OTOLITHS AND THEIR APPLICATIONS

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Summary

Otolith science continues to make substantial contributions to traditional fisheries management and stock assessment efforts while expanding its role in ecological research and conservation applications. Certain intrinsic properties of otoliths, including continual growth, deposition of distinct daily and seasonal increments, ability to permanently record chemical aspects of the environment, and species-specific shape, combine to create one of the most versatile vertebrate structures used in science and management. Although historically used to study age and growth and for stock assessment applications, otoliths are currently used to address a wide variety of research questions. Otolith research has made major contributions to our understanding of population structure, demography, foraging ecology, early life history of fishes, and migratory patterns (Campana *1999*, *2005*; Campana and Thorrold, *2001*; Elsdon et al., *2008*). Researchers worldwide continue to seek novel methodologies for extracting diverse information from otoliths.

The purpose of this paper is to provide an overview of the traditional and current applications of the otoliths.

Keywords: otolith, fish, age determination, growth, developments, composition, identification

Introduction

Otoliths are structures located in the inner ear cavity of all teleost fish. They are isolated within a semi permeable membrane and bathed in an endolymphatic fluid. These structures serve as a balance organ and also aid in hearing (Campana, 1999; Campana and Thorrold, 2001). The structure of the otoliths is three dimensional but they do not necessarily grow at the same rate equally in all dimensions. Also, the size and shape vary considerably among species (Campana and Thorrold, 2001).

Otoliths are composed mainly of calcium carbonate ($CaCO_3$) mostly in the form of aragonite. Aragonite is also found in coral and sclerosponge skeletons, bivalve shells, and squid statoliths. The other 10 % of the otolith is minor and trace elements within the aragonite matrix that are derived from the water surrounding the fish. These impurities reflect the water chemistry, as well as the fish's metabolism (Telmer, 2004; Campana and Thorrold, 2001).

The pattern in the otolith is composed of a number of concentric shells with different radii. Depending on the amount of organic material in each shell or zone, its appearance will vary from extremely opaque to complete hyaline (transparent). The first zone is the nucleus of the otolith. The opaque zones in the otoliths are formed during the period of greatest growth and the hyaline zone is laid down usually during the period of slowest growth.

Any major change in the environment in which a fish lives is likely to produce a ring. In the earth's temperate zones differences between summer and winter are marked by both changes in water temperatures and amounts of food available. Equally marked changes occur also in the tropics, for example, many rivers are subject to seasonal floods. During the floods, food is abundant and the fish grow rapidly. In the dry season the food becomes scarce and the fish very often starve. Still, some fish live in uniform environments, notably in the polar and tropical regions and consequently no rings whatsoever are laid down in their skeletal structures (Holden and Raitt, 1974).

Daily increments of growth have been detected on fish otoliths. The width of these increments averages 1 to 2 μm in larval anchovies (*Engraulis mordax*) and 3 to 4 μm in the larger hake (*Merluccius spp*.), (Moyle and Cech, 2004). In a study, daily increments were also found in juvenile skipjack tuna (*Katsuwonus pelamis*) otoliths, measuring 15–40 μm (Tanabe et al., 2003).

One of the most appreciated characteristics of the otoliths is the lack of resorption. This means that once the material has been deposited, the organism will not use again these minerals even in periods of starvation. Lack of resorption is not shared with any other calcified structure (like scales and bones) in fish or other vertebrates (Bilton, 1974 cited by Campana and Thorrold, 2001). Another special characteristic of otoliths is that they form the only calcified structures known to grow throughout the lifetime of the fish in a continual way.

All the facts mentioned before provide the basis on which otoliths can be used as powerful tools for age determination. This is very important because age, growth rate, and mortality rate (growth and mortality are both based on age information) are three of the most influential life history characteristics controlling the productivity of fish populations.

Besides age and growth determination, otoliths have been the object of study in many and different fields, such as, fish biology (hearing and balance in fishes), larval fish ecology, species identification, fish stock identification, environmental reconstruction of the fish habitat (Campana,1999; Brazner et al., 2004; Campana, 2005; Humphreys et al., 2005; Popper et al., 2005).

Thus, the purpose of this paper is to provide an overview of the traditional and current applications of the otoliths.

There are six important thematic areas regarding otoliths applications: (1) age and growth; (2) chemistry; (3) life history and management; (4) back-calculation analysis; (5) physiology and morphology; and (6) sclerochronology and the environment.

1. Age and growth

The need for accurate ageing remains an important component of otolith studies around the world (Campana, *2001*). Representative examples include efforts to further refine methodologies, such as bomb radiocarbon analyses to validate ages of both long-lived (Armsworthy and Campana, *2010*) and short-lived (Melvin and Campana, *2010*) species, as well as to use shape analysis in ageing applications (Beyer and Szedlmayer, *2010*). The expanding application of otolith studies to conservation science is highlighted by the use of traditional age and growth analyses to examine temporal changes in the demography of species of concern (Fowler and King, *2010*; Terwilliger et al., *2010*).

Otoliths are a focus of attention by fisheries scientists because of the precision of age estimates based on annuli and the relative ease of otolith preparation and annuli enumeration. Indeed, the clarity of annual increments in the otoliths of fish species from both marine (e. g., *Pogonias cromis*; Campana and J o n e s, 1998) and freshwater habitats (*Aplodinotus grunniens*; P e r e i r a et al., 1995) can be quite remarkable (Campana and Thorrold, 2001).

The exact age determination of fish is one of the most important elements in the study of their population dynamics. It forms the basis for calculations leading to knowledge of the growth, mortality, recruitment and other fundamental parameters of their populations (Holden and Raitt, 1974).

Thus, otoliths provide some of the best examples of biochronologies in the animal kingdom, combining annual sequences of up to 110 years in adult fishes with daily chronologies of up to a year during the larval and juvenile stages (Campana and Thorrold, 2001).

Age estimation of most larval and juvenile fishes is possible thanks to the presence of easily discernable daily increments in otoliths, making a remarkably accurate and precise method (Campana and Neilson, 1985; Tanabe et al., 2003).

These data have been used to determine growth rates during early life history, estimate pelagic larval durations of reef–associated species, and examine the effects of physical processes on larval survival through back–calculation of hatch date distributions. It is also possible to reconstruct instantaneous daily growth rates of larval fish from increment width trajectories, based on an empirical relationship between otolith size and fish size (Campana and Jones, 1992).

In a study a sample of 20 metamorphosing conger eel (*Conger conger leptocephali*) were collected from the Minho River, Portugal, in February 1999 and their sagittal otoliths were analyzed by scanning electron microscopy. Four different etching agents were applied along both sagittal and frontal sections during otolith preparation to examine the microstructural growth in this species. Otolith growth increments were visible throughout the increment countable zone using all four treatments, but a permanent peripheral diffuse zone, where the daily increments were unclear, appeared on all otoliths, preventing accurate age estimation. To understand more about the nature of the diffuse zone, otoliths of 10 other metamorphosing leptocephali reared in aquaria were marked by immersion in tetracycline hydrochloride. The distance between the fluorescent marks and otolith edge, measured over a fixed period of time, was used to estimate the otolith growth rate. The application of this technique led to an anomalously high estimated otolith growth rate, probably as a result of the capture, marking and handling stress (Correia et al., 2006).

Another important step when determining age of fish is age validation. Age validation studies are required for accurate age determination and are essential for fisheries catch–at–age models, to assess the health of a fishery resource or to correctly interpret the dynamics of a fish population. Validation methods include chemical marking, using oxytetracycline, length–frequency, and identification of the first annulus, marginal increment analysis, tag–recapture analysis and bomb radiocarbon assays to assess the accuracy of these age determination methods and examine growth (D w y e r et al., 2003).

One of the most used methods (because of its relative simplicity), is the marginal increment method (MI). It is based on estimating the marginal increment of the otolith of each fish for age class and estimates the profile of the mean monthly marginal increment. The marginal increment is measured as the distance from the inner margin of the outermost translucent ring to the periphery of the otolith. Measurements are always made along the longest axis.

2. Chemistry

Studies on the elemental and isotopic composition of otoliths continue to be a major component of recent research. Given that otolith chemical analyses draw from several sub-disciplines, including ecology, fisheries science, and analytical chemistry, the need for consistent and accurate presentation of relevant analytical procedures continues to be particularly important. Furthermore, in many instances our limited understanding of the mechanisms underlying otolith chemical composition calls for conservative data interpretation. Contributions in this field include continued efforts to use otolith chemistry to elucidate population structure (Newman et al., *2010*; Steer et al., *2010*), but studies that incorporate other natural markers, including microsatellite DNA (Svedang et al., *2010*) and parasite communities (Niklitschek et al., *2010*), are becoming more common. Examples of laboratory experiments aimed at identifying regulating mechanisms and factors that influence otolith chemical composition were done (DiMaria et al. *2010*; Walther et al., *2010*). Additionally, a study that inferred ancient trophic relationships in the Gulf of California based on the isotopic composition of archaeological otolith collections (Rowell et al., *2010*) represents an interesting application of otolith chemistry that is likely to expand in the future.

Studies have used the elemental composition of the otoliths to infer the timing of day–to–day environmental changes and changes in the physical habitat. For example; the change in the calcium/strontium ratio can be used in combination with increment number to estimate the dates of migration of anadromous and catadromous species (Kennedy et al., 2002). Other trace elements, such as K, Mn, Li, Mg and Ba have also proved their utility as natural tags of the nursery ground origins of juvenile fish and spacio–temporal distribution of stocks, as for the American shad (*Alosa sapidissima*) inhabiting the Connecticut, Hudson and Delaware rivers in North America (T h o r r o l d et al., 1998), the Pacific swordfish (*Xiphias gladius*) (Humphreys et al., 2005) and the Atlantic cod stocks (*Gadus morhua*) along the north shore of Quebec in Canada (Methot et al., 2005).

In a study of the migration patterns of the European flounder *Platichthys flesus (Linnaeus, 1758)* the interpretation of otolith strontium distribution patterns from flounder specimens collected in the freshwater tidal area of the Minho estuary and in the lower estuary suggests that the flounders hatched in the estuary, while only 6.7% of those captured in the coastal area hatched in the coastal area. Ultimately, studies aimed at collecting larval stages and adult flounders must be made to confirm that flounders spawn in the estuary and to define new and better scientifically supported fishing policies, or simply to confirm the existing ones regarding temporal and spatial closures for each gear used in the Minho estuary (Morais et al, 2011).

3. Life history and management

The use of otoliths to address population-level questions continues to be labor intensive and presents challenges in terms of generating adequate sample sizes for robust statistical evaluation. There are several studies that combined field collections with genetic, otolith microstructural and/or chemical analyses to address questions on early life history, migratory pattern, natal origin, and habitat use. For example, Kerr and Secor (*2010*) combined field collections with otolith microstructural analysis to identify proximate causes of partial migration in an estuarine-dependent species. Highlighting conservation applications of otolith science, other researchers identified key habitats for species of concern based on variation in otolith isotopic composition (Engstedt et al. *2010*; Hobbs et al., *2010*). Other contributions, which highlight potential management applications, build upon the use of otolith isotopic composition to determine natal origins in Chinook salmon (Barnett-Johnson et al., *2010*) and identify anadromous and non-anadromous ecotypes of sockeye salmon (Godbout et al., *2010*).

4. Back-calculation analysis

Back–calculation analysis is often used to estimate fish length at a previous age or date. Growth back–calculations can be derived from a series of growth increments (either daily or yearly) and represent one of the most powerful applications of the otolith. Since the fish length: otolith length relationship can be determined, the widths of the daily (or yearly) growth increments in an otolith reflect the daily (or yearly) growth rates of the fish at that age and on those dates (C amp a n a, 2004a). For example, one of the most used methods for doing this is based on a body proportional hypothesis applied to a power relationship between the fish length and the otolith radius (F r a n c i s, 1990 cited by P a j u e l o and L o r e nz o, 2003). Other methods use regression and the Fraser–Lee equation.

In the method used by F r a n c i s (1990), the radius of the *i*th band (Ri) and the radius of the otolith at capture (Rc) are measured for the analysis. The first one (Ri) is the distance from the center of the otolith to the outer margin of the translucent ring. The second is the distance from the center of the otolith to the periphery.

The data can be fitted by regression of the log T L and log of the otolith radius (R). The length of an individual when the *i*th band was laid down (Li, mm) is calculated using Li=(Ri/Rc)v Lc, where Lc is the length at capture and *v* is a constant derived from the power function which describes the relationship of between the fish length and the otolith radius. Then again, the data can be fitted to the von Bertalanffy curve and compute the growth parameters.

In a study of the growth of the common two banded seabream (*Diplodus vulgaris*), P a j u e l o and Lo r e n z o (2003) estimated the growth parameters by the two methods described above (direct otolith reading and back–calculation methods) which gave similar results in this species. Still, some constraints appear to most procedures in back–calculations. One of the most important is the assumption that the fish–otolith relationship is not only linear, but does not vary systematically with the growth rate of the fish. Many studies have demonstrated that otoliths of slow–growing fish tend to be larger and heavier than those of fast–growing fish of the same size, whether at the daily or yearly scale. Such a systematic variation implies that growth back–calculations made with any of the traditional equations will tend to underestimate previous lengths at age, with the degree of error varying with the range of growth rates that are present in the population (Campana, 2004a).

5. Physiology and morphology

Contributions to this field include the development of a novel statistical methodology for shape analysis (Reig-Bolano et al., *2010*) along with a direct comparison of otolith chemistry and shape analyses to examine spatial structure in a deep-sea species (Longmore et al., *2010*). There is also an examination of otolith ecomorphological trends in relation to the phylogeny of Nototheniid fishes (Lombarte et al., *2010*).

Otoliths have a distinct shape, which is often characteristic of the species of fish. Thus fish, seal and seabird biologists, as well as taxonomists and archaeologists, often rely on the shape and size of preserved or undigested otoliths to reconstruct the species and size composition of the diet of fish predators. (Campana and Casselman, 1993; Campana, 2004a). The basis for these types of studies is either a reference collection of otoliths from the local fish species or published atlases (Campana, 2005). For example, Harvey et al. (2000) presented a compilation of images of otoliths of 63 fish species of the North Pacific Ocean, including the relationship between the otolith length and fish length, Assis (2003) published a comprehensive examination of asteriscal otoliths in teleosts and Campana (2004b) published a photographic atlas of otoliths from fishes of the northwest Atlantic.

Otolith appearance and shape often vary geographically within a species, although there are mixed reports concerning the potential for stock discrimination. In a comprehensive examination of the shape of all 3 otolith pairs, Campana and Casselman (1993) concluded that otolith shape did indeed vary among some stocks, although the stock variation appeared to be environmentally induced rather than genetically induced.

Another example using otolith morphometry was presented by Begg et al. (2001). In this case, the internal structures of the otolith where used for stock differentiation in the Georges Bank haddock (*Melanogrammus aeglefinus*). They found out differences in growth rates (and hence, otolith structure) of eastern and western Georges Bank haddock which appeared to derive mainly from differences in the stock's environmental conditions (water temperature and diet), showing the usefulness of otoliths in stock studies. Cardinale et al. (2004) used hatchery releases of cod into the wild, recaptured after several years, to demonstrate that both genetic and environmental influences control otolith shape. Several laboratory experiments confirmed that the relationship between otolith and somatic growth is mediated by temperature and/or other modifiers of growth rate, such that slow–growing fish produce relatively large otoliths (Oozeki and Watanabe, 2000; Strelcheck et al., 2003 cited by Campana, 2005).

6. Sclerochronology and the environment

Many aspects of otolith science fall within the field of sclerochronology, which involves the study of physical and chemical variations in the accretionary structures of organisms (Panfili et al., *2002)*. Cross-dating originated in forest science (Douglass, *1941*) and has been applied to terrestrial ecosystems and, more recently, aquatic systems (e.g., Black, *2009*). Several presentations demonstrated how, through the use of methodologies originally applied in dendrochronology, otolith aging error can be minimized and time series can be more confidently aligned to elucidate patterns of growth within and among species and examine climate relationships. The potential to apply aspects of sclerochronology to improve interpretation of otolith structural and chemical data as well as generate environmental proxies is not yet fully realized, but it is evident that contributions will grow as otolith science moves into the future.

Conclusion

Otoliths are one of the most reliable tools for determining the age of a fish. Since age is used to establish growth rates of a fish species and age compositions of a certain population, otoliths are a powerful tool in fisheries management. Otolith chemistry and microstructure analysis have developed greatly in the recent years and have showed a wide range of applications for stock identification and other environmental studies concerning fish habitats. However, there are still several areas of otolith research that need further development. For example, to avoid overfishing, the management of deep sea fishes requires the development of new methods to validate the age determined from the otoliths, because many species appear to have long life spans and grow slowly. Also, the development of otolith growth models, apart from improving the accuracy of back–growth calculations, would simplify the methods for reconstructing life histories and environmental exposures.

It is evident that otolith science will continue to make significant contributions to fundamental and applied research. Researchers continue to successfully extract highly relevant information from otoliths that is essentially inaccessible through other research approaches. Otolith science currently encompasses a broad range of research approaches that will likely continue to expand as new and improved technologies are developed.

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