuronectiform species from Europe, in which the translucent zones are formed during the summer in the Mediterranean but in winter in Northern European waters. The time of translucent-zone formation in *Sebastes entomelas* from the U.S. Pacific coast has been found to vary with sex, geographical area and year (Pearson, 1996). In this species a link between temperature and translucent zone formation is apparent although other temperature-related factors, such as food availability or nutrient content of the prey, may also be important. Further evidence of temperature-related zone formation was found for several species of Acanthurids from eastern Australia (Choat & Axe, 1996). Recapture of tetracycline-marked fish showed that the formation of opaque zones corresponds to the rise in water temperature in the summer.

3.3. Influences on accretion rate

Temperature can enhance otolith accretion beyond the point at which somatic growth is adversely affected (Mosegaard *et al.*, 1988), although high temperatures can also have a negative effect on increment width (Gutiérrez & Morales-Nin, 1986; May & Jenkins, 1992; Ralston & Howard, 1995). Experiments have shown that otoliths continue to accrete even when somatic growth has ceased naturally (Wright, 1990; Mugiya & Tanaka, 1992) or has been artificially restricted (Mosegaard *et al.*, 1988). This continuity results in slow-growing individuals having relatively large otoliths. In order to explain this phenomenon, Secor & Dean (1989) suggested that otolith accretion may be determined by the interaction of two components: the daily periodicity of increment formation, which continues even during periods of no somatic growth, and an amplitudinal component that varies with somatic growth. However, a number of experimental studies have found that the increase in otolith accretion rate with temperature is much more similar to the increasing trend in metabolic rate than to the optimum curve of somatic growth rate (Mosegaard *et al.*, 1987; Mosegaard *et al.*, 1988; Hoff & Fuiman, 1993). Moreover, studies by Wright (1991a) and Yamamoto *et al.* (1998) have shown that individual differences in increment width correlated with resting metabolic rate rather than somatic growth. Mosegaard *et al.* (1988) suggested an isometric relationship between resting metabolic rate and otolith growth based on the relationship between changes in the otolith weight of groups of fish and the temperature at which they were held, and extrapolated from general temperature-resting metabolism relationships for fish. However, recent measurement of individual changes in oxygen consumption and increment size indicates that otolith accretion responds more conservatively to a change in temperature than in resting metabolic rate (Wright *et al.*, 2001). The specific dynamic action potential, the metabolic response associated with food intake, also appears to have an influence on otolith accretion rate (Fallon-Cousins, 1999). The process governing accretion rate thus appears to be related to components of the metabolic rate. Given the influence of these components on otolith accretion rate, periods of starvation would only be expected to lead to a gradual decline in increment widths. Evidence for such a response has been found in a number of experimental studies (Neilson & Geen, 1985; Eckmann & Rey, 1987; Molony & Choat, 1990; Umezawa & Tsukamoto, 1991; Bradford & Geen, 1992; Zhang & Runham, 1992a; Molony, 1996).

3.4. Physiological regulation of otolith formation at the sacculus level

Wilbur (1980) suggested that biomineralisation systems had three properties in common:

- all systems involve the transport of ions and provide supersaturated concentrations of ions (i.e. which exceed the solubility product) at the mineralising surface. This enables the formation of crystalline *nuclei* and the growth of crystals;
- a sufficiently alkaline pH must be maintained so that, once begun, mineralisation can continue;
- crystal formation is often intimately associated with organic material.

The observation that otolith increments are composed of a mineral-rich and a mineral-deficient zone (L- and D-zones, respectively) suggests that one or more of the above properties must vary. The periodic deposition of mineral-rich zones may therefore (i) be related to a diurnal physico-chemical limitation in crystallisation (involving either a lowering of the calcium and hydrogen carbonate ion concentration at the otolith surface or a decline in endolymph pH), or (ii) involve the organic matrix. An analogy with the mineralisation of mollusc shells suggests that either the insoluble organic matrix deposited in the mineral deficient layer (D zone) acts as a barrier to crystallisation or crystal growth-inhibiting compounds within the soluble organic matrix are secreted on to the mineral-deficient layer (Wheeler *et al.*, 1981; Wilbur & Saleuddin, 1983). These possible forms of regulation have been considered for otoliths during the past three decades.

The otolith is precipitated from the fluid of the endolymphatic sac of the inner ear. Otolith calcium carbonate is in the form of twinned aragonite, although abnormal crystalline otoliths are composed of calcite (Morales-Nin, 1985) or vaterite (Gauldie, 1986). Calcium reaches the endolymph primarily from the blood plasma (Kalish, 1989, 1990, 1991a; Wright *et al.*, 1992). Otolith calcification is rate-limited by the number of nucleation sites provided by the insoluble matrix (Crenshaw, 1982; Mann *et al.*, 1983) as well as by physico-chemical conditions at the otolith surface. The rate of insoluble matrix production will therefore be the ultimate determinant of the rate of otolith calcification (Saitoh & Yamada, 1989; Wright, 1990). This matrix is also a significant factor controlling the shape of the otolith (Degens *et al.*, 1969; Dunkelberger *et al.*, 1980; Mugiya, 1987; Gauldie, 1991, 1993; Zhang & Runham, 1992b; Payan *et al.*, 1999). As in mollusc shell, otoliths possess a soluble proteinaceous matrix that is capable of regulating the rate of mineral deposition (Wright, 1991b). Variations in the rate of production of this protein may therefore regulate the rate of mineralisation. The less soluble otolith matrix is composed of a collagen-type protein (Degens *et al.*, 1969). The matrix is denser in the early development phase and its amino acid composition changes with age (Morales-Nin, 1986a,b).

Investigations of isolated *sacullae* have indicated that active, regulated ionic transport occurs through the epithelia (fig. II.A.14b). Endolymph calcium ion concentration is influenced by intracellular active transport that is sensitive to plasma calcium concentration. Similarly, proton secretion through the *sacculus* is driven by an energy-dependent (Na-ATPase) mechanism that is sensitive to plasma pH (Payan *et al.*, 1999). Changes in plasma ion concentration would therefore be expected to have a direct effect on that of the endolymph. However, the precise mechanism by which plasma calcium and pH induce changes in the physico-chemical conditions at the otolith surface is not clear. This is because the sensory kinocilia bathed by the endolymph are sensitive to changes in Ca^{2+} concentrations well below the solubility product needed for calcification (Mugiya, 1987; Wright *et al.*, 1992). The seasonal variation in free Ca^{2+} ions in the endolymph of rainbow trout ranges from 65.4% of total calcium levels during fast growth to 79.1% during slow growth (Mugiya, 1966), which probably represents the range over which Ca^{2+} can vary without causing physiological malfunction of the neural mechanisms of the *macula* (Gauldie, 1990). It is thus necessary to explain how ion levels are elevated at the otolith surface above the background concentrations found in the endolymph. Proximo-distal gradients of ion concentration have been detected in the endolymph, a condition which will favour the biomineralisation process (fig. II.A.14b) (Payan *et al.*, 1999). Calcareous spherules have been observed in close association with the otolith surface of a number of fish species and these may be involved in elevating ion concentration at the otolith surface (Dale, 1976; Wright, 1990). These spherules are formed and secreted from the otolithic membrane and are transported to the surface of the otolith within the fibrous sub-cupular meshwork (Dale, 1976; Wright, 1990). Diurnal rhythmicity in otolith calcification may be mediated by a diel variation in plasma chemistry, as Mugiya (1984) and Wright *et al.* (1992) found a parallel diel decline in otolith calcification and total and free plasma calcium concentration. Mugiya (1984) also found a seasonal reversal in the rhythm of otolith calcification associated with a reversal in the diurnal plasma calcium cycle. However, similar cycles in plasma and endolymph composition in *Pleuronectes platessa* (Edeyer *et al.*, 2000) were not associated with changes in the ionic gradients within the endolymph (Payan *et al.*, 1999). Nevertheless, Wright *et al.* (1992) found that an induced depression in plasma calcium led to a net loss of calcium from the mineralising otolith increment, which indicates that calcium ion concentration at the otolith surface is sensitive to plasma concentration. While there may be a periodical ionic limitation to otolith calcification, however, this alone cannot explain reports of a diel variation in matrix secretion (Mugiya, 1987; Wright *et al.*, 1990) or the formation

of matrix-rich layers (Watabe *et al.*, 1982; Morales-Nin, 1987a; Mugiya, 1987). The distribution of matrix and mineral in the otolith appears to occur in two phases. The first is associated with the twinning plane of the basic aragonite crystal (Gauldie & Xhie, 1995). Twinning is a complex process (Bloss, 1971) which stabilises crystal polymorphism and increases the growth rate of the crystal (Smith, 1974; Davey *et al.*, 1993). The second phase of the matrix-mineral association appears in the form of the dense band of fibres that corresponds in size and orientation to the D-zone of the primary increment (Dunkelberger *et al.*, 1980; Morales-Nin, 1987a). This observation is consistent with the diel variation in insoluble matrix protein indicated by radio-labelling experiments (Mugiya, 1987). The two phases of the protein matrix may play different roles, the first being to

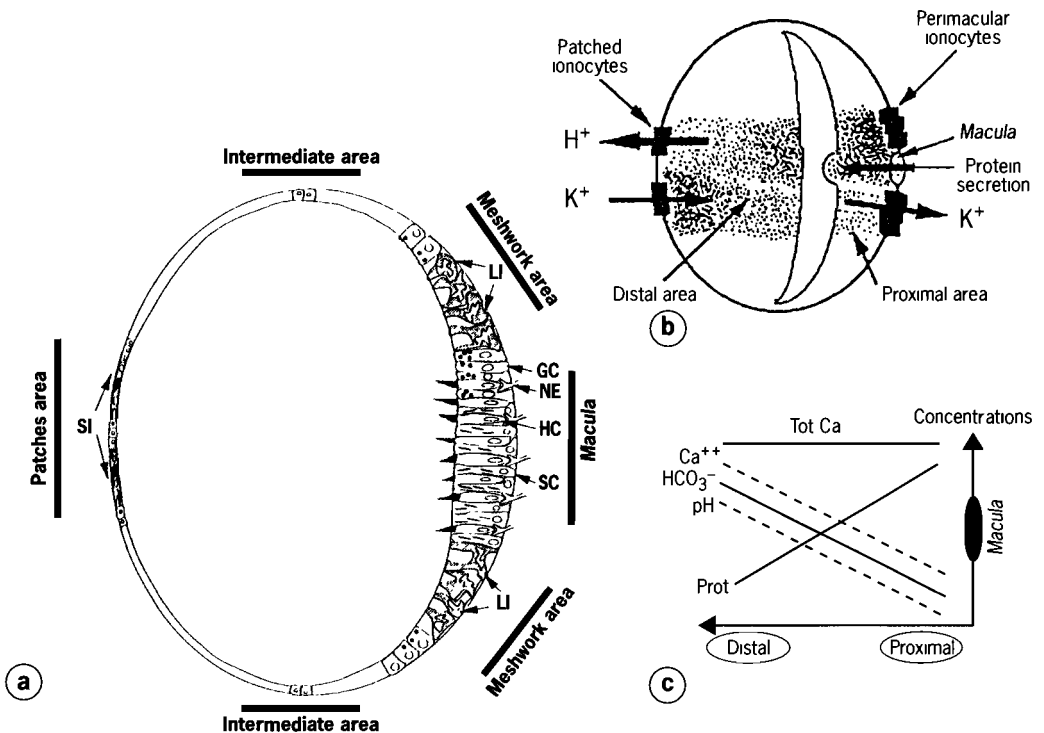


Figure II.A.14 - Schematic representation of the saccular epithelium (transverse section of a sacculus) of the inner ear of a Teleost and the hypothetical model of elemental transport across the epithelium (modified from Pisam *et al.*, 1998; Payan *et al.*, 1999). a) Map of the cell distribution within the saccular epithelium. The **macula** consists of hair cells (HC), which are in contact with nerve endings (NE), supporting cells (SC) and, at its periphery, granular cells (GC). It is surrounded by a "meshwork area" containing large ionocytes (LI). The "patches area" contains small ionocytes (SI).

b) Hypothetical model of elemental transport across the saccular epithelium. Note that overall movement of H^+ results in net excretion of H^+ .

c) Schematic representation of chemical concentrations in the proximal and distal zones. The Y-axis shows the concentrations while the X-axis shows the proximal-distal otolith axis. Proteins (Prot), total calcium (TotCa) and HCO_3^- concentration were directly measured, whereas Ca^{2+} and pH concentrations were estimated.

provide a template for crystal growth and the second to stabilize the otherwise soluble (Wright *et al.*, 1992) and thermo-dynamically unstable aragonite morph (Mann *et al.*, 1983; Gauldie & Xhie, 1995). It is therefore necessary to consider the regulation of both ion concentrations and matrix production in the periodic deposition of L- and D-zones. Given the correlation between otolith calcification and plasma ion concentration, the concentration of certain ions in the plasma may have a direct effect on cellular secretions of matrix or may covary with some other signalling factor. In addition, the calcification neurosecretory activity in the *macula* has a daily cycle which is related to the deposition of daily increments (Gauldie & Nelson, 1990b; Edeyer *et al.*, 2000). A number of recent studies have identified the function of the different regions within the saccular epithelium and the importance of these regions to otolith growth (Payan *et al.*, 1997; Pizam *et al.*, 1998; Payan *et al.*, 1999) (fig. II.A.14). The secretory cells are mostly located in the macular area. Within the endolymph, proteins are more concentrated in the proximal region, while total CO₂ is higher in the distal region (fig. II.A.14c).

In summary, the evidence to date indicates that the formation of the calcium carbonate-rich L-zone is influenced by intracellular active transport of calcium ions (Mugiya, 1986) and protons through the *sacculus* (Payan *et al.*, 1997; Payan *et al.*, 1999) which in turn are sensitive to plasma calcium concentration and pH (Wright *et al.*, 1992; Payan *et al.*, 1997). Secretion of the proteinaceous matrix varies diurnally with a peak during the formation of D-zone (Mugiya, 1987; Edeyer *et al.*, 2000). Production of the protein matrix template and the soluble protein inhibitor must also have a role in limiting the rate of mineral accretion during the formation of the L-zone.

3.5. Hormonal regulation of otolith formation

The mechanisms that determine periodic mineralisation are probably under endocrine control (Campana & Neilson, 1985; Mugiya, 1987), either directly or indirectly via metabolic influences (Geffen, 1983; Mosegaard *et al.*, 1988). Growth hormone (STH) may also be involved, since hypophysectomy has been found to produce a reduction in otolith growth (Mugiya, 1990) and otolith demineralisation (Simkiss, 1974), and otolith mineralisation in hypophysectomised fish can be restored by injections of pituitary extract (Simkiss, 1974). Such hormonal regulation could influence both ion transport and matrix production in the *sacculus*. Wright *et al.* (1992) suggested that as plasma calcium concentration is regulated by hyper- and hypo-calcemic hormones, diel changes in the plasma concentration of these hormones may be indirectly responsible for the periodic decline in otolith calcification. Moreover, carbonate crystallisation in molluscs involves neu-

ral control (Zylstra *et al.*, 1978). Neural control of calcium concentration in the *sacculus* provides a physiological explanation for the direct tracking of seasonal and daily total calcium levels of the blood plasma by the endolymph (Mugiya & Yoshida, 1995).

Entrainment to light-dark cycles suggests the involvement of the pineal-hypophysial complex. Endocrine secretion displays a circadian periodicity in many animals and through the intermediary of metabolic rate, ultimately controls most physiological processes (Simpson, 1978). Endocrinological studies have demonstrated diurnal variations in the levels of several hormones in the plasma of Teleosts (Matty, 1985). These include thyroxine (T₄) (Eales *et al.*, 1981) a hormone known to influence skeletal growth and calcification in rainbow trout (La Roche *et al.*, 1966).

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