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Why did solid otoliths evolve in the ears of modern bony fishes?

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ABSTRACT

Modern bony fishes (Teleostei), which comprise almost one-half of all extant vertebrate species, show a remarkable diversity in ear morphology, including otolith shape. Fish bioacousticians are still puzzling over the question of why solid calcareous otoliths with species-specific shapes evolved, whereas most "non-teleost" vertebrates possess numerous tiny otoconia. This question is linked to when and how often a switch from loosely aggregated material towards a solid structure—and from apatite to calcium carbonate—occurred during the vertebrate radiation and whether those character shifts are related to altered hearing. In a recent study, we constructed a hypothetical framework for otolith evolution by compiling the available information on the structure of otoliths and otoconia in > 160 species covering all major vertebrate groups. We concluded that solid teleost otoliths may have initially evolved as a selectively neutral by-product of other key innovations, and that the teleost-specific genome duplication event may have facilitated their subsequent diversification. Differences in otolith mass and shape might have enabled the perception of different ranges of acoustic information and may have evolved during the diversification of auditory abilities. In some teleost groups, otolith morphology may have co-evolved with ancillary hearing structures, especially if ears are closely connected to a gas bladder.

Keywords: otolith, otoconia, aragonite, hearing, teleost-specific genome duplication event

1. Hypothetical framework

Fossil and extant species of modern bony fishes (Teleostei) show a remarkable diversity of otolith morphology (regarding size and shape) in their inner ears (e.g. (1, 2)). Sciaenids (drums or croakers), for example, possess large saccular otoliths that are often characterized by pronounced humps and protuberances whereas all otophysans, such as zebrafish, goldfish and catfishes, display a thin and needle-shaped saccular otolith (2). In most other vertebrates, the sensory epithelia of the otolith end organs, namely the utricle, saccule and—if present—the lagena, are overlain by numerous loosely attached tiny otoconia (3). The questions arise of why solid calcareous otoliths with species-specific shapes evolved in teleost fishes and if this shape-diversity is correlated with (improved) hearing across species. Based on a recent compilation on data related to the morphology, number, and mineralized material of the inertial masses in vertebrate inner ears (3), we provide a brief overview of hypotheses on the origin of teleost otoliths (Fig. 1). Initially, solid otoliths may have been a by-product of differences in biomineralization processes (see the evolution of cycloid scales, (4)) or of the emergence of other key innovations in teleost fishes. Hence, the initial emergence of solid otoliths in teleosts may have been selectively neutral, or solid otoliths with rather simple shapes might have provided a slightly positive selective advantage. They may have improved the detection of linear accelerations and sound stimuli and were thus retained and even diversified during the subsequent radiation of teleosts. Species-specific otolith shapes may have evolved later, closely correlated to different auditory demands and abilities in different species together with the diversification of swim bladder morphology during the radiation of teleosts. Studies on the formation of otoliths and the swim bladder walls during early larval development in zebrafish found genes like Sparc and Starmaker to

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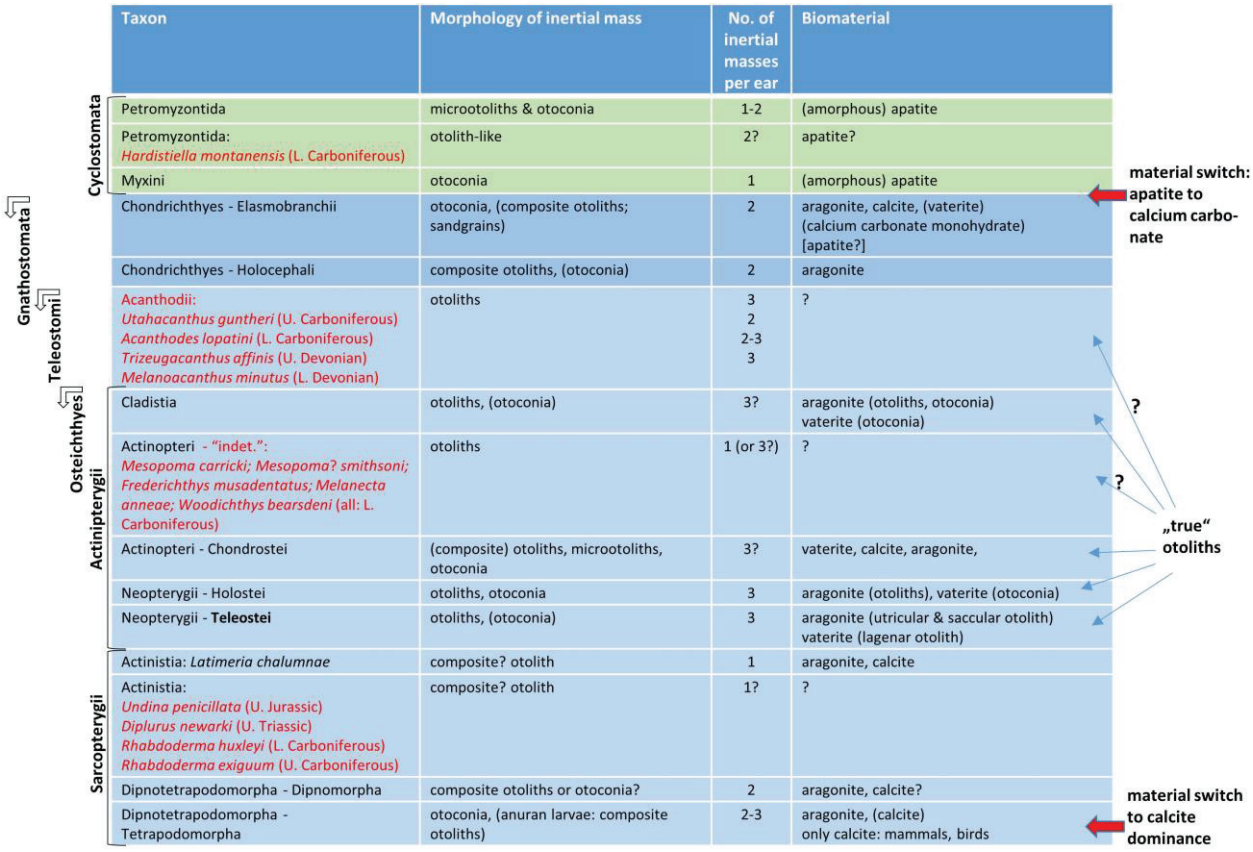
play crucial roles during the development of both otoliths and the swim bladder (5, 6). In addition, in vocal fish taxa, otolith morphology may also have co-evolved with acoustic communication because larger otoliths were found in taxa producing sounds compared to closely related non-vocal taxa (7). In addition, the emergence of solid otoliths might have been as well favoured due to a better surface area-to-volume ratio in a solid inertial mass compared to a (loose) aggregate of numerous otoconia. Due to this improved surface area-to-volume ratio the biomineralization of otoliths might be more "efficient" by saving energy, i.e. less organic matrix might need to be produced overall than in the case of an otoconial mass.



Taxon	Morphology of inertial mass	No. of inertial masses per ear	Biomaterial
Petromyzontida	microotoliths & otoconia	1-2	(amorphous) apatite
Petromyzontida: *Hardistiella montanensis* (L. Carboniferous)	otolith-like	2?	apatite?
Myxini	otoconia	1	(amorphous) apatite
Chondrichthyes - Elasmobranchii	otoconia, (composite otoliths; sandgrains)	2	aragonite, calcite, (vaterite) (calcium carbonate monohydrate) [apatite?]
Chondrichthyes - Holocephali	composite otoliths, (otoconia)	2	aragonite
Acanthodii: *Utahacanthus guntheri* (U. Carboniferous) *Acanthodes lopatini* (L. Carboniferous) *Trizeugacanthus affinis* (U. Devonian) *Melanoacanthus minutus* (L. Devonian)	otoliths	3 2 2-3 3	?
Cladistia	otoliths, (otoconia)	3?	aragonite (otoliths, otoconia) vaterite (otoconia)
Actinopteri - "indet.": *Mesopoma carricki; Mesopoma? smithsoni; Frederichthys musadentatus; Melanecta anneae; Woodichthys bearsdeni* (all: L. Carboniferous)	otoliths	1 (or 3?)	?
Actinopteri - Chondrostei	(composite) otoliths, microotoliths, otoconia	3?	vaterite, calcite, aragonite,
Neopterygii - Holostei	otoliths, otoconia	3	aragonite (otoliths), vaterite (otoconia)
Neopterygii - **Teleostei**	otoliths, (otoconia)	3	aragonite (utricular & saccular otolith) vaterite (lagenar otolith)
Actinistia: *Latimeria chalumnae*	composite? otolith	1	aragonite, calcite
Actinistia: *Undina penicillata* (U. Jurassic) *Diplurus newarki* (U. Triassic) *Rhabdoderma huxleyi* (L. Carboniferous) *Rhabdoderma exiguum* (U. Carboniferous)	composite? otolith	1?	?
Dipnotetrapodomorpha - Dipnomorpha	composite otoliths or otoconia?	2	aragonite, calcite?
Dipnotetrapodomorpha - Tetrapodomorpha	otoconia, (anuran larvae: composite otoliths)	2-3	aragonite, (calcite) only calcite: mammals, birds

Figure 1 – Overview of the characteristics of inertial masses found in vertebrate inner ears (for details see (3)). Fossil taxa are indicated in red. L. = Lower, U. = Upper.

All non-tetrapod vertebrates and some aquatic anuran larvae (3) display a trend towards a compact, inertial mass overlying the sensory epithelium in the form of amorphous microotoliths (Petromyzontida) or "composite otoliths" (Sarcopterygii, some chondrichthyan species). "Composite otoliths" are formed by fused otoconia (8, 9) whereas "true otoliths", as found in actinopterygians, especially teleosts, are formed by daily accreted layers of organic and inorganic material (10). Gauldie (11) proposed that solid otoliths may have been initially formed by a fusion of otoconia. Comparisons of otoconial masses dissected from fresh and formalin-fixed ears of lungfishes and lizards, however, indicated that otoconia are rather loosely attached in fresh material whereas compact otolith-like structures were only found in animals stored in fixative (12). Especially in lungfishes and cartilaginous fishes, a (re-)examination of these otoconial masses is needed to evaluate their rigidity and the degree of species-specificity of their shapes and to characterize their motion patterns relative to the underlying sensory epithelium.

The fossil record (13) as well as crystallographic studies on extant otoliths and otoconia (14, 15) indicated that calcareous otoliths may represent the ancestral otolith type of gnathostome fishes (13) and that three pairs of solid otoliths may have already been present in the ancestor of bony fishes (Teleostomi *sensu* (16)). The latter assumption might be supported by fossil Acanthodii, in which three different otolith types might have already been present. The fossil record of teleosts (including specimens with their otoliths *in situ*) further suggests that the overall shape of saccular otoliths was mainly oval with a straight sulcus in Early Jurassic taxa, followed by radiations and diversification of

otolith shapes in the Late Cretaceous and Paleogene (17). The fossil record provides important information on otolith evolution; however, limitations with respect to incompleteness, misinterpretations, and material re-crystallization—when the preserved material found in otoliths or otolith-like structures does not represent the initial condition, which may even affect the overall morphology of the otolith—have to be considered. Rather loosely attached otoconia are more prone to disintegration during taphonomic and diagenetic processes than solid otoliths. Thus, the likelihood to find fossil specimens with otoconial masses *in situ* is extremely low. As a consequence, it remains unknown what the diversity in otoconial masses or "composite otoliths" may have looked like in extinct cartilaginous and bony fishes, or in basal (Cambrian) vertebrates. Even though solid otoliths of fossil taxa have a greater likelihood to be found *in situ*, the fossil record can be incomplete because not all otolith types of the left and right inner ear were preserved (18). It is difficult to interpret findings of only one otolith or otolith pair in basic actinopterygians (19) or extinct sarcopterygians (20) and to draw conclusions regarding the evolution of otolith numbers. In taxa that possess—or are assumed to possess—open endolymphatic ducts (such as cartilaginous fishes) and show poor preservation, while the fossil "otoliths" lack characteristic features, the remains may simply represent *post-mortem* sedimentary infillings. These infillings may indeed have been misinterpreted as fossil otoliths *in situ* (21). The unambiguous identification of the original biominerals is another issue when examining fossil specimens. The otolith-like structures detected in a Carboniferous lamprey appear to be made up by apatite (22), which may support the idea that both fossil and extant Petromyzontida possess apatite inertial masses in their ears. However, fossil hard tissue is prone to re-crystallization due to diagenetic effects (23), which becomes a more severe problem the older the fossils are. To overcome these limitations and to formulate hypotheses on otolith evolution, one needs to include a genetic (i.e., "EvoDevo") perspective so as to identify 1) genes/gene families that play important roles in otoconia and otolith formation and 2) which genes might be differentially expressed in teleost or actinopterygian inner ears during the initial stages of otolith mineralization as well as during later increment formation.

2. What may have triggered the evolution of solid otoliths?

2.1 The potential role of whole genome duplication events

The high evolvability of otolith size and shape may be related to the teleost-specific whole genome duplication event (TGD, (24)), or even the preceding vertebrate-specific whole genome duplication event(s) (25). Both genome duplications events provided the basis for a sub- or neo-functionalization of duplicated genes (26), probably including genes related to ear and otolith formation. Especially the TGD may have paved the way—in the sense of a genomic exaptation (27)—for the evolution of taxon-specific otolith shapes.

Claudin genes may represent one example for the importance of gene/genome duplications in the context of ear evolution. Some of these genes encode proteins that are assumed to reduce the paracellular membrane permeability to water and dissolved ions (28). Teleost-specific claudins, such as claudin 8-like (claudin j, (29)) and claudin 7a ((28); but termed claudin 7b in (30)) are indispensable during the early formation of the ear and otoliths. Mutations in claudin 8-like and claudin 7a result in smaller or no otoliths (29, 30). While both claudin 7a and claudin 7b are expressed in several organs in zebrafish, such as the kidney, spleen, and testes, only claudin 7a is also expressed in the brain and the eyes. It appears as if the teleost-specific duplication of claudin 7 into claudin 7a and 7b enabled a subsequent sub-functionalization of the duplicated gene (28).

Following the TGD, otolith diversification was likely driven by different forms of natural selection arising from the variety of habitat types that teleost fishes were able to colonize (31), whereby some habitats types may be imposed specific selection on improved hearing. Examples of different (eco-acoustical) environmental conditions imposing different selection on hearing capacities are, for instance, the "cut-off frequency phenomenon" observed in shallow waters (e.g. 0.4-2 kHz at a total water depth of 1 m (32)). Moreover, noisy versus quiet habitats (eco-acoustical constraints hypothesis (33)) have been hypothesized to select for an improved ability to detect higher frequencies in shallow waters—as found in otophysans, such as goldfish or carp—and have triggered higher auditory sensitivities in quiet water bodies (31). Extreme habitats, such as the deep-sea, can be characterized by high water pressure, which may hamper efficient sound pressure transmission to the ears through the gas-filled swim bladder, while permanent darkness renders non-visual senses (including hearing) more important, for example, for navigation and communication (34).

2.2 Are solid otoliths related to improved hearing and/or acoustic communication?

The "vestibular first hypothesis" (35) assumes that the vestibular sense evolved during early stages of vertebrate evolution, followed by the evolution of the auditory sense. Otoconia are already found in the basal bauplan of vertebrates and probably evolved first, mainly in the form of loose aggregates, even though lampreys (Petromyzontida) already show a trend toward more solid inertial masses (36). Hence, otoconia and "composite otoliths" may initially have served for gravity perception, similar to the statoconia and statoliths found in many invertebrates (37). More elaborate auditory abilities probably required derived morphological structures such as 1) tympana, auditory ossicles, and tectorial membranes in tetrapods (38) or 2) gas-filled bladders in close proximity (or connected) to the ears (which function similar to tympana), along with solid otoliths, displaying species-specific shapes in teleosts (39). In this way, solid otoliths with daily growth patterns instead of numerous loosely aggregated otoconia or "composite otoliths" may have enabled the evolution of improved hearing abilities in teleosts (40). Sound detection might be improved by teleost otoliths compared to otoconia because otoliths represent rigid bodies with a specific shape which are likely to result in mass- and shape-specific motion patterns (11, 39, 41). "Composite otoliths" either lack a uniform shape and/or may not be rigid enough to efficiently transduce frequency-specific movement patterns to the sensory epithelia (11, 40). Otoliths may vibrate at—and, therefore, enable perception of—higher frequencies up to several kHz (41). It seems that frequencies above approx. 1 kHz cannot be perceived by fishes possessing otoconia and/or "composite otoliths" (e.g. sharks and rays, see (42)). This idea receives support from the observation that teleost species possessing ancillary auditory structures and enhanced audition usually also display strongly divergent otolith shapes. These include tetrahedral utricular otoliths in clupeiform fishes (43) or the needle-shaped saccular and star-like lagenar otoliths in otophysans (2). In addition, mathematical modelling (41) and recent experimental studies using synchrotron radiation-based imaging ((44), unpublished data) suggest that otolith motion patterns strongly depend on otolith shape and the type of ancillary auditory structures (i.e. a Weberian apparatus in otophysans versus anterior swim bladder extensions contacting the inner ears in other groups).

Although enhanced hearing in teleosts was probably not triggered by the ability to communicate acoustically (45), otolith morphology may have co-evolved with acoustic communication in vocal fish taxa. Otolith size might also be evolving under constraints imposed by sound production: vocal species, such as *Nezumia aequalis* (Macrouridae), appear to display larger and heavier (saccular) otoliths than non-vocal species like *Nezumia parini* (46). Heavier otoliths may avoid over-stimulation of the ears when the fish produces sound using, for example, its swim bladder (47).

3. Is the "choice" of biominerals related to otolith/otoconia function?

A better understanding of the evolution of solid, mainly aragonitic otoliths is also linked to the question of why different types of biominerals occur in vertebrate inertial masses. Differences in the mineralized material may be related to the function of the inertial mass in inner ear physiology and/or to metabolic and biomineralization processes.

3.1 Apatite versus calcium carbonate

A comparison of the chemical composition of otoconia and otoliths indicates a switch from apatite in cyclostomes (hagfishes and lampreys) to calcium carbonate in gnathostomes (14, 15). The apatite-to-calcium carbonate switch may be related to improved inner ear function. However, apatite has a higher density (e.g. hydroxyapatite: 3.16 g cm^{-3}, (48)) than aragonite (2.93 g cm^{-3}, (10)) which would mean a greater inertial mass per given otolith size and thus may result in improved sensitivity to low frequency sounds (cf. (14)). From this viewpoint, an inertial mass consisting of apatite should have been favoured over calcareous otoconia or otoliths in aquatic habitats. Alternatively, using apatite for the formation of bones and teeth but calcium carbonate in the inertial masses of the ears may have been advantageous as it allows decoupling the homeostatic control of the mineralization of skeletal biominerals from those in the ears (49). In the ears of bony fishes, the ionic composition of the endolymphatic fluid is more strictly regulated than that of the blood plasma (50) and decoupling of somatic and otolith growth rates is partly possible (51). Under stressful conditions like starvation (52) this decoupling may allow for the maintenance of continuous otolith growth—and thus, proper ear functioning—even when somatic growth rates are reduced or come to a halt, which may be important if the sensory epithelium also continues to grow (53).

In contrast to earlier findings of purely calcareous endogenous otoconia/"composite otoliths" in cartilaginous fishes (54), some species seem to possess apatitic inertial masses (55). If this holds true, two hypotheses can be formulated that should be tested in future studies. 1) Calcareous inertial masses evolved at least twice in parallel, i.e. in bony fishes (Osteichthyes) and in some cartilaginous fishes. 2) Some cartilaginous species secondarily developed apatitic otoconia/"composite otoliths".

3.2 Aragonite versus calcite

The switch from a predominance of aragonitic otoliths and otoconia in "fishes" to calcitic otoconia in birds and mammals may be related to the functional role of an aragonitic inertial mass in the inner ear. In aquatic habitats, the greater density of aragonite compared to calcite may be advantageous if the inertial mass acts as an accelerometer (36). Moreover, certain features related to the growth of aragonite, namely aragonite twinning, have been hypothesized to be more suitable for the formation of solid otoliths, as it may facilitate a daily incremental growth pattern and ultimately, taxon-specific otolith shapes (56). The mode of thermoregulation may play an additional role because inertial masses made up by aragonite and, in part, vaterite are found in poikilothermic vertebrates, whereas only calcitic inertial masses are present in homoeothermic vertebrates (14). Note, however, that calcitic otoconia also occur in cartilaginous fishes (14, 54), but these otoconia show a cuboidal crystal habit, which is distinctly different from the barrel-shaped (cylindrical) calcitic otoconia found in tetrapods. As some squamates possess both aragonitic and calcitic otoconia (3), it is unlikely that the mode of thermoregulation is the only factor accounting for the predominance of calcite in mammals and birds (12). The transition from an aquatic to a terrestrial lifestyle may have been another important driver of this material switch. Support for this assumption may come from studies on different genes and gene products involved in otoconia and otolith formation. Even though similar molecular mechanisms are involved in the formation of mouse otoconia and zebrafish otoliths and despite the fact that both possess homologues in their organic matrix like Sparc and Otoconin-90 (Oc90, (57)), eight genes involved in ear development were lost in tetrapods when compared to *Latimeria* and zebrafish (58). One of those genes is otolith matrix protein 1 precursor (otomp). The corresponding otolith matrix protein 1 (OMP-1) is part of the organic matrix of zebrafish otoliths but not of the matrix of mammalian otoconia (57) and might determine the mineralized calcium carbonate polymorph (i.e. aragonite). Likewise, Starmaker (Stm) is part of the zebrafish otolith matrix but is not found in mouse otoconia (57). Stm was shown to be essential for the normal development of spherical to hemispherical aragonitic otoliths, while silencing gene expression results in star-like, calcitic otoliths (59). An experimental study on aragonite biomineralization focussed on the potential roles of other matrix proteins, namely OMM-64 and otolin-1, and hypothesized that either the entire protein aggregate of the otolith matrix induces aragonite mineralization or that the studied aggregate contained an aragonite inducer other than OMM-64 or otolin-1 (60). Hence, OMP-1 and/or Stm might be potential candidates that act (alone or in concert) as inducers of aragonite mineralization and were lost in tetrapods either due to genetic drift or selection related to the transition from an aquatic to a terrestrial lifestyle (OMP-1, (58)): OMP-1 binds calcium and is important for the recruitment of other proteins into the otolith matrix (61), whereas Starmaker represents a so-called "intrinsically disordered" protein which potentially interacts with multiple other proteins (62).

4. CONCLUSIONS

Solid teleost otoliths and especially the diversity in otolith shapes seems to be correlated with the diversification of hearing abilities. Solid, continuously growing otoliths with species-specific shapes might have enabled the perception of different ranges of acoustic information in diverse habitats, such as shallow versus deep or quiet versus noisy waters. However, this diversity might also have arisen as adaptations to specific demands of the vestibular sense and the ability to manoeuver, or as a by-product of other evolutionary adaptations. The teleost-specific whole genome duplication event might have provided a genomic "toolbox" for the evolutionary diversification of otolith size and shape, as it allows sub- or neo-functionalization of duplicated genes.

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