CSIRO PUBLISHING

www.publish.csiro.au/journals/mfr *Marine and Freshwater Research*, 2005, **56**, 485–495

Review

Otolith science entering the 21st century

Steven E. Campana

Marine Fish Division, Bedford Institute of Oceanography, PO Box 1006, Dartmouth,
Nova Scotia, Canada B2Y 4A2. Email: campanas@mar.dfo-mpo.gc.ca

Abstract. A review of 862 otolith-oriented papers published since the time of the 1998 Otolith Symposium in Bergen, Norway suggests that there has been a change in research emphasis compared to earlier years. Although close to 40% of the papers could be classifed as 'annual age and growth' studies, the remaining papers were roughly equally divided between studies of otolith microstructure, otolith chemistry and non-ageing applications. A more detailed breakdown of subject areas identified 15 diverse areas of specialisation, including age determination, larval fish ecology, population dynamics, species identification, tracer applications and environmental reconstructions. For each of the 15 subject areas, examples of representative studies published in the last 6 years were presented, with emphasis on the major developments and highlights. Among the challenges for the future awaiting resolution, the development of novel methods for validating the ages of deepsea fishes, the development of a physiologically-based otolith growth model, and the identification of the limits (if any) of ageing very old fish are among the most pressing.

Introduction

Research involving otoliths continues to evolve and expand as we enter the 21st century. A search of the literature indicates that the number of primary publications reporting research on, or application of, otoliths has increased almost linearly over the past 35 years. Papers involving otoliths are now being published at five times the rate that they were in the 1970s, with annual rates approaching 200 papers per year in the last few years. Of greater interest is the changing balance in the manner in which the otoliths are being used. In an earlier perspective, Campana and Thorrold (2001) noted that publications reporting the use of otoliths for annual age and growth estimates have long dominated the field, but that the use and study of otolith microstructure and otolith chemistry had increased markedly through the 1980s and 1990s respectively. A review of the literature since the time of the 1998 Otolith Symposium in Bergen suggests that the balance continues to shift. In the present paper, I will briefly review the change in research emphasis that has been evident in the field of otolith research and application since the time of the last Otolith Symposium. I will make no attempt to summarise all research progress over the past 6 years; the objective of the current paper is not a comprehensive review of the field. Rather, I will attempt to highlight what I consider to be the major developments in each of the otolith disciplines, noting those areas that seem particularly ripe for rapid advancement. Finally, I will close with a view of the challenges awaiting resolution in the near future.

Recent changes in research emphasis

Papers which reported the study or use of otoliths, and published between 1999 and the early months of 2004, were split between four major otolith disciplines: annual age and growth, chemistry, microstructure and other non-ageing applications (Fig. 1*a*). Of these, age and growth papers at the annual level made up close to 40% of the 862 publications surveyed. However, this represents a substantial shift in balance among disciplines, since annual age and growth papers accounted for 80% of all otolith papers before 1999 (S. E. Campana, unpublished data). The remaining disciplines each now account for 15–20% of the total. The increased emphasis on microstructure, chemistry and non-ageing applications reflects the increased interest in their fields, rather than any marked decline in annual ageing studies.

A more detailed breakdown of the papers surveyed reveals some intriguing patterns in otolith research (Fig. 1*b*). The specific categories shown are to some extent arbitrary, since most (but not all) are application-oriented. Nevertheless, the fact that there are at least 15 areas of otolith specialisation clearly indicates that otoliths are used in a very diverse range of applications. In what follows next, I will briefly highlight examples of the type of research being conducted in each of the areas of specialisation shown in Fig. 1*b*, along with the percentage of recent papers published in that specialisation. Then, I will attempt to summarise the major developments which have occurred in each of these areas.

10.1071/MF04147 1323-1650/05/050485

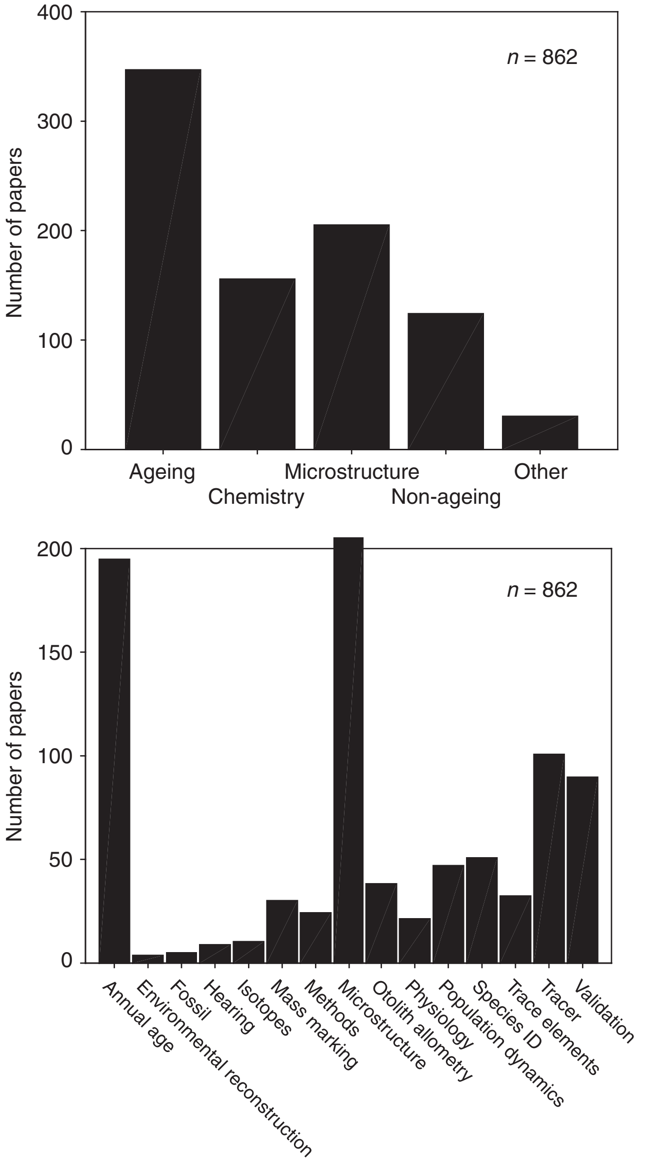

Fig. 1. Breakdown by subject area of papers reporting the study or use of otoliths and published between 1999 and early 2004. (*a*) Breakdown by major otolith discipline; (*b*) detailed breakdown by subject area.

Recent trends in research

Otolith microstructure (24% of recent papers)

Studies involving otolith microstructure have become the most applied approach in otolith science. Recent applications continue to be most represented by studies in which the sequence of daily growth increments were used to describe the early life history of a fish species, particularly the hatch date and growth rate (e.g. Takahashi *et al.* 2001), or to determine the duration of residency as a pelagic larva (Pasten *et al.* 2003). However, increasingly common were studies which compared and contrasted the growth rate and hatch date of different cohorts of young fish, perhaps as a function of environmental conditions (Anderson and Dalley 2000; Meekan *et al.* 2003). The developing maturity of the field is reflected in the relative scarcity of routine daily increment validation studies, and the increasing use of the microstructure (representing daily growth structures) to validate the interpretation of the first-formed annulus (representing annual growth structures) (Panfili and Tomas 2001).

The major developments in this field have used otolith microstructure for more than just early life history descriptions or routine age validation. Of particular note were a number of studies which used larval age composition and/or daily increment width series to infer population exposure to regional or size-selective mortality events (Searcy and Sponaugle 2001; Pepin *et al.* 2002; Wilson and Meekan 2002), or to link growth and mortality rates in a population (Bunnell *et al.* 2003). Brophy and Danilowicz (2002) used microstructural growth patterns to identify and track patches of drifting larvae. However, there were also developments in the conceptual basis for these applications. Several experimental studies examined the factors influencing the production or interpretation of problematic daily increment sequences under adverse or low-growth conditions (Fey 2002; Fox *et al.* 2003). The practical limitations of inferring growth from incremental width sequences were examined by others (Barber and Jenkins 2001; Pepin *et al.* 2001). Several reviews focusing on specific features or applications of otolith microstructure were published (Campana and Thorrold 2001; Panfili *et al.* 2002; Thorrold and Hare 2002).

Annual age and growth (23% of recent papers)

The use of otoliths for determining annual age and growth continues to be a ‘bread and butter’ application, and the frequency of its use appears unlikely to decline in the near future. Included in this category were a broad suite of studies that used the annual growth pattern in the otolith to estimate the longevity and growth rate of a population or species of fish (Wilson and Nieland 2001; Laidig *et al.* 2003). If this category had been broadened to include papers that integrated the development of ageing methods or age validation with estimates of population longevity or growth rate, this category would have ranked as the most common of all otolith studies. However, the two categories were kept distinct so as to differentiate between applications of established ageing methods, and attempts to develop, improve or validate an ageing method. Although it was difficult to identify any major developments in the *Annual Age and Growth* category, the importance of this class of information is difficult to ignore, as it forms the basis for most comparisons of growth rate and survival among environments and populations, not to mention fish stock assessments and life history studies (Campana and Thorrold 2001).

Age validation and ageing method comparisons (10% of recent papers)

This category includes those papers that compare among ageing methods, or provide the results of an age validation study, either with or without accompanying estimates of population longevity or growth rate. Examples of this type of study included age validation studies which combined several different approaches, such as tetracycline mass marking, mark-recapture and length frequency analysis (Brown *et al.* 2004). Other studies reported both the development and validation of an ageing method, followed by its application to a population for estimation of longevity and growth rate. For example, Dwyer *et al.* (2003) compared ages derived from whole and thin-sectioned otoliths, then applied bomb radiocarbon, tag-recapture analysis and length frequency analysis to validate the accuracy of the method across all age groups before assessing growth rate. Similarly, Brouwer and Griffiths (2004) evaluated the accuracy of whole and sectioned otoliths through comparison of tetracycline mark-recaptures and marginal increment analysis before estimating the growth rate of the population. Although still uncommon, studies using known-age fish had the advantage of allowing specific recommendations to be made concerning optimum age interpretation approaches (Buckmeier 2002).

Major advances in this research category most often centred around improved or more rigorous methods for age validation. Clear *et al.* (2000) demonstrated the successful use of a novel otolith marker (strontium chloride) in a large-scale tag-recapture study of southern bluefin tuna, *Thunnus maccoyii*. The applicability of bomb radiocarbon for validating the ages of long-lived species around the world was broadened when radiocarbon histories for the northwest Pacific (Kerr *et al.* 2004) and northeast Atlantic (Kalish *et al.* 2001) demonstrated that the years during which the bomb signal increased were similar in other areas of the world, even if the radiocarbon levels themselves were not. However, the single largest methodological advance was associated with the development of an improved radiochemical assay for ^{226}Ra, which improved the precision of radiometric age estimates by a factor of 2–10 (Andrews *et al.* 1999). These and other advances in the field were covered in two substantial reviews of age validation methods (Campana 2001; Panfili *et al.* 2002).

Population dynamics (6% of recent papers)

Although arguably a subset of annual age and growth papers, this category was defined to include studies which made more sophisticated use of otolith annual growth patterns, such as growth back-calculation. Recent years continued to see studies where growth back-calculations were used to provide length-at-age sequences for populations or species, which were then related to the ambient environmental conditions of the time (Dutil *et al.* 1999). In addition, there were continuing efforts to improve upon existing growth back-calculation models through addition of an age effect to the model (Morita and Matsuishi 2001; Finstad 2003). Klumb *et al.* (2001) implemented a controlled experiment in which oxytetracycline was used to mark the otolith at periodic intervals during the experiment, and thus evaluate the relative accuracy of competing back-calculation models without having to invoke assumptions of ageing accuracy.

The most significant developments in this research category revolved around the use of growth back-calculations to assess the influence of size-selective mortality on previous life history stages. Good *et al.* (2001) compared the radius of the hatch check in newly-emerged and older salmon to infer the extent of year-to-year variation in size-selective mortality at the fry stage. Using a similar principle, Gronkjaer and Schytte (1999) used the radius of the otolith hatch check as an unambiguous marker to demonstrate that larvae which were smaller at hatch were less likely to survive to the first few weeks of life. However, the most comprehensive study of this type was one based on more than 13 000 back-calculations of cod otoliths, which were used to estimate both the direction and magnitude of size-selective mortality as a result of fishing (Sinclair *et al.* 2002).

Hearing and balance (1% of recent papers)

There were only a handful of papers published on otolith function since 1998, which is ironic given that hearing and balance are the two central functions of the fish otolith. Several descriptive studies explored form and function in the otolith or inner ear (Parmentier *et al.* 2001; Aguirre 2003). However, it was a pair of experimental studies that provided the most insight into otolith function. Taking advantage of a developmental anomaly, Riley and Moorman (2000) demonstrated that the lapilli, but not the sagittae, are required by the fish for balance and survival. And in an unrelated study, the removal of one sagitta reduced hearing sensitivity, while the removal of both sagittae resulted in robust hearing losses, thereby demonstrating that the saccule plays an important role in directional hearing and frequency responses (Lu and Xu 2002). Synthesising many years of hearing-related research, Popper (2003) provided an important review on the effects of anthropogenic sounds on fishes.

Otolith allometry (4% of recent papers)

Included in this group of papers were those dealing with the factors influencing either otolith shape or the allometry between otolith growth and somatic growth. Since otolith shape has continued to be examined for its utility for distinguishing among fish stocks (see *Tracer Applications*), 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). Applications of this principle were illustrated in studies where the life history of the fish was reconstructed on the basis of the fish : otolith relationship (Shafer 2000). Perhaps more telling however was a study in which the growth rate effect on the fish-otolith allometry was so strong that traditional methods of growth back-calculation could not be applied to fish that had changed sexes (Munday *et al.* 2004). Applications that rely on this allometry, such as age estimation based on the relationship between fish age and otolith weight, continue to be improved (Pilling *et al.* 2003).

Species identification (6% of recent papers)

A surprising number of predation studies rely upon the identification of otoliths recovered from stomachs or droppings to reconstruct the species of the prey. In this type of study, the otolith is not the subject of the study per se, but a tool to answer a predation question. For example, the extent of predation of shrimp on juvenile plaice in a shallow-water nursery was assessed by identifying plaice otoliths in shrimp stomachs (Wennhage and Pihl 2001). The basis for these types of studies is either a reference collection of otoliths from the local fish species, or published atlases of otolith shape across a wide range of species. Two such atlases were published in recent years: the first comprehensive examination of asteriscal otoliths in teleosts (Assis 2003), and the first photographic atlas of otoliths from fishes of the northwest Atlantic (Campana 2004). Since studies of seal predation on fish rely extensively on otoliths recovered from stomachs, Bowen (2000) experimentally assessed the rate of loss of otoliths as a result of digestion, and then calculated the impact on perceived predation rates.

Tracer applications (12% of recent papers)

Although the metabolic stability of otoliths has been recognised for many years, natural tracer applications have become a very active area for research only over the past decade. The approach is based on natural structures or chemical features formed in response to environmental or genetic factors which leave a permanent natural marker recorded in the otolith of the fish experiencing the same condition. Thus the marker serves as a natural tag or tracer of that same group of fish throughout its life. Although most of the recent research effort has been focused on chemical tracers, alternative and novel markers such as those owing to unusual microstructural growth patterns formed in the early life history have been used (Quinn *et al.* 1999). Otolith shape is not a permanent marker, but several studies reported success in using it to distinguish among populations (Begg *et al.* 2001).

Assays of Sr : Ca along otolith transects continues to be a common means of reconstructing migrations among environments with different salinities. This type of study is most applicable to diadromous fishes where the signal is most marked, such as the detection of catadromy in eels (Tsukamoto and Arai 2001) and anadromy in salmonids (Howland *et al.* 2001). Where elements other than Sr alone have been used, the objective has usually been to distinguish among groups or populations of a species. For example, Bastow *et al.* (2002) took advantage of the distinct stable oxygen isotope ratio recorded in otoliths by the hypersaline conditions in Shark Bay, Australia to identify pink snapper (*Pagrus auratus*) which grew up in the bay. Although this and other studies used stable isotope ratios as the population marker, the trace element composition of the otolith has more often been used in this type of study. For example, Brazner *et al.* (2004) observed distinct elemental fingerprints in juvenile yellow perch growing up in different wetland nurseries, while Secor *et al.* (2002) noted significant differences in the elemental fingerprint among bluefin tuna (*Thunnus thynnus*) growing up on opposite sides of the Atlantic Ocean. Studies which used a combination of trace elements and stable isotopes to distinguish among populations often achieved the greatest discrimination (Thorrold *et al.* 2001).

Taking the tracer approach to its logical conclusion involves identifying or classifying a group of fish to their source group. An increasing number of studies have been successful in identifying unknown fish in this manner, basing their classifications on maximum likelihood statistical methods and careful sampling of all possible source groups (Campana *et al.* 1999; Gillanders 2002).

An exciting breakthrough in this area of research has been the identification of stable strontium isotopes as a valuable otolith marker in freshwater fishes (Kennedy *et al.* 2000). Although not suited to marine fishes (since seawater shows a uniform stable Sr signal), stable isotopes of Sr appear to vary considerably and consistently among freshwater drainages, and are precisely reflected in the otolith composition. As a result, the movement patterns and origin of individual fishes can often be reconstructed with high fidelity (Kennedy *et al.* 2002; Milton and Chenery 2003). These and other elemental tracers were explored in more detail in various reviews of the field (Campana 1999; Lecomte-Finiger 1999; Thresher 1999).

Mass marking (4% of recent papers)

Mass marking applications use either induced temperature fluctuations or calcium-binding chemicals in the water to induce distinct visual or chemical marks in the otolith at the time of application. The power of the approach lies in the ability to tag thousands or even millions of fish at the same time at minimal cost. Thermal marks continue to be most popular in mass marking young salmonids in a hatchery before their release into the wild (Negus 1999), although one study took advantage of the high proportion of vateritic otoliths formed in hatcheries to distinguish between wild and hatchery-reared fish (Bowen *et al.* 1999). In their review of the thermal marking approach, Volk *et al.* (1999) provided many recommendations for optimising mark detection and producing date-specific marks. Blick and Hagen (2002) made further progress by developing a statistical test for quantifying the results from a thermal marking study.

Chemical marks have been successfully applied to a broad range of species in mass marking studies, with the most popular being alizarin red, alizarin complexone, oxytetracycline and strontium (Skov *et al.* 2001). However until recently, the chemical tags had to be applied in a laboratory setting. In a major breakthrough, Jones *et al.* (1999) demonstrated the first large-scale chemical mass marking of wild fish. By enclosing the egg masses of a coral reef fish in a plastic bag for 1 h at a time, they were able to expose the embryos and their developing otoliths to a tetracycline solution, thus marking the otoliths permanently for later detection during a dispersal study. It seems likely that similar *in situ* marking studies will follow in other habitats.

Trace elements (4% of recent papers)

Although Tracer Applications accounted for most of the research on otolith trace elements, considerable research effort was focused on the factors that influence the uptake and incorporation of trace elements into the otolith. Several studies examined the relationship between water composition and the resulting composition of the otolith, concluding that the incorporation of only selected trace elements was influenced by water composition (Bath *et al.* 2000; Elsdon and Gillanders 2003). The most striking example of this was seen along a pollution gradient, where relatively few of the otolith trace elements changed in response to increasing pollution levels (Hanson and Zdanowicz 1999). The physiology (de Pontual *et al.* 2003) and habitat (Kingsford and Gillanders 2000) of the fish often played a substantive role. Fortunately, preservation in ethanol was shown not be an influential factor, producing little effect on otolith concentrations of either Sr or Ba (Hedges *et al.* 2004).

Analytically, the production of the first certified otolith reference material provided a standard against which all otolith assays could be compared (Yoshinaga *et al.* 2000). In addition to a review on otolith chemistry and composition (Campana 1999), a review on laser-based assay techniques was published (Jones and Chen 2003).

Isotopes (1% of recent papers)

Stable isotopes play a central role in both Tracer Applications and Environmental Reconstructions, and radioisotopes are a central component of Age Validation, but the emphasis here is on studies examining the incorporation of stable isotopes and radioisotopes into otoliths, plus some new applications. Loss, not incorporation, was the subject of a study which reported that the depuration rate of gaseous radon from halibut otoliths was too low to substantially affect age estimates derived from $^{226}\mathrm{Ra}:^{210}\mathrm{Pb}$ radiochemical dating (Kastelle and Forsberg 2002). However, most of the remaining studies in this category dealt with stable isotopes. Factors influencing the incorporation of stable carbon and oxygen isotopes were examined in both field (Blamart *et al.* 2002) and laboratory studies (Høie *et al.* 2004), confirming that oxygen isotopes are deposited in equilibrium with that of the water while carbon isotopes are not. In a unique perspective, Sherwood and Rose (2003) suggested that otolith $\delta^{13}\mathrm{C}$ could serve as a useful proxy for swimming form. Meanwhile, a new stable isotope marker, sulfur, was added to the inventory of otolith isotopes (Weber *et al.* 2002). Although applications for its use need to be better defined, initial examinations suggest that sulfur isotope ratios may provide a proxy for feeding history.

Environmental reconstruction (<1% of recent papers)

A long-standing goal of fisheries science has been to take advantage of our understanding of the environmental factors which influence the elemental and isotopic composition of otoliths to reconstruct the previous (and unknown) environmental history of the fish. Experiments to better define environmental influences on otolith composition continue to demonstrate an environmental effect, but with a significant interaction between temperature and salinity that complicates the extraction of the temperature or salinity effect by itself (Elsdon and Gillanders 2002). Challenges such as these may explain why prehistoric environmental reconstructions have provided most of the high-profile successes, since the absence of alternative approaches means that the underlying assumptions of the approach must be accepted. Stable oxygen isotopes played the most significant role here, having been used to reconstruct the ambient temperatures millions of years ago to within several degrees celsius (Ivany *et al.* 2000; Andrus *et al.* 2002). In each case, otoliths of fish species with narrow salinity preferences were selected so as to minimise interpretation difficulties owing to the unknown water composition. In the most ambitious application of this type, Carpenter *et al.* (2003) used otolith strontium and oxygen stable isotope ratios to reconstruct the movements of a Cretaceous fish between freshwater and estuarine environments, and then used oxygen isotope ratios to constrain its temperature environment.

Fossils (<1% of recent papers)

Whether a fossil otolith can be distinguished from an unaltered old otolith is not always clear, since otoliths hundreds of years old can appear unaltered from their original state. Ignoring this distinction for the moment, and as discussed in the previous section, many of the studies which reconstructed paleoenvironments were based upon fossil otoliths. However, it is also possible to reconstruct historic fishing patterns and pre-fishing growth rates using fossil otoliths. In one such study, the recent growth rates of four commercially fished species were compared with the growth rates of several hundred years ago, based on archaeological excavations of otoliths from European fishing communities at a time when overall fishing effort was minimal (Bolle *et al.* 2004). Somewhat surprisingly, not all of the species showed large increases in growth rate as a result of heavy exploitation. The ability to estimate past growth rates and season of fishing from growth patterns visible in fossil otoliths was discussed in a review by Woydack and Morales-Nin (2001).

Methods (3% of recent papers)

In a field with such a high reliance on methodology, it is not surprising that methodological advances have played a significant role in otolith research. Some of this research has been directed at better methods for visualising growth increments, whether through application of dyes and stains (Green *et al.* 2002) or through the development of semi-automated image analysis methods for identifying and counting increments (Takashima *et al.* 2000). Truly novel approaches have also been developed, such as a method for carrying out three-dimensional otolith analysis (Hamrin *et al.* 1999). Rapid instrumental development has also played a major role in studies of otolith chemistry (Arslan and Paulson 2002), particularly with the latest generation of high-resolution inductively-coupled plasma mass-spectrometry instruments coupled to lasers, capable of analysing both trace elements and isotopic ratios at a temporal scale of weeks (Thorrold and Shuttleworth 2000). The development of computerised micromilling machines has greatly simplified the ability to micro-sample seasonal or annual growth increments for either trace element or stable isotope assays (Wurster *et al.* 1999).

Along with the technical advances have come improved statistical techniques for analysis of otolith-derived data. In a welcome advance, Cappo *et al.* (2000) developed a rigorous statistical method for quantifying the state of development of the marginal growth increment, well suited to assessing the timing and periodicity of growth increment formation. A statistical method for relating mark-recapture data to otolith increments was also reported (Govender 1999). Powerful statistical methods for analysing annulus-based size-at-age data (Schaalje *et al.* 2002) and for estimating individual growth variability (Pilling *et al.* 2002) should provide an improved ability to extract information from data, something that is warranted given the effort that goes into obtaining the growth data.

Physiology (2% of recent papers)

Some of the more exciting developments in basic research on otoliths occurred in the fields of physiology and biomineralisation. Laboratory studies suggested that glycogen may serve an important role in the initial seeding of the otolith polycrystal (Pisam *et al.* 2002). In addition, the 'starmaker' gene has now been identified as the most likely controller of crystal size and lattice formation in otoliths (Soellner *et al.* 2003). Several other studies examined the role of endolymph chemistry on otolith growth (Payan *et al.* 1999; Takagi 2002; Tohse *et al.* 2004), reinforcing the view that endolymph pH is a driving force behind otolith biomineralisation. Even at the daily level, endolymph composition is tightly correlated with diel variations in otolith growth (Edeyer *et al.* 2000; Tohse and Mugiya 2002). However, not all aspects of otolith growth are under chemical control: in a neurological study, Anken *et al.* (2000) reported that an intact vestibular nerve was required for calcium incorporation into the growing otolith.

Interface of otolith science with other disciplines

The broad range of research and applications being carried out with otoliths in recent years points to an active and influential field of science. With areas of study as diverse as age determination, larval fish ecology, population dynamics, fish physiology, population tracking and environmental reconstruction, it is clear that much of fish and fisheries science is affected by, or dependent on, otoliths. The growth or vitality of a research field however must be measured in a different manner. Obviously, publication rate is one such measure, and the increasing rate of otolith-oriented publications indicates that the field is growing. An additional measure of the vitality of a field is the extent to which it interacts with other disciplines. Currently, otolith research is working at the interface of several scientific disciplines. Most prominent among these is geochemistry, where much of the technology and some of the principles are shared with otolith science. Although the development of otolith chemistry through the 1990s saw a largely one-way transfer of information and technology from geochemistry into otolith studies, the information transfer route now occurs in both directions. This is most evident in the environmental reconstructions of paleoenvironments based on isotopic assays of fossil fish otoliths, and in the development of laser-based and microsampling instrumentation.

Areas where otolith research is impacting the scientific study of organisms other than fish are also increasing in numbers. Recent examples include the use of elemental fingerprints to reconstruct the migration patterns of squid in the southern ocean (Arkhipkin *et al.* 2004), and the use of bomb radiocarbon to determine the age of sea urchins (Ebert and Southon 2003). Of interest though, are two examples where the expected interdisciplinary interchange has not occurred: age determination of non-aquatic organisms and dendrochronology. In the case of dendrochronology, it appears that relatively little research to develop or improve methods is now being conducted. Thus, tree rings continue to be important tools for science (Briffa *et al.* 1990), but only in an applied sense. One would think that research that better linked the two disciplines would be mutually beneficial. In contrast, age determinations of non-aquatic organisms would appear to be an area where, logically, the advanced state of otolith ageing methods would be expected to be influential. The less-developed approaches applied in age determinations of terrestrial vertebrates and other non-aquatic organisms may reflect the fact that terrestrial zoologists pay relatively little attention to the aquatic journals where fish biologists typically publish. Perhaps this is an indication that some otolith-oriented science needs (or deserves) to be published for a more diverse audience.

Challenges for the future

Although the search for the ideal ageing method, chemical tracer, etc. will undoubtedly continue for the foreseeable future, there are several areas of otolith research that are in urgent need of study. In contrast, there are other areas where all of the required elements appear to be in place, and thus poised to answer existing questions in the very near future. Here I present a few illustrative examples of these research areas.

Methods for validating the ages of deepsea fishes are urgently required. Many deepsea fishes appear to be very long-lived and slow-growing, and thus very susceptible to overfishing. Turbot (*Scophthalmus maximus*), oreo (*Pseudocyttus* spp.), and in particular, orange roughy (*Hoplostethus atlanticus*) are prime examples of species which may live to ages of 100 years or more (Tracey and Horn 1999) with exceedingly slow growth rates, yet are now being fished commercially. Standard mark-recapture methods are impractical for species which live at depths of >500 m, with swimbladders that rupture long before they reach the surface. Bomb radiocarbon age validation, which is so well suited to many other long-lived fishes, is of little value at a depth where the bomb signal has either not yet penetrated, or did so at some unknown date. And even the oft-abused marginal increment analysis is of questionable value in species where the annuli are poorly defined and the age interpretations challenging. This is one area of otolith research where a creative solution is urgently required.

Also of relevance to age determination is the challenge of recognising the limits of ageing very old fish, if indeed there are any. Several reports suggest that such limits exist. For example, Beamish and McFarlane (2000) recaptured several tetracyline-injected sablefish (*Anoplopoma fimbria*) who showed far fewer annuli than were expected based on the number of years at liberty after tagging. Similarly, Dwyer *et al.* (2003) noted a consistent age underestimation of 2–4 years in very old yellowtail flounder (*Limanda ferruginea*) whose minimum age was known based on bomb radiocarbon. Are these cases merely unusual anomalies, or are they reflective of a more general phenomenon whereby traditional ageing methods can fail in extremely slow-growing or old fishes? And if the latter, is it just the method that is at fault? For instance, age determinations from high quality otolith sections of 40+ year-old lake trout (*Salvelinus namaycush*) consistently underestimated the bomb validated age until the importance of using a compound microscope to observe extremely narrow annuli near the margin was recognised (S. E. Campana, C. Jones and J. Casselman, unpublished data).

One area that seems poised for rapid advancement in the near future involves the development of more efficient methods for production ageing, taking advantage of the frequently-noted relationship between otolith weight and age. Several studies have documented this relationship, whereby the otoliths of slow-growing fish weigh more than do faster-growing fish of the same length (Templeman and Squires 1956). Despite repeated demonstrations that the relationship between otolith weight and age can be used to distinguish among age groups better than other somatic measures (Pilling *et al.* 2003), a practical framework through which this relationship can be used to reduce the cost of age determination has yet to be proposed. The recent development of a statistical framework for linking otolith weight, fish length and age information (Francis and Campana 2004) suggests that it may soon be possible to reduce the cost of production ageing for at least some species.

The development of an otolith growth model would not only improve the accuracy of growth back-calculations, it could lead to simplified methods for reconstructing life histories and environmental exposures, among other things. For example, recent experimental work has indicated that the translucency of the otolith is at least partially a function of temperature and somatic growth rate (Hüssy *et al.* 2004). Would it be possible then to use measurements of otolith growth rate and translucency, without more expensive assays of otolith composition, to infer growth temperature and scope for growth? And can a growth back-calculation method be developed, which avoids the 10–15% uncertainty that often accompanies existing growth back-calculations? A robust model of otolith growth that takes advantage of recent physiological advances is almost certainly required to meet these goals.

Although challenges such as the development of an otolith growth model may not be solved in the near future, there are several specific goals that should be more tractable. For instance, an increasing number of otolith chemistry studies are using trace elements or isotopes for which the sole existing certified reference material is not suited (Yoshinaga *et al.* 2000). A more fully characterised otolith reference material is very much required. Of course, laser-based otolith assays lack any form of a standard whatsoever, necessitating the use of glass-based assay standards or pressed otolith powders with very different ablation characteristics than real otoliths (Campana 1999). A very different type of challenge faces those attempting to validate the age determinations of a species using bomb radiocarbon, for which otoliths of fish born in the 1958–1967 period can be invaluable. An unknown but large number of otolith collections exist around the world, yet most of these collections remain known only to those institutions that collected them. Better documentation concerning the existence and contents of these archives would help not only those seeking to validate age determinations, but provide the material for genetic reconstructions of historic populations using the small amounts of dried tissue which often adheres to otoliths after collection (Hutchinson *et al.* 1999). Once again, this is an example of relatively simple applied work that could quickly lead to tangible benefits in the near term.

References

Aguirre, W. E. (2003). Allometric growth of the sulcus in *Cynoscion* spp. (Sciaenidae). *Journal of Fish Biology* **63**, 1341–1346. doi:10.1046/J.1095-8649.2003.00238.X

Anderson, J. T., and Dalley, E. L. (2000). Interannual differences in hatching times and growth rates of pelagic juvenile cod in Newfoundland waters. *Fisheries Research* **46**, 227–238. doi:10.1016/S0165-7836(00)00148-X

Andrews, A. H., Coale, K. H., Nowicki, J. L., Lundstrom, C., Palacz, Z., Burton, E. J., and Cailliet, G. M. (1999). Application of an ion-exchange separation technique and thermal ionization mass spectrometry to ^{226}Ra determination in otoliths for radiometric age determination of long-lived fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1329–1338. doi:10.1139/CJFAS-56-8-1329

Andrus, C. F., Crowe, D. E., Sandweiss, D. H., Reitz, E. J., and Romanek, C. S. (2002). Otolith δ^{18}O record of mid-Holocene sea surface temperatures in Peru. *Science* **295**, 1508–1511. doi:10.1126/SCIENCE.1062004

Anken, R. H., Edelmann, E., and Rahmann, H. (2000). Fish inner ear otoliths stop calcium incorporation after vestibular nerve transection. *Neurological Report* **11**, 2981–2983.

Arkhipkin, A. I., Campana, S. E., Fitzgerald, J., and Thorrold, S. R. (2004). Spatial and temporal variation in elemental signatures of statoliths from the Patagonian longfin squid (*Loligo gahi*). *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1212–1224. doi:10.1139/F04-075

Arslan, Z., and Paulson, A. J. (2002). Analysis of biogenic carbonates by inductively-coupled plasma mass-spectrometry (ICP-MS). Flow injection on-line solid-phase preconcentration for trace element determination in fish otoliths. *Analysis and Bioanalysis Chemistry* **372**, 776–785. doi:10.1007/S00216-002-1274-2

Assis, C. A. (2003). The lagenar otoliths of teleosts: their morphology and its application in species identification, phylogeny and systematics. *Journal of Fish Biology* **62**, 1268–1295. doi:10.1046/J.1095-8649.2003.00106.X

Barber, M. C., and Jenkins, G. P. (2001). Differential effects of food and temperature lead to decoupling of short-term otolith and somatic growth rates in juvenile King George whiting. *Journal of Fish Biology* **58**, 1320–1330. doi:10.1006/JFBI.2000.1539

Bastow, T. P., Jackson, G., and Edmonds, J. S. (2002). Elevated salinity and isotopic composition of fish otolith carbonate: stock delineation of pink snapper, *Pagrus auratus*, in Shark Bay, Western Australia. *Marine Biology* **141**, 801–806. doi:10.1007/S00227-002-0884-8

Bath, G. E., Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W., and Lam, J. W. H. (2000). Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* **64**, 1705–1714. doi:10.1016/S0016-7037(99)00419-6

Beamish, R. J., and McFarlane, G. A. (2000). Reevaluation of the interpretation of annuli from otoliths of a long-lived fish, *Anoplopoma fimbria. Fisheries Research* **46**, 105–111. doi:10.1016/S0165-7836(00)00137-5

Begg, G. A., Overholtz, W. J., and Munroe, N. J. (2001). The use of internal otolith morphometrics for identification of haddock (*Melanogrammus aeglefinus*) stocks on Georges Bank. *Fishery Bulletin* **99**, 1–14.

Blamart, D., Escoubeyrou, K., Juillet-Leclerc, A., Ouahdi, R., and Lecomte-Finiger, R. (2002). Composition isotopique δ^{18}O-δ^{13}C des otolithes des populations de poissons récifaux de Taiaro (Yuamotu, Polynésie francaise): implications isotopiques et biologiques. *Comptes Rendu Biologies* **325**, 1–8 [In French]. doi:10.1016/S1631-0691(02)01420-8

Blick, D. J., and Hagen, P. T. (2002). The use of agreement measures and latent class models to assess the reliability of classifying thermally marked otoliths. *Fishery Bulletin* **100**, 1–10.

Bolle, L. J., Rijnsdorp, A. D., van Neer, W., Millner, R. S., van Leeuwen, P. I., Ervynck, A., Ayers, R., and Ongenae, E. (2004). Growth changes in plaice, cod, haddock and saithe in the North Sea: a comparison of (post-)medieval and present-day growth rates based on otolith measurements. *Journal of Sea Research* **51**, 313–328. doi:10.1016/J.SEARES.2004.01.001

Bowen, W. D. (2000). Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 898–905. doi:10.1139/CJFAS-57-5-898

Bowen, C. A., II, Bronte, C. R., Argyle, R. L., Adams, J. V., and Johnson, J. E. (1999). Vateritic sagitta in wild and stocked lake trout: applicability to stock origin. *Transactions of the American Fisheries Society* **128**, 929–938. doi:10.1577/1548-8659(1999)128<0929:VSIWAS>2.0.CO;2

Brazner, J. C., Campana, S. E., and Tanner, D. K. (2004). Habitat fingerprints for Lake Superior coastal wetlands derived from elemental analysis of yellow perch otoliths. *Transactions of the American Fisheries Society* **133**, 692–704. doi:10.1577/T03-091.1

Briffa, K. R., Bartholin, T. S., Eckstein, D., Jones, P. D., Karlen, W., Schweingruber, F. H., and Zetterberg, P. (1990). A 1,400-year treering record of summer temperatures in Fennoscandia. *Nature* **346**, 434–439. doi:10.1038/346434A0

Brophy, D., and Danilowicz, B. S. (2002). Tracing populations of Atlantic herring (*Clupea harengus*) in the Irish and Celtic Seas using otolith microstructure. *International Council for the Exploration of the Sea Journal of Marine Science* **59**, 1305–1313.

Brouwer, S. L., and Griffiths, M. H. (2004). Age and growth of *Argyrozona argyrozona* (Pisces: Sparidae) in a marine protected area: an evaluation of methods based on whole otoliths, sectioned otoliths and mark-recapture. *Fisheries Research* **67**, 1–12. doi:10.1016/J.FISHRES.2003.08.007

Brown, P., Green, C., Sivakumaran, K. P., Stoessel, D., and Giles, A. (2004). Validating otolith annuli for annual age determination of common carp. *Transactions of the American Fisheries Society* **133**, 190–196. doi:10.1577/T02-148

Buckmeier, D. L. (2002). Assessment of reader accuracy and recommendations to reduce subjectivity in age estimation. *Fisheries* **27**, 10–14. doi:10.1577/1548-8446(2002)027<0010:AORAAR>2.0.CO;2

Bunnell, D. B., Gonzalez, M. J., and Stein, R. A. (2003). Zooplankton biomass enhances growth, but not survival, of first-feeding *Pomoxis* spp. larvae. *Canadian Journal of Fisheries and Aquatic Sciences* **60**, 1314–1323. doi:10.1139/F03-112

Campana, S. E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* **188**, 263–297.

Campana, S. E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59**, 197–242. doi:10.1006/JFBI.2001.1668

Campana, S. E. (2004). 'Photographic Atlas of Fish Otoliths of the Northwest Atlantic Ocean.' (NRC Research Press: Ottawa.)

Campana, S. E., and Thorrold, S. R. (2001). Otoliths, increments and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 30–38. doi:10.1139/CJFAS-58-1-30

Campana, S. E., Chouinard, G. A., Hanson, J. M., and Fréchet, A. (1999). Mixing and migration of overwintering cod stocks near the mouth of the Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1873–1881. doi:10.1139/CJFAS-56-10-1873

Cappo, M., Eden, P., Newman, S. J., and Robertson, S. (2000). A new approach to validation of periodicity and timing of opaque zone formation in the otoliths of eleven species of *Lutjanus* from the central Great Barrier Reef. *Fishery Bulletin* **98**, 474–488.

Cardinale, M., Doering-Arjes, P., Kastowsky, M., and Mosegaard, H. (2004). Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 158–167. doi:10.1139/F03-151

Carpenter, S. J., Erickson, J. M., and Holland, F. D., Jr (2003). Migration of a late Cretaceous fish. *Nature* **423**, 70–74. doi:10.1038/NATURE01575

Clear, N. P., Gunn, J. S., and Rees, A. J. (2000). Direct validation of annual increments in the otoliths of juvenile southern bluefin tuna, *Thunnus maccoyii*, by means of a large-scale mark-recapture experiment with strontium chloride. *Fishery Bulletin* **98**, 25–40.

de Pontual, H., Lagardère, F., Amara, R., Bohn, M., and Ogor, A. (2003). Influence of ontogenetic and environmental changes in the otolith microchemistry of juvenile sole (*Solea solea*). *Journal of Sea Research* **50**, 199–210. doi:10.1016/S1385-1101(03)00080-7

Dutil, J.-D., Castonguay, M., Gilbert, D., and Gascon, D. (1999). Growth, condition, and environmental relationships in Atlantic cod (*Gadus morhua*) in the northern Gulf of St. Lawrence and implications for management strategies in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1818–1831. doi:10.1139/CJFAS-56-10-1818

Dwyer, K. S., Walsh, S. J., and Campana, S. E. (2003). Age determination, validation and growth of Grand Bank yellowtail flounder (*Limanda ferruginea*). *International Council for the Exploration of the Sea Journal of Marine Science* **60**, 1123–1138.

Ebert, T. A., and Southon, J. R. (2003). Red sea urchins (*Strongylocentrotus franciscanus*) can live over 100 years: confirmation with A-bomb 14carbon. *Fishery Bulletin* **101**, 915–922.

Edeyer, A., de Pontual, H., Payan, P., Troadec, H., Sévère, A., and Mayer-Gostan, N. (2000). Daily variations of the saccular endolymph and plasma compositions in the turbot *Psetta maxima*: relationship with the diurnal rhythm in otolith formation. *Marine Ecology Progress Series* **192**, 287–294.

Elsdon, T. S., and Gillanders, B. M. (2002). Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 1796–1808. doi:10.1139/F02-154

Elsdon, T. S., and Gillanders, B. M. (2003). Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecology Progress Series* **260**, 263–272.

Fey, D. P. (2002). Formation of daily increments in otoliths of larval and juvenile herring (*Clupea harengus*) and early juvenile smelt (*Osmerus eperlanus*) in low-food conditions. *Archives of Fisheries and Marine Research* **49**, 189–197.

Finstad, A. G. (2003). Growth backcalculation based on otoliths incorporating an age effect: adding an interaction term. *Journal of Fish Biology* **62**, 1222–1225. doi:10.1046/J.1095-8649.2003.00102.X

Fox, C. J., Folkvord, A., and Geffen, A. J. (2003). Otolith micro-increment formation in herring *Clupea harengus* larvae in relation to growth rate. *Marine Ecology Progress Series* **264**, 83–94.

Francis, R. I. C. C., and Campana, S. E. (2004). Inferring age from otolith measurements: a review and a new approach. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1269–1284. doi:10.1139/F04-063

Gillanders, B. (2002). Connectivity between juvenile and adult fish populations: do adults remain near their recruitment estuaries? *Marine Ecology Progress Series* **240**, 215–223.

Good, S. P., Dodson, J. J., Meekan, M. G., and Ryan, D. A. J. (2001). Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1187–1195. doi:10.1139/CJFAS-58-6-1187

Govender, A. (1999). A statistical procedure for validating age estimates from otolith bands using mark-recapture data, with an illustration for *Pomatomus saltatrix*. *South African Journal of Marine Science* **21**, 1–8.

Green, B. S., Reilly, S. M., and McCormick, M. I. (2002). A cost-effective method of preparing larval fish otoliths for reading using enzyme digestion and staining. *Journal of Fish Biology* **61**, 1600–1605. doi:10.1006/JFBI.2002.2177

Gronkjaer, P., and Schytte, M. (1999). Non-random mortality of Baltic cod larvae inferred from otolith hatch-check sizes. *Marine Ecology Progress Series* **181**, 53–59.

Hamrin, S. F., Arneri, E., Doering-Arjes, P., Mosegaard, H., Patwardhan, A., Sasov, A., Schatz, M., van Dyck, D., Wickstrom, H., and van Heel, M. (1999). A new method for three-dimensional otolith analysis. *Journal of Fish Biology* **54**, 223–225. doi:10.1006/JFBI.1998.0855

Hanson, P. J., and Zdanowicz, V. S. (1999). Elemental composition of otoliths from Atlantic croaker along an estuarine pollution gradient. *Journal of Fish Biology* **54**, 656–668. doi:10.1006/JFBI.1998.0903

Hedges, K. J., Ludsin, S. A., and Fryer, B. J. (2004). Effects of ethanol preservation on otolith microchemistry. *Journal of Fish Biology* **64**, 923–937. doi:10.1111/J.1095-8649.2004.00353.X

Høie, H., Otterlei, E., and Folkvord, A. (2004). Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua*). *International Council for the Exploration of the Sea Journal of Marine Science* **61**, 243–251.

Howland, K. L., Tonn, W. M., Babaluk, J. A., and Tallman, R. F. (2001). Identification of freshwater and anadromous inconnu in the Mackenzie River system by analysis of otolith strontium. *Transactions of the American Fisheries Society* **130**, 725–741. doi:10.1577/1548-8659(2001)130<0725:IOFAAI>2.0.CO;2

Hüssy, K., Mosegaard, H., and Jessen, F. (2004). Effect of age and temperature on amino acid composition and the content of different protein types of juvenile cod (*Gadus morhua* L.) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1012–1020. doi:10.1139/F04-037

Hutchinson, W. F., Carvalho, G. R., and Rogers, S. I. (1999). A non-destructive technique for recovery of DNA from dried fish otoliths for subsequent molecular genetic analysis. *Molecular Ecology* **8**, 893–894.

Ivany, L. C., Patterson, W. P., and Lohmann, K. C. (2000). Cooler winters as a possible cause of mass extinctions at the Eocene/Oligocene boundary. *Nature* **407**, 887–890. doi:10.1038/35038044

Jones, C. M., and Chen, Z. (2003). New techniques for sampling larval and juvenile fish otoliths for trace element analysis with laser-ablation sector-field inductively-coupled plasma mass spectrometry (SF-ICP-MS). In 'The Big Fish Bang. Proceedings of the 26th Annual Larval Fish Conference'. (Eds H. I. Browman and A. B. Skiftesvik.) pp. 432–443. (Institute of Marine Science: Bergen.)

Jones, G. P., Milicich, M. J., Emslie, M. J., and Lunow, C. (1999). Self-recruitment in a coral reef fish population. *Nature* **402**, 802–804. doi:10.1038/45538

Kalish, J. M., Nydal, R., Nedreaas, K. H., Burr, G. S., and Eine, G. L. (2001). A time history of pre- and post-bomb radiocarbon in the Barents Sea derived from Arcto-Norwegian cod otoliths. *Radiocarbon* **43**, 843–855.

Kastelle, C. R., and Forsberg, J. E. (2002). Testing for loss of ^{222}Rn from Pacific halibut (*Hippoglossus stenolepis*) otoliths. *Fisheries Research* **57**, 93–98. doi:10.1016/S0165-7836(01)00339-3

Kennedy, B. P., Blum, J. D., Folt, C. L., and Nislow, K. H. (2000). Using natural strontium isotopic signatures as fish markers: methodology and application. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 2280–2292. doi:10.1139/CJFAS-57-11-2280

Kennedy, B. P., Klaue, A., Blum, J. D., Folt, C. L., and Nislow, K. H. (2002). Reconstructing the lives of fish using Sr isotopes in otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 925–929. doi:10.1139/F02-070

Kerr, L. A., Andrews, A. H., Frantz, B. R., Coale, K. H., Brown, T. A., and Cailliet, G. M. (2004). Radiocarbon in otoliths of yelloweye rockfish (*Sebastes ruberrimus*): a reference time series for the coastal waters of southeast Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 443–451. doi:10.1139/F04-009

Kingsford, M. J., and Gillanders, B. M. (2000). Variation in concentrations of trace elements in otoliths and eye lenses of a temperate reef fish, *Parma microlepis*, as a function of depth, spatial scale and age. *Marine Biology* **137**, 403–414. doi:10.1007/S002270000304

Klumb, R. A., Bozek, M. A., and Frie, R. V. (2001). Validation of three back-calculation models by using multiple oxytetracycline marks formed in the otoliths and scales of bluegill × green sunfish hybrids. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 352–364. doi:10.1139/CJFAS-58-2-352

Laidig, T. E., Pearson, D. E., and Sinclair, L. L. (2003). Age and growth of blue rockfish (*Sebastes mystinus*) from central and northern California. *Fishery Bulletin* **101**, 800–808.

Lecomte-Finiger, R. (1999). L'otolithe: la "boite noire" des Téléostéens. *Année Biologiques* **38**, 107–122 [In French]. doi:10.1016/S0003-5017(99)80029-4

Lu, Z., and Xu, Z. (2002). Effects of saccular otolith removal on hearing sensitivity of the sleeper goby (*Dormitator latifrons*). *Journal of Comparative Physiology* **188A**, 595–602.

Meekan, M. G., Carleton, J. H., McKinnon, A. D., Flynn, K., and Furnas, M. (2003). What determines the growth of tropical reef fish larvae in the plankton: food or temperature? *Marine Ecology Progress Series* **256**, 193–204.

Milton, D. A., and Chenery, S. R. (2003). Movement patterns of the tropical shad hilsa (*Tenualosa ilisha*) inferred from transects of ^{87}Sr/^{86}Sr isotope ratios in their otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **60**, 1376–1385. doi:10.1139/F03-133

Morita, K., and Matsuishi, T. (2001). A new model of growth back-calculation incorporating age effect based on otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1805–1811. doi:10.1139/CJFAS-58-9-1805

Munday, P. L., Hodges, A. L., Choat, J. H., and Gust, N. (2004). Sex-specific growth effects in protogynous hermaphrodites. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 323–327. doi:10.1139/F04-057

Negus, M. T. (1999). Thermal marking of otoliths in lake trout sac fry. *North American Journal of Fisheries Management* **19**, 127–140. doi:10.1577/1548-8675(1999)019<0127:TMOOIL>2.0.CO;2

Oozeki, Y., and Watanabe, Y. (2000). Comparison of somatic growth and otolith increment growth in laboratory-reared larvae of Pacific saury, *Cololabis saira*, under different temperature conditions. *Marine Biology* **136**, 349–359. doi:10.1007/S002270050693

Panfili, J., and Tomas, J. (2001). Validation of age estimation and back-calculation of fish length based on otolith microstructures in tilapias (Pisces, Cichlidae). *Fishery Bulletin* **99**, 139–150.

Panfili, J., de Pontual, H., Troadec, H., and Wright, P. J. (2002). 'Manual of Fish Sclerochronology.' (Ifremer–IRD: Brest, France.)

Parmentier, E., Vandewalle, P., and Lagardère, F. (2001). Morpho-anatomy of the otic region in carapid fishes: eco-morphological study of their otoliths. *Journal of Fish Biology* **58**, 1046–1061. doi:10.1006/JFBI.2000.1511

Pasten, G. P., Katayama, S., and Omori, M. (2003). Timing of parturition, planktonic duration, and settlement patterns of the black rockfish, *Sebastes inermis*. *Environmental Biology of Fishes* **68**, 229–239. doi:10.1023/A:1027388215711

Payan, P., Edeyer, A., de Pontual, H., Borelli, G., Boeuf, G., and Mayer-Gostan, N. (1999). Chemical composition of saccular endolymph and otolith in fish inner ear: lack of spatial uniformity. *The American Journal of Physiology* **277**, R123–R131.

Pepin, P., Dower, J. F., and Benoit, H. P. (2001). The role of measurement error on the interpretation of otolith increment width in the study of growth in larval fish. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 2204–2212. doi:10.1139/CJFAS-58-11-2204

Pepin, P., Dower, J. F., Helbig, J. A., and Leggett, W. C. (2002). Estimating the relative roles of dispersion and predation in generating regional differences in mortality rates of larval radiated shanny (*Ulvaria subbifurcata*). *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 105–114. doi:10.1139/F01-198

Pilling, G. M., Kirkwood, G. P., and Walker, S. G. (2002). An improved method for estimating individual growth variability in fish, and the correlation between von Bertalanffy growth parameters. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 424–432. doi:10.1139/F02-022

Pilling, G. M., Grandcourt, E. M., and Kirkwood, G. P. (2003). The utility of otolith weight as a predictor of age in the emporer *Lethrinus mahsena* and other tropical fish species. *Fisheries Research* **60**, 493–506. doi:10.1016/S0165-7836(02)00087-5

Pisam, M., Jammet, C., and Laurent, D. (2002). First steps of otolith formation of the zebrafish: role of glycogen? *Cell and Tissue Research* **310**, 163–168. doi:10.1007/S00441-002-0622-Z

Popper, A. N. (2003). Effects of anthropogenic sounds on fishes. *Fisheries* **28**, 24–31.

Quinn, T. P., Volk, E. C., and Hendry, A. P. (1999). Natural otolith microstructure patterns reveal precise homing to natal incubation sites by sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Zoology* **77**, 766–775. doi:10.1139/CJZ-77-5-766

Riley, B. B., and Moorman, S. J. (2000). Development of utricular otoliths, but not saccular otoliths, is necessary for vestibular function and survival in zebrafish. *Journal of Neurobiology* **43**, 329–337.

Schaalje, G. B., Shaw, J. L., and Belk, M. C. (2002). Using nonlinear hierarchical models for analyzing annulus-based size-at-age data. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 1524–1532. doi:10.1139/F02-120

Searcy, S. P., and Sponaugle, S. (2001). Selective mortality during the larval-juvenile transition in two coral reef fishes. *Ecology* **82**, 2452–2470.

Secor, D. H., Campana, S. E., Zdanowicz, V. S., Lam, J. W. H., Yang, L., and Rooker, J. R. (2002). Inter-laboratory comparison of Atlantic and Mediterranean bluefin tuna otolith microconstituents. *International Council for the Exploration of the Sea Journal of Marine Science* **59**, 1294–1304.

Shafer, D. J. (2000). Evaluation of periodic and aperiodic otolith structure and somatic-otolith scaling for use in retrospective life history analysis of a tropical marine goby, *Bathygobius coalitus. Marine Ecology Progress Series* **199**, 217–229.

Sherwood, G. D., and Rose, G. A. (2003). Influence of swimming form on otolith δ^{13}C in marine fish. *Marine Ecology Progress Series* **258**, 283–289.

Sinclair, A. F., Swain, D. P., and Hanson, J. M. (2002). Measuring changes in the direction and magnitude of size-selective mortality in a commercial fish population. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 361–371. doi:10.1139/F02-015

Skov, C., Gronkjaer, P., and Nielsen, C. (2001). Marking pike fry otoliths with alizarin complexone and strontium: an evaluation of methods. *Journal of Fish Biology* **59**, 745–750. doi:10.1006/JFBI.2001.1664

Soellner, C., Burghammer, M., Busch-Nentwich, E., Berger, J., Schwarz, H., Riekel, C., and Nicolson, T. (2003). Control of crystal size and lattice formation by starmaker in otolith biomineralization. *Science* **302**, 282–286. doi:10.1126/SCIENCE.1088443

Strelcheck, A. J., Fitzhugh, G. R., Coleman, F. C., and Koenig, C. C. (2003). Otolith-fish size relationship in juvenile gag (*Mycteroperca microlepis*) of the eastern Gulf of Mexico: a comparison of growth rates between laboratory and field populations. *Fisheries Research* **60**, 255–265. doi:10.1016/S0165-7836(02)00171-6

Takagi, Y. (2002). Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ. *Marine Ecology Progress Series* **231**, 237–245.

Takahashi, M., Watanabe, Y., Kinoshita, T., and Watanabe, C. (2001). Growth of larval and early juvenile Japanese anchovy, *Engraulis japonicus*, in the Kuroshio-Oyashio transition region. *Fisheries Oceanography* **10**, 235–247. doi:10.1046/J.1365-2419.2001.00160.X

Takashima, Y., Takada, T., Matsuishi, T., and Kanno, Y. (2000). Validation of auto-counting method by NIH image using otoliths of white-spotted char *Salvelinus leucomaenis. Fisheries Science* **66**, 515–520. doi:10.1046/J.1444-2906.2000.00081.X

Templeman, W., and Squires, H. J. (1956). Relationship of otolith lengths and weights in the haddock *Melanogrammus aeglefinus* (L.) to the rate of growth of the fish. *Journal of the Fisheries Research Board of Canada* **13**, 467–487.

Thorrold, S. R., and Hare, J. A. (2002). Otolith applications in reef fish ecology. In 'Advances in the Ecology of Fishes on Coral Reefs'. (Ed. P. F. Sale.) pp. 243–264. (Elsevier Science: New York.)

Thorrold, S. R., and Shuttleworth, S. (2000). In situ analysis of trace elements and isotope ratios in fish otoliths using laser ablation sector field inductively coupled plasma mass spectrometry. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1232–1242. doi:10.1139/CJFAS-57-6-1232

Thorrold, S. R., Latkoczy, C., Swart, P. K., and Jones, C. M. (2001). Natal homing in a marine fish metapopulation. *Science* **291**, 297–299. doi:10.1126/SCIENCE.291.5502.297

Thresher, R. E. (1999). Elemental composition of otoliths as a stock delineator in fishes. *Fisheries Research* **43**, 165–204. doi:10.1016/S0165-7836(99)00072-7

Tohse, H., and Mugiya, Y. (2002). Diel variations in carbonate incorporation into otoliths in goldfish. *Journal of Fish Biology* **61**, 199–206. doi:10.1006/JFBI.2002.2033

Tohse, H., Ando, H., and Mugiya, Y. (2004). Biochemical properties and immunohistochemical localization of carbonic anhydrase in the sacculus of the inner ear in the salmon *Oncorhynchus masou. Comparative Biochemistry and Physiology* **137A**, 87–94.

Tracey, D. M., and Horn, P. L. (1999). Background and review of ageing orange roughy (*Hoplostethus atlanticus*, Trachichthyidae) from New Zealand and elsewhere. *New Zealand Journal of Marine and Freshwater Research* **33**, 67–86.

Tsukamoto, K., and Arai, T. (2001). Facultative catadromy of the eel *Anguilla japonica* between freshwater and seawater habitats. *Marine Ecology Progress Series* **220**, 265–276.

Volk, E. C., Schroder, S. L., and Grimm, J. J. (1999). Otolith thermal marking. *Fisheries Research* **43**, 205–219. doi:10.1016/S0165-7836(99)00073-9

Weber, P. K., Hutcheon, I. D., McKeegan, K. D., and Ingram, B. L. (2002). Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life history. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 587–591. doi:10.1139/F02-038

Wennhage, H., and Pihl, L. (2001). Settlement patterns of newly settled plaice (*Pleuronectes platessa*) in a non-tidal Swedish fjord in relation to larval supply and benthic predators. *Marine Biology* **139**, 877–889. doi:10.1007/S002270100648

Wilson, D. T., and Meekan, M. G. (2002). Growth-related advantages for survival to the point of replenishment in the coral reef fish *Stegastes partitus* (Pomacentridae). *Marine Ecology Progress Series* **231**, 247–260.

Wilson, C. A., and Nieland, D. L. (2001). Age and growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. *Fishery Bulletin* **99**, 653–664.

Woydack, A., and Morales-Nin, B. (2001). Growth patterns and biological information in fossil fish otoliths. *Paleobiology* **27**, 369–378.

Wurster, C. M., Patterson, W. P., and Cheatham, M. M. (1999). Advances in micromilling techniques: a new apparatus for acquiring high-resolution oxygen and carbon stable isotope values and major/minor elemental ratios from accretionary carbonate. *Computers & Geosciences* **25**, 1159–1166. doi:10.1016/S0098-3004(99)00052-7

Yoshinaga, J., Nakama, A., Morita, M., and Edmonds, J. S. (2000). Fish otolith reference material for quality assurance of chemical analyses. *Marine Chemistry* **69**, 91–97. doi:10.1016/S0304-4203(99)00098-5

Manuscript received 13 July 2004; revised 22 December 2004; and accepted 28 January 2005.