

## REVIEW

# Chemistry and composition of fish otoliths: pathways, mechanisms and applications

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**ABSTRACT:** The fish otolith (earstone) has long been known as a timekeeper, but interest in its use as a metabolically inert environmental recorder has accelerated in recent years. In part due to technological advances, applications such as stock identification, determination of migration pathways, reconstruction of temperature and salinity history, age validation, detection of anadromy, use as a natural tag and chemical mass marking have been developed, some of which are difficult or impossible to implement using alternative techniques. Microsampling and the latest advances in beam-based probes allow many elemental assays to be coupled with daily or annual growth increments, thus providing a detailed chronological record of the environment. However, few workers have critically assessed the assumptions upon which the environmental reconstructions are based, or considered the possibility that elemental incorporation into the otolith may proceed differently than that into other calcified structures. This paper reviews current applications and their assumptions and suggests future directions. Particular attention is given to the premises that the elemental and isotopic composition of the otolith reflects that of the environment, and the effect of physiological filters on the resulting composition. The roles of temperature, elemental uptake into the fish and the process of otolith crystallization are also assessed. Drawing upon recent advances in geochemistry and paleoclimate research as points of contrast, it appears that not all applications of otolith chemistry are firmly based, although others are destined to become powerful and perhaps routine tools for the mainstream fish biologist.

**KEY WORDS:** Otolith · Element · Isotope · Composition · Stock identification · Temperature history

## INTRODUCTION

Otoliths (earstones) are paired calcified structures used for balance and/or hearing in all teleost fishes. While they are clearly more important to the fish than to the fish biologist, that point is easily forgotten. The use of otoliths as indicators of fish age has now reached the century landmark, starting with Reibisch's observations of otolith annuli in 1899. In 1971, Pannella's discovery of daily growth increments helped propel the interpretation of otolith microstructure into the mainstream of fish biology. Since that time, interest in the otolith as a metabolically inert timekeeper and environmental recorder has accelerated, culminating in 2 recent international symposia devoted solely to fish otoliths (Secor et al. 1995a, Fossum et al. 1999). In

addition to the continued and widespread use and research on the timekeeping properties of the otolith, a new and largely independent sub-discipline of otolith chemistry has emerged which promises to address a suite of questions that have proven difficult or impossible to resolve through other means. Discussed in only 6 papers prior to 1980, the otolith chemistry field has since entered a period of exponential growth, resulting in 157 papers on the topic having been published by the end of 1998. It is the objective of this review to highlight the basis for this rapidly growing interest, and to critically assess the emerging suite of applications involving otolith composition and chemistry.

The same 2 properties of the otolith which allow it to form and retain daily growth increments are also responsible for its ability to record aspects of the environment to which the fish is exposed. The fact that the otolith is acellular and metabolically inert means that

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any elements or compounds accreted onto its growing surface are permanently retained, whereas the continued growth of the otolith from before the time of hatch to the time of death implies that the entire lifetime of the fish has been recorded (Campana & Neilson 1985). In principle then, the otolith may contain a complete record of exposure to both the temperature and composition of the ambient water. When coupled with age- or date-structured examination of otolith growth increments (either annual or daily), the potential for a detailed chronological record of the environment to which the fish was exposed is obvious. In practice, the assay and interpretation of this chemical record is less than clear-cut, in part due to physiological filters between the water and the otolith (Kalish 1989) and in part due to technical difficulties. Nevertheless, there are numerous examples to date of successful reconstructions of the fish's life history, ranging from temperature history (Patterson et al. 1993), detection of anadromy (Secor 1992), determination of migration pathways (Thresher et al. 1994), age validation (Kalish 1993), stock identification (Edmonds et al. 1989), use as a natural tag (Campana et al. 1995) or metabolic indicator (Schwarcz et al. 1998), and for rapid, inexpensive mass marking (Tsukamoto 1985). Other applications exist, and many others have been proposed. Several are not currently possible using other approaches.

This review begins with current hypotheses concerning the pathways and mechanisms underlying the incorporation of natural and anthropogenic elements and isotopes into the otolith. Subsequent sections deal with the extent to which environmental effects (primarily temperature and water composition) are reflected in otolith composition. In light of the fact that otolith assays often require sophisticated instrumentation, some attention is given to the diversity, advantages and disadvantages of the suite of available methodologies. Much of the latter half of the review is then devoted to otolith chemistry applications, both successful and unsuccessful, concluding with speculations concerning future directions. Wherever possible, recent developments in allied fields, particularly geochemical and paleoclimate studies, have been discussed as points of contrast. The overall intent is not only to review existing and potential applications of otolith chemistry to fisheries biology and management, but to critically assess how well otolith composition reflects and records the environmental exposure history of the fish.

## COMPOSITION OF THE OTOLITH

Otolith composition is relatively pure compared to most biological and mineralogical structures, being dominated by calcium carbonate in a non-collagenous organic matrix. A total of 31 elements have been detected in otoliths to date, not including radioactive elements such as Th and Ra. The elemental composition is dominated by the major elements calcium, oxygen and carbon, which make up the calcium carbonate ( $\text{CaCO}_3$ ) matrix. However, the majority of the elements are present at the minor (>100 ppm) and trace (<100 ppm) levels, with many more trace elements presumably awaiting detection. The minor elements are represented by Na, Sr, K, S, N, Cl and P, while the bulk of the trace elements are present at concentrations of less than about 10 ppm (Fig. 1). On the basis of a comprehensive suite of assays, Campana et al. (1997) reported that otoliths of the Atlantic croaker *Micropogonias undulatus* were composed 96.2% by weight of calcium carbonate, with an additional 0.73% present as non-organic trace impurities. By differencing, the organic matrix made up the remaining 3.1%. Other broad spectrum elemental assays of otoliths have also resulted in trace and minor element concentrations of less than 1% (Edmonds et al. 1992, Sie & Thresher 1992, Proctor et al. 1995, Severin et al. 1995) and protein concentrations of about 3 to 4% (range of 1 to 8%) (Degens et al. 1969, Asano & Mugiya 1993, Hoff & Fuiman 1993). While the relative purity of the otolith poses some analytical problems, it also highlights the fact that elemental incorporation into the otolith is not a simple function of environmental availability.

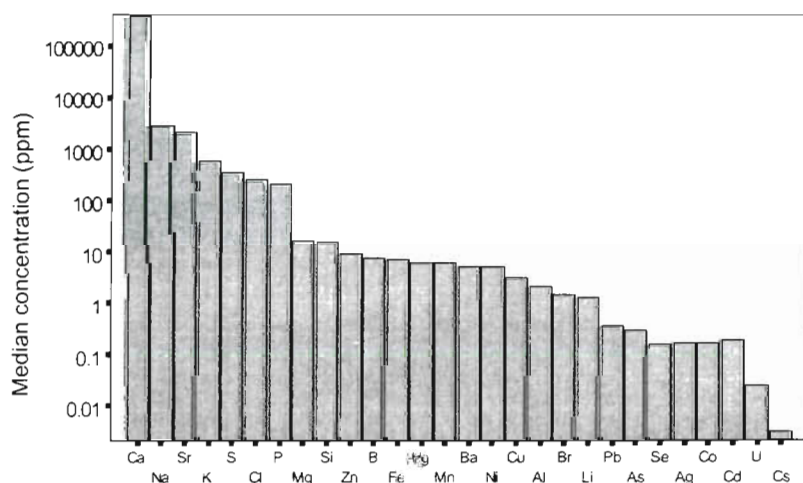


Fig. 1. Summary of published elemental concentrations (ppm or  $\mu\text{g g}^{-1}$ ) in fish otoliths across all species. Not included are the major elements C, O and N, as well as the trace radioactive elements Ra and Th

## OTOLITH MINERALOGY AND CRYSTALLIZATION

A mechanism for otolith growth is beyond the scope of this review, but some details are important for understanding the incorporation of trace elements. In brief, biomineralization of otoliths differs somewhat from that of vertebrate bone, molluscan shell and coral skeleton in that the otolith epithelium is not in direct contact with the region of calcification. As a result, the calcification process is heavily dependent upon the composition of the endolymphatic fluid surrounding the otolith, and to a certain extent, can be described on the basis of purely physical principles. The key physical, regulating factor appears to be the pH of the endolymph, which is determined by the concentration of bicarbonate ions in the endolymph (bicarbonate is one of the ion products of carbon dioxide in solution) (Romanek & Gauldie 1996, Payan et al. 1997, 1998). Reduced alkalinity in the endolymph is regulated by proton secretion through the saccular epithelium, which then reduces the rate of calcification (Payan et al. 1997). Temperature influences calcification rate as well. By itself however, a solely inorganic process cannot account for many of the features of biomineralization in general, or otolith growth in particular (Wheeler & Sikes 1984), suggesting that the small percentage of the otolith composed of proteins plays a pivotal role in otolith calcification.

Recent findings suggest that the composition and orientation of otolith proteins conform to those in other biomineralized structures, including squid statoliths (Morris 1991). Approximately half of the otolith proteins are water-insoluble (Asano & Mugiya 1993), and presumably make up the structural framework for subsequent calcification. These proteins are characterized by a high abundance of acidic amino acids, and were termed 'otolin' by Degens et al. (1969). However, it is the more recently characterized water-soluble proteins which appear to be the most influential in regulating the rate of calcification. These calcium-binding glycoproteins are also characterized by acidic amino acids, and appear to serve as regulators of calcification rate (Asano & Mugiya 1993), perhaps through inhibition of crystal nucleation (Wright 1991). Support for this suggestion comes from observations of molluscan shell formation, in which a soluble matrix rich in acidic amino acids binds calcium and forms an anti-parallel  $\beta$ -pleated sheet in the polymerized state (Morris 1991). In such a conformation, all of the amino acid side chains lie on 1 side of the corrugated layer and project in the same direction; the spacing between these side chains closely matches that of the calcium atoms in aragonite crystals, suggesting that the soluble matrix could act to either encourage or inhibit crystal nucleation (Morris 1991, Falini et al. 1996). While the linkage between the soluble and insoluble proteins needs

to be clarified, periodic secretion of the soluble proteins to the mineralization front could result in an association which would then either initiate or inhibit crystal growth (Wheeler & Sikes 1984).

Calcium carbonate can crystallize as any 1 of 3 crystal polymorphs: calcite, aragonite or vaterite. Aragonite and vaterite are normally considered to be crystallographically unstable, reverting to the calcite polymorph, although the transformation of aragonite to calcite is slow (Carlström 1963). Yet unlike the calcitic otoconia of mammals, X-ray diffraction has confirmed that the vast majority of sagittal otoliths are composed of polycrystalline aragonite (Irie 1955, Carlström 1963, Degens et al. 1969, Mann et al. 1983, Morales-Nin 1986, Maisey 1987, Lecomte-Finiger 1992, Oliveira et al. 1996). The orientation of the individual crystal faces is sometimes referred to as being twinned (Degens et al. 1969, Gauldie & Nelson 1988). Until recently, an explanation for the overwhelming predominance of aragonite over calcite in otoliths was unclear; despite the fact that calcite is rhombohedral and aragonite is orthorhombic, the 2 polymorphs have very similar crystal structures, differing primarily in the organization of the carbonate molecules sandwiched between layers of calcium (Falini et al. 1996). Small quantities of divalent ions such as Sr and Mg favour formation of aragonite over calcite (Carlström 1963), but it was not clear that such conditions were by themselves sufficient to explain the aragonitic otolith. However, a recent study indicates that selection of the polymorph of calcium carbonate to be precipitated is mediated, and perhaps controlled, by organic molecules (Falini et al. 1996). Under identical experimental conditions, macromolecules extracted from aragonitic shell layers induced aragonite formation, while macromolecules extracted from calcitic shell layers induced calcite formation (Falini et al. 1996). These results provide the best evidence to date for organic control of crystal formation in organisms, and suggest that both the rate and type of calcium carbonate crystals formed in otoliths is regulated by proteins.

Curiously, different polymorphs of calcium carbonate appear to be linked to the different otolith organs. While aragonite is the norm for sagittae and lapilli, most asteriscii are made of vaterite, thus accounting for their glassy appearance (Lowenstam & Weiner 1989, Oliveira et al. 1996). Vaterite is also the principal polymorph in many aberrant, or 'crystalline', otoliths (Mugiya 1972, Gauldie et al. 1997, but see Strong et al. 1986). There is no accepted explanation for the formation of vateritic regions within a largely aragonitic otolith (Strong et al. 1986), but vaterite is known to precipitate in supersaturated solutions which are far from equilibrium (Carlström 1963). Alternatively, vaterite formation may be mediated by matrix proteins in the

same way described earlier for calcite and aragonite (Falini et al. 1996). Calcitic regions in otoliths are much rarer (Davies et al. 1988), and are probably a product of anomalous inorganic precipitations or slip planes (Oliveira et al. 1996).

The exact location of elemental impurities in the otolith matrix has not been studied, but would be expected to be similar to that of other aragonitic carbonates. Three sites are possible: within the crystal lattice as a substitute for calcium, as an inclusion in the interstitial spaces, or in association with the proteinaceous matrix. The first of the three (the within-lattice inclusion) can occur through a simple ion substitution for Ca or through co-precipitation of another carbonate (e.g.  $MgCO_3$ ). High correlations (positive or negative) among elements such as Sr, Mg, Li and Ba suggest a common mode of inclusion, while uncorrelated elements such as Mn and Zn probably differ in their inclusion site. Of the 3 possible sites, the calcium substitution/co-precipitation is most studied, and appears directly applicable to divalent metal ions of comparable size. Strontium carbonate is virtually isostructural with aragonite, thus explaining strontium's affinity for aragonite, but in light of the lower Sr concentration in otoliths compared to corals, substitution of Sr ions for Ca may be more likely than co-precipitation of  $SrCO_3$  (Greegor et al. 1997). Ions slightly larger than calcium (such as Ba and Pb) can also be expected to be substituted or co-precipitated (Morse & Mackenzie 1990). For such elements, the extent of incorporation can often be predicted with temperature-sensitive partition coefficients, although the rate of precipitation can also be influential. Anions such as chloride and sulfate can also be co-precipitated, but less predictably. The spacing between calcium ions in the crystal lattice is important to both substitution and co-precipitation, explaining the very different affinities of vateritic otoliths for some elements compared to aragonitic otoliths (Gauldie 1996b). In contrast, ions such as those of sodium do not behave as normal co-precipitants and are probably found at crystal defects (Morse & Mackenzie 1990). In general, ion impurities trapped in interstitial spaces are difficult to predict or model in terms of partition coefficients. Otoliths are well known for their micro-channel architecture (Gauldie et al. 1998), and the presence of poorly bound elemental inclusions in these interstitial regions could well explain why elements such as Na, Cl, Zn and K are so easily leached out (Proctor & Thresher 1998, Campana et al. 1999a).

Elements incorporated into a calcium carbonate matrix as a result of covalent bonding or other associations with organic molecules are virtually unstudied, but are expected to be present. Aside from the major (C, H, O, N) and minor elements (S) that make up the constituent amino acids, it is not clear what other ele-

ments might be included. However, the rate of otolith precipitation might have a different influence on these elements than those more directly associated with calcium carbonate crystals. Perhaps more importantly, to the extent that a change in temperature changes the balance between organic matrix and calcium carbonate formation, it might also be expected to change the ratio of elements which is incorporated.

## PATHWAYS AND BARRIERS OF ELEMENTS INTO THE OTOLITH

The pathway of any given element or ion from the environment into the otolith is a multi-stage process, and is characterized by a sequence of more or less independent barriers. Elemental and ionic barriers are an obvious prerequisite of a highly osmoregulated organism such as a fish, but the pathway into the otolith is even more regulated than is the case for other tissues. For example, calcified tissues such as bone (Ishikawa et al. 1991, Schmitz et al. 1991, Miller et al. 1992) and scale (Johnson 1989, Pender & Griffin 1996) contain higher concentrations of most elements than do otoliths (Sr is a conspicuous exception), while uncalcified structures such as the eye lens are often characterized by a different suite of elements altogether (Dove & Kingsford 1998). This high degree of regulation is most evident in a phylogenetic comparison, in which the aragonitic skeleton of the most primitive animals (e.g. corals) tends to reflect the composition of the ambient water, while animals of increasing complexity show increasing discrimination against the most abundant elements (Table 1). Interestingly, there appears to be relatively little discrimination against the incorporation of at least some of the trace elements into the otolith.

The basic pathway of the bulk of inorganic elements into the otolith is from the water into the blood plasma via the gills or intestine, then into the endolymph, and finally into the crystallizing otolith. Water passing over the gills (branchial uptake) is the primary source of

Table 1. Phylogenetic comparison of representative trace element concentrations in marine biogenic aragonite of corals, bivalves, squid statoliths and fish otoliths. Elemental discrimination tends to be least for the most primitive animals (e.g. corals) and for physiologically unimportant trace elements (e.g. Ba). All concentrations (in  $\mu\text{mol mol}^{-1}$ ) are approximate, and are for illustration only

Element	Coral skeleton	Bivalve shell	Squid statolith	Otolith
Mg/Ca	4400	1750	38	114
Sr/Ca	8750	2200	1800	2500
Ba/Ca	2.7	3.0	2.8	2.8

most elements in freshwater fish, while the continual drinking of marine fish is the main source of water-borne elements for assimilation via the intestine (Olsson et al. 1998). A small but unknown proportion of elements is undoubtedly assimilated from food sources; for example, at least some of the Sr in the diet can be incorporated into the otolith (Limburg 1995, Farrell & Campana 1996, Gallahar & Kingsford 1996). However, other experiments suggested that otolith uptake of a suite of minor elements from food was minimal (Hoff & Fuiman 1995). The majority of the inorganic otolith elements are probably derived from the water: 80 to 90% in the case of Ca and Sr, respectively, in freshwater fishes (Simkiss 1974, Farrell & Campana 1996).

As shown schematically in Fig. 2, elemental discrimination can occur at any or all of the 3 interfaces: water-gill, blood-endolymph and endolymph-crystal. For most elements, the element:Ca ratio in the otolith is far lower than that in the blood plasma or ambient water, but there are large differences in the degree of discrimination among elements, and where that discrimination occurs. Using the distribution coefficient between water and otolith as a measure of elemental discrimination (where  $D = (\text{element:Ca})_{\text{otolith}} / (\text{element:Ca})_{\text{water}}$ ,

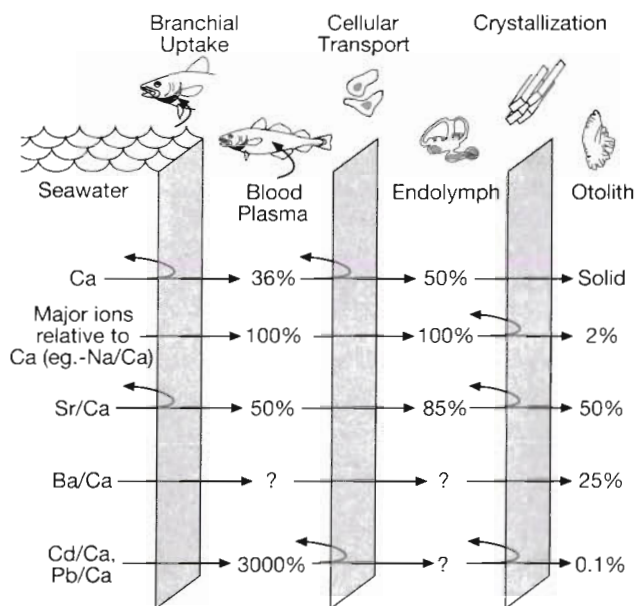


Fig. 2. Overview of elemental pathways and barriers between seawater and the otolith, with coarse estimates of transfer rates (distribution coefficients) for selected elements at each physiological barrier. Elemental concentrations have been referenced to calcium concentration, since the initial uptake of many elements is often inversely proportional to the relative calcium concentration. Elemental discrimination is greatest for major and physiologically regulated ions, and least for trace elements, but the site of maximum discrimination is often unpredictable

values of  $D = 1$  would be expected when there is no elemental discrimination, and values of  $D = 0$  when none of the element is incorporated into the otolith. Observed values for many of the major ions (e.g. Na, K, Cl) are less than 0.05, while that for Sr is about 0.14. Distribution coefficients for many of the trace elements exceed 0.25, and may even approach 1.0.

Undoubtedly, one of the biggest barriers to elemental uptake occurs at the gill-water (in freshwater fishes) or intestine-water (in saltwater fishes) interface, where osmoregulation regulates movement of water-borne ions into the fish. Plasma concentrations of Ca and other major ions are approximately one-third of that of marine waters, but flux rates between water and blood are even lower, since only excreted ions are replaced. In saltwater fishes, trace elements are probably assimilated from the intestine in direct proportion to their relative concentration in the water, albeit with low efficiency (Olsson et al. 1998). Salinity, pH, dissolved oxygen concentration and other factors can also influence elemental uptake into the fish (Mayer et al. 1994). In freshwater fishes, however, it is calcium concentration, or water hardness, which has one of the largest effects on elemental uptake through the gills. While the mechanism remains unclear, it appears that quite a few elements (particularly the divalent elements) are taken up through chloride cell calcium channels in the gill (McKim 1994). The net result is that branchial uptake of metals generally decreases as the relative concentration of calcium in the water increases (Mayer et al. 1994). Where the ambient calcium concentration is low, a greater proportion of both the calcium and the other elements will be taken up. For this reason, the absolute concentration of a dissolved element is often an unreliable indicator of environmental availability to the freshwater fish; the element:Ca ratio is more relevant for such elements. In the case of salt water, Ca concentration is highly correlated with salinity, implying that either the element:Ca or element:salinity ratio can be used as an indicator of environmental availability. This effect is shown in Fig. 2, in which the concentration of major ions (such as Na and K) in the water differs substantially between estuarine and marine environments, yet uptake into the blood is constant in the 2 environments due to the fact that the ion:Ca ratio in the water remains unchanged. Such is not the case for trace elements such as Pb, whose ratio with Ca varies widely among locations or with salinity.

Elemental discrimination also occurs during the movement from plasma to endolymph (Fig. 2), resulting in an endolymph composition which is depleted in all major ions other than K (Fänge et al. 1972, Mugiya & Takahashi 1985, Kalish 1991a, Gauldie & Romanek 1998). In general, the composition of the endolymph appears to be closer to the composition of the otolith

than is the water or blood plasma. While the factors influencing endolymph composition remain poorly understood, the transfer of calcium and other ions into the endolymph may occur via a transcellular route, thus ensuring significant regulation of both the selection of elements and their concentrations in the endolymph (Mugiya & Yoshida 1995). Recent experiments by Payan et al. (1998) indicate that the elemental composition of the endolymph is less affected by starvation than is the plasma.

The final stage of the elemental pathway from environment to otolith occurs during the otolith crystallization process, as discussed above in the section 'Otolith mineralogy and crystallization'. Even without regulation by otolith proteins, significant discrimination against some elements could be expected at this stage. For example, the distribution coefficient of Sr:Ca between water and coral or inorganic aragonite is typ-

ically between 1.06 and 1.15 (de Villiers et al. 1994, Stecher et al. 1996). However, the  $D_{Sr}$  between endolymph and otolith is close to 0.25 (Kalish 1989). In light of the similarity between the coefficients for water-otolith and endolymph-otolith (Fig. 2), it appears that most of the discrimination against Sr occurs during otolith crystallization, rather than during Sr uptake into the blood plasma from the water. A similar argument may explain the observed discrimination against Sr in bivalves (Stecher et al. 1996).

The extent to which otolith trace element concentration reflects (or fails to reflect) that of the ambient water can be inferred from a comparison of water, blood and otolith composition from 2 very different chemical environments: fresh water and salt water. For example, the concentrations of many of the most common elements (e.g. Ca, Na, K, Mg, Cl) differ substantially between fresh and salt water, even when normalized to Ca con-

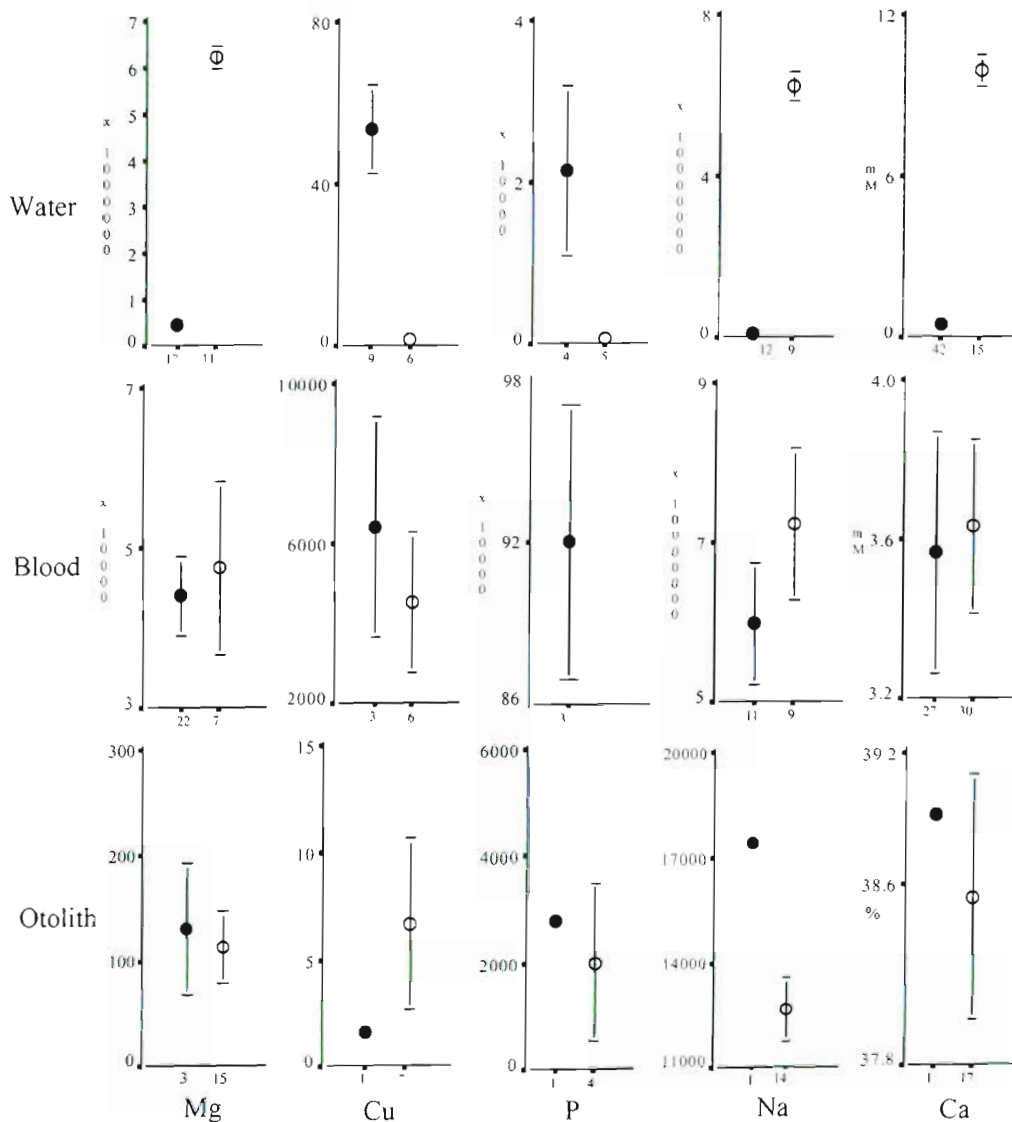


Fig. 3. Examples of otolith trace elements unlikely to be influenced by concentrations in the water, as indicated by inconsistencies between the published composition of the water (top panels), blood plasma (middle panels), or otolith (bottom panels). All elemental concentrations other than that of Ca are in units of pmoles element per mole Ca. (●) Freshwater species; (○) marine species. Error bar = 1 SE. Numbers below x-axis indicate number of papers upon which the elemental concentration was based

centration, yet those differences do not appear to be reflected in the otolith (Fig. 3). Other physiologically important elements, such as P, Cu and S, also appear to remain uninfluenced by the relative concentration in the environment (Fig. 3). The lack of correspondence between environmental and otolith differences in fresh and saltwater fishes becomes more understandable when the corresponding blood concentrations are examined (Fig. 3). For most of the elements noted above, there is little or no difference in plasma concentrations between freshwater and marine fishes. This stability in blood plasma composition is well documented in the physiological literature (Evans 1993), and is completely consistent with the strict osmoregulatory control re-

quired of the fish's survival. Since the elements deposited in the otolith are derived penultimately from the blood plasma, it is clearly unrealistic to expect the otolith content of physiologically regulated elements to reflect environmental abundance.

Based on differences in concentration between fresh water and salt water, there are several elements whose environmental abundance may well be reflected in the otolith. Although the data are limited, the relative concentration of trace elements such as Sr, Zn, Pb, Mn, Ba and Fe in freshwater and marine otoliths is consistent with an environmental effect (Fig. 4). These are also elements whose uptake is more likely to be unregulated compared to those of the common salts. Other

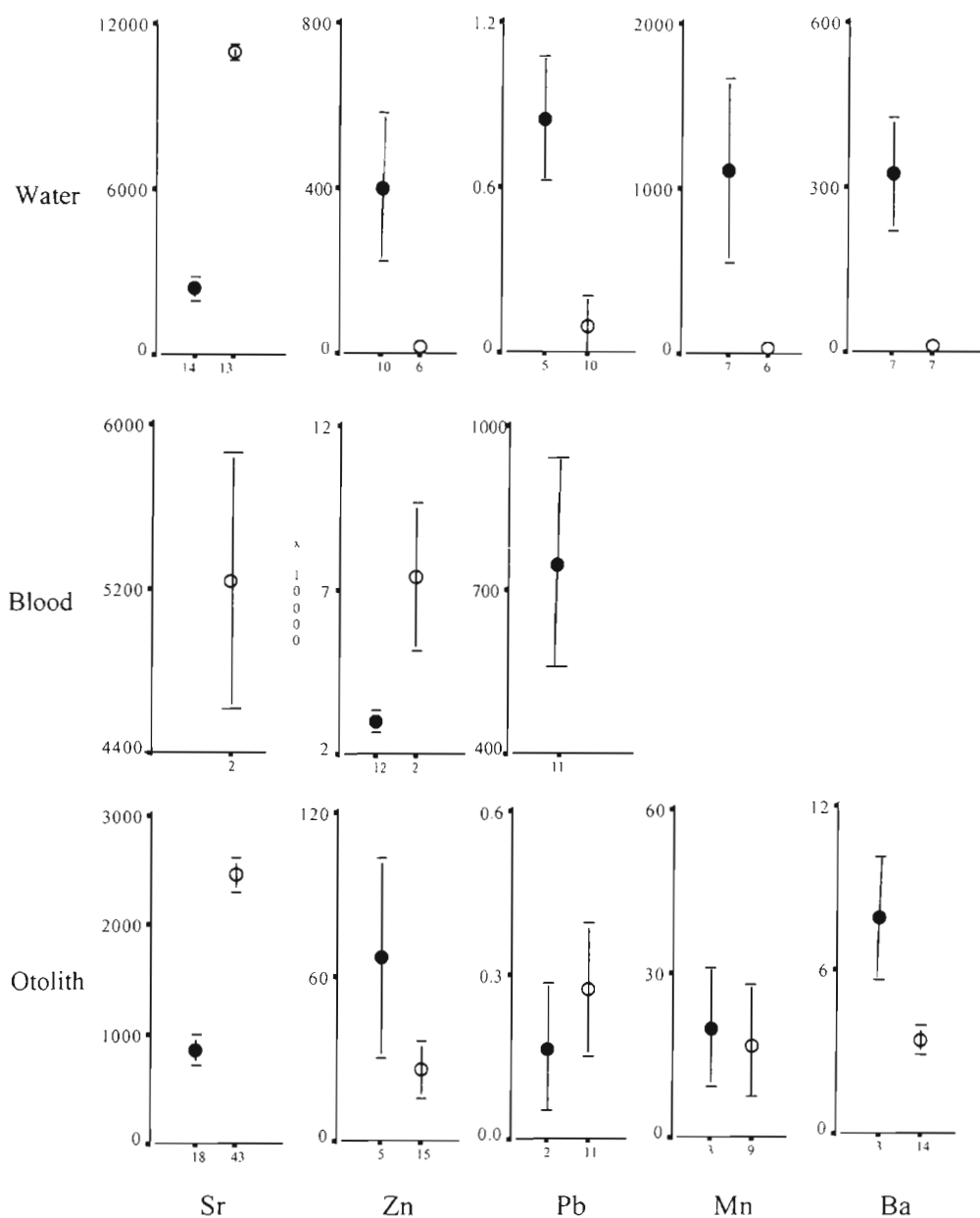


Fig. 4. Examples of otolith trace elements which are probably influenced by concentrations in the water, as indicated by consistency between the published composition of the water (top panels), blood plasma (middle panels), and/or otolith (bottom panels). All elemental concentrations are in units of  $\mu\text{moles element per mole Ca}$ . (●) Freshwater species; (○) marine species. Error bar = 1 SE. Numbers below x-axis indicate number of papers upon which the elemental concentration was based

Table 2. Summary of published otolith trace element compositions ( $\mu\text{g g}^{-1}$ ) from 3 major habitat types

Element	Marine species			Freshwater species			Estuarine species		
	Mean	SE	N	Mean	SE	N	Mean	SE	N
Ag	0.16	–	1	–	–	–	–	–	–
Al	2.0	–	1	–	–	–	–	–	–
As	0.3	0.2	2	–	–	–	–	–	–
B	7.3	2.2	2	0.20	–	1	5.0	–	1
Ba	3.7	0.60	14	11.0	2.8	3	8.2	2.7	3
Br	1.4	0.6	2	–	–	–	–	–	–
Ca	380176	4981	17	407232	18232	2	–	–	–
Cd	0.18	0.17	2	–	–	–	–	–	–
Cl	231	27	4	–	–	–	–	–	–
Co	0.5	0.2	4	–	–	–	0.10	–	1
Cs	0.003	–	1	–	–	–	–	–	–
Cu	4.0	1.9	7	1.0	–	1	2.6	1.5	2
Fe	640	396	8	30.0	–	1	–	–	–
Hg	2.7	1.7	3	–	–	–	–	–	–
K	571	55	13	–	–	–	1105	415	2
Li	1.0	0.3	6	0.05	–	1	1.0	–	1
Mg	27	5.8	15	32	9.2	3	33	7.5	4
Mn	9.6	5.8	9	11.1	7.5	3	8.8	7.3	2
Na	2859	178	14	3900	–	1	1866	285	3
Ni	9.5	3.3	6	–	–	–	2.0	–	1
P	610	464	4	846	–	1	50.0	–	1
Pb	0.6	0.2	11	0.32	0.27	2	–	–	–
S	379	70	10	–	–	–	290	–	1
Se	0.15	–	1	–	–	–	–	–	–
Si	15.0	–	1	–	–	–	8.0	–	1
Sr	2137	127	43	698	111	17	1937	70	4
U	0.024	–	1	–	–	–	–	–	–
Zn	15.6	5.8	15	45.8	18.3	5	9.1	1.1	2

trace elements such as Li, Cd, Ni and the less abundant elements may well fall into this category as well, although data for these elements are even more scarce (Table 2). Note, however, that the element:calcium ratios in the plasma (excluding Sr) tend to be much higher than in the otolith (Figs. 3 & 4), indicating that otolith composition is not merely a passive reflection of plasma composition, even if correlated.

### BASIS FOR TRACE ELEMENT VARIATIONS IN THE OTOLITH

#### Environmental availability

For those elements where the composition of the otolith reflects the elemental composition of the water, a broad range of otolith concentrations can be ex-

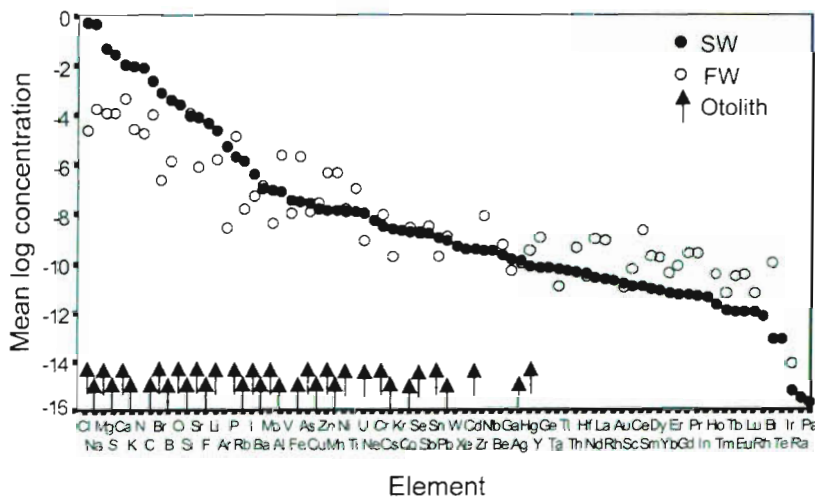


Fig. 5. Mean elemental composition ( $\text{mol l}^{-1}$ ) of seawater (SW) and fresh water (FW) as drawn from the literature. Only recent (1987 to present) publications ( $n = 80$ ) were used so as to take advantage of improvements in analytical accuracy. Elements which have been reported as being detected in otoliths are indicated by an arrow



pected (Johnson et al. 1992). Elemental concentrations in seawater range over at least 16 orders of magnitude for the 78+ elements that have been quantified (Fig. 5). With the exception of the common marine salts, freshwater concentrations are reasonably similar (Fig. 5); the apparent abundance of the rarer elements in fresh water compared to seawater may reflect recent advances in marine analytical procedures more than true differences (Benoit et al. 1997). Many of the more abundant elements have also been detected in otoliths (Fig. 5), suggesting that the less common elements simply await detection in the otolith. For those otolith elements which have already been detected, the variance around the mean values is reasonably large (Table 2), suggesting the presence of inter-specific differences, methodological problems and heterogeneity in environmental concentrations. Of course, it is the geographic variation in water-borne concentrations which is of greatest interest to those using otolith composition as an indicator of past location. Such geographic variations in water concentration are the norm for many elements (Balls et al. 1993, Benoit et al. 1997), although variability in coastal and inland waters is often much larger than that of the open ocean. With elements such as Ca, Sr and Mg showing conservative profiles (reflecting salinity) and others such as Ba, Zn and Cd showing nutrient-type profiles (surface depletion with enrichment at greater water depths), broad patterns of relative concentration are often predictable (Bruland 1983). Elements such as Hg and Pb are more generally associated with anthropogenic activity. However, regional variability associated with river discharge, upwelling, volcanic activity, pollution, biological activity and inter-annual differences can all interact to confound any expected relationship. Variability of a factor of 10 or more is not unusual when comparing aqueous elemental concentrations among locations (Balls et al. 1993, Benoit et al. 1997).

It is important to note that the ambient concentration of an element, even when referenced to calcium, is not necessarily a good indication of its availability to either the otolith or the fish. In general, dissolved ions which are free of ligands are the only chemical species available for uptake by the gills (Knezovich 1994). Elemental concentrations referenced to calcium concentrations are most relevant for such elements. As an example, the absolute concentration of Sr ranges from about  $9 \times 10^{-7}$  M in fresh water to about  $8.7 \times 10^{-5}$  M in salt water, an almost 100-fold difference. However, the molar ratio of Sr:Ca in fresh water ( $1.8 \times 10^{-3}$ ) is only 4.8 times less than that of salt water ( $8.6 \times 10^{-3}$ ). It is this latter ratio that is more relevant to the question of relative rates of Sr uptake in fish between fresh water and salt water.

Do the elemental concentrations in the otolith reflect the elemental concentrations of the ambient water?

The answer is an unequivocal 'sometimes'. While several experiments have been carried out to test the response of otolith composition to the composition of the water, the majority have been limited to simple dilutions of seawater so as to modify absolute elemental concentrations. These experimental salinity treatments have resulted in modest changes in the otolith composition (somewhat greater for Sr), but not of the scale which might have been expected based on the change in absolute concentration (Fowler et al. 1995a, Hoff & Fuiman 1995, Secor et al. 1995b, 1998, Tzeng 1996). In retrospect, this is not surprising given that the element:Ca ratio in the experimental water did not differ among treatments. Experiments which have manipulated ambient elemental concentrations independent of Ca have largely been restricted to Sr, but have shown much more pronounced effects on otolith composition (Ennevor & Beames 1993, Brown & Harris 1995, Schroder et al. 1995, Farrell & Campana 1996, Gallahar & Kingsford 1996, Dove 1997, Geffen et al. 1998). Increased concentrations of trace elements such as La, Sm, Ce, Ba, Hg and Mn have all been reported in the otolith after exposure to elevated concentrations in fresh water (Ennevor & Beames 1993, Dove 1997, Geffen et al. 1998). Single-element additions of Sr, Cd and Ba to salt water also resulted in significant incorporation into the otolith (S. R. Thorrold, G. Bath, C. Jones & S. E. Campana unpubl.). Similarly, Sr isotope ratios in the otolith increased with the ratios present in the surrounding water (Kennedy et al. 1997). In each of 5 independent experiments which manipulated Sr:Ca ratios in the water, ambient Sr:Ca was well correlated with otolith Sr:Ca (Brown & Harris 1995, Schroder et al. 1995, Farrell & Campana 1996, Gallahar & Kingsford 1996, Dove 1997). When the data from all of these studies are pooled, the overall relationship also shows a significant positive relationship between ambient and otolith Sr:Ca (Fig. 6); this experimental relationship is consistent with observed differences between freshwater and saltwater fish otoliths (Fig. 6). However, the linear increase in the otolith Sr:Ca ratio relative to the ratio in ambient water is not proportional; the distribution coefficient,  $D_{Sr}$ , of 0.14 from Fig. 6<sup>1</sup> is significantly less than 1, in keeping with the earlier discussion of physiological discrimination. Distribution coefficients between water and otolith have not yet been calculated for elements other than Sr. Otolith calcium, however, does not respond to variability in water concen-

<sup>1</sup>The  $D_{Sr}$  reported here, as well as that of Kalish (1989), is based on the slope of the relationship between ambient and otolith Sr:Ca, since the regression intercept was significant. However,  $D$  is usually calculated as  $(\text{element:Ca})_{\text{otolith}} / (\text{element:Ca})_{\text{solution}}$

trations, at least under normal conditions (Ichii & Mugiya 1983, Farrell & Campana 1996).

The presence of geographic variations in water chemistry, combined with partial correspondence between environmental availability and the incorporation rate into the otolith, bodes well for those applications of otolith elemental analysis that reconstruct environmental history. However, there are caveats to this statement. The first such caveat is that 5 of the 6 minor elements which are most easily measured with the widely available electron microprobe (Gunn et al. 1992) are likely to be under strict physiological regulation, and thus unsuitable for use as environmental indicators. The only exception is Sr. The second caveat concerns the analysis of the less abundant trace elements, which appear to be more suitable as environmental indicators. While their lower concentrations makes them less likely to be osmoregulated by the fish, it also makes them more difficult to assay with accuracy and without contamination during handling. Finally, few if any trace elements (even when normalized to Ca) are likely to be incorporated into the otolith in direct proportion to availability in the environment. Both temperature and growth rate are known to be at least as influential as ambient concentration in modifying otolith elemental composition, as is discussed in the following section.

### Temperature

Perhaps more than any other variable, the influence of temperature on the rate of trace element incorporation into calcified tissues has been studied and debated. The driving force behind much of this interest is the potential application of trace element concentrations as paleoindicators of oceanographic conditions and for reconstructing the temperature exposure history of individual organisms. Several elements have been reported to vary systematically with temperature in aragonitic corals, including Mg (Mitsuguchi et al. 1996) and U (Shen & Dunbar 1995). But aside from  $^{18}\text{O}:^{16}\text{O}$  ratios (which are discussed below under 'Stable isotopes'), it is Sr:Ca ratios that have been considered to be the most promising. The underlying basis of the Sr:Ca temperature dependence is presumed to be a kinetic effect, since the rate of Sr:Ca incorporation into inorganic aragonite varies inversely with temperature according to the following equation (Kinsman & Holland 1969):

$$(\text{Sr:Ca}) \text{ (in mmol mol}^{-1}\text{)} = 10.66 - 0.039 \times \text{Temperature}$$

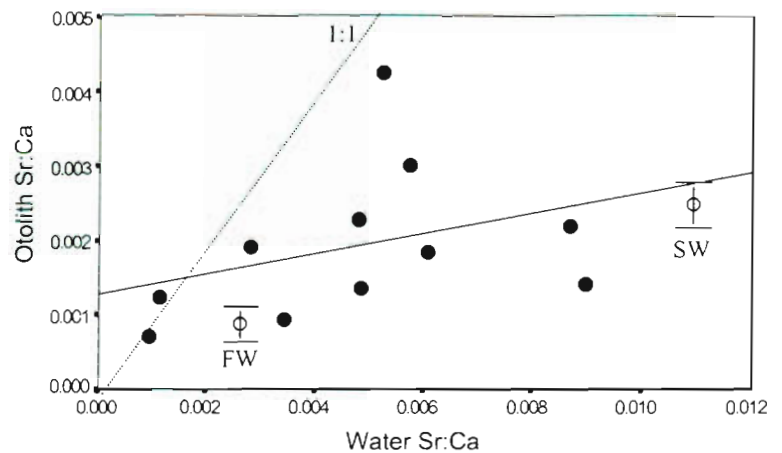


Fig. 6. Summary of published molar Sr:Ca values as a function of the Sr:Ca ratio in the water (●). The fitted regression is statistically significant. Also shown are the mean ( $\pm 95\%$  CI) ratios for freshwater (FW) and saltwater (SW) otoliths/water as drawn from the literature

This equation does not apply to aragonitic corals, where some form of biological fractionation is presumed to influence the underlying kinetic relationship. Nevertheless, some form of temperature dependence in coral Sr:Ca seems to be well accepted, despite concerns over the nature of the Sr incorporation (Greeger et al. 1997) and the likelihood of confounding physiological effects (de Villiers et al. 1994, Hart & Cohen 1996). Shen et al. (1996) presented the following equation for generalized use with the *Porites* genus of aragonitic corals:

$$\text{Sr:Ca}_N \text{ (in mmol mol}^{-1}\text{)} = 10.286 - 0.0514 \text{ SST}$$

where SST = sea surface temperature and N = Sr:Ca normalized to that of Hawaiian seawater. Note that here, as in all published temperature calibrations for corals, the Sr:Ca of the coral is directly proportional to that of the seawater, independent of any temperature effect. Even in the open ocean, variability in the Sr:Ca of the water among locations or throughout the year can introduce variability in the Sr:Ca of the coral equivalent to temperature variations of 0.2 to 0.7°C (de Villiers et al. 1994, Shen et al. 1996). Variability in coastal waters would, of course, be much larger.

In light of the strong correlation between temperature and element:Ca concentrations in corals, it is understandable that similar relationships were expected to be uncovered in otoliths. Indeed, significant and often substantial temperature effects have been reported in many otolith studies, although the relationships have not necessarily been consistent among studies. Examples of otolith elements influenced by temperature include Mg (Fowler et al. 1995a, Hoff & Fuiman 1995), K (Hoff & Fuiman 1995), Na (Kalish

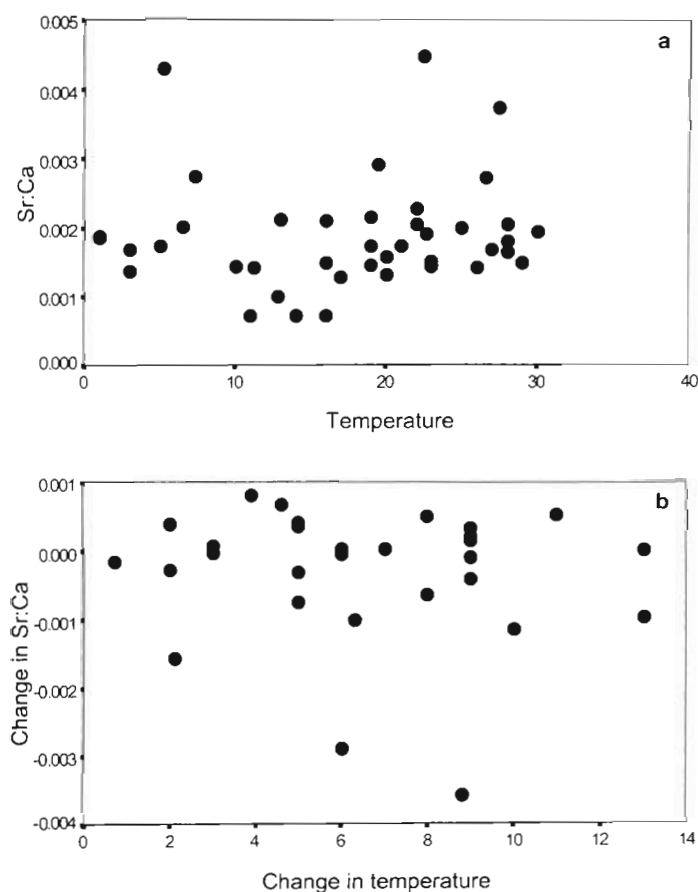


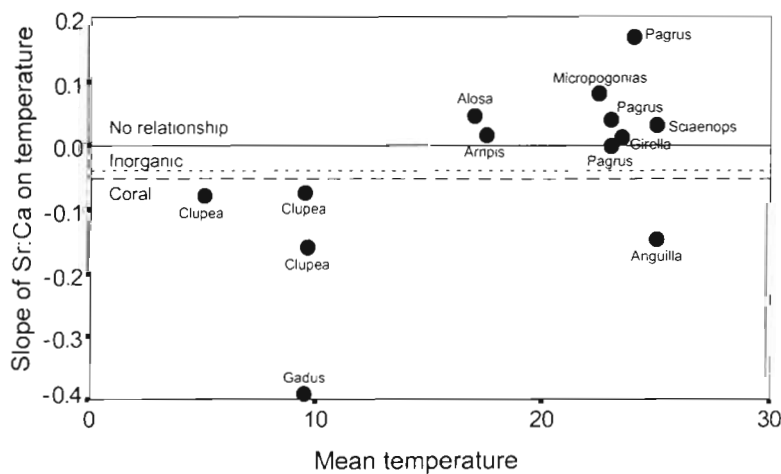
Fig. 7. Relationship between marine otolith Sr:Ca ( $\mu\text{mol mol}^{-1}$ ) and temperature. Each point represents the results of an experimental treatment, as drawn from the literature. Neither relationship is statistically significant. (a) Observed values. (b) Within-study change in Sr:Ca across within-study temperature treatments

1989, Hoff & Fuiman 1995), Mn and Zn (Arai et al. 1995, Fowler et al. 1995a), and Fe (Gauldie et al. 1980, Arai et al. 1995, Fowler et al. 1995a). However, particular attention has been focused on Sr:Ca, in which pos-

itive (Kalish 1989, Arai et al. 1995, 1996, Fowler et al. 1995a, Hoff & Fuiman 1995, Limburg 1996), negative (Townsend et al. 1989, 1992, 1995, Radtke et al. 1990, Sadovy & Severin 1992, Secor et al. 1995b), and non-existent (Gallahar & Kingsford 1996, Tzeng 1996) correlations with temperature have been reported. A meta-analysis of the existing literature does not support an overall relationship between otolith Sr:Ca and temperature for either saltwater or freshwater fish (Fig. 7a). Of course, interspecies differences could mask such a relationship. Therefore, the analysis was repeated using only the within-study difference in Sr:Ca ratio versus the within-study difference in temperature, so as to maximize statistical power (Fig. 7b). Once again, no relationship was evident. While this result does not prove that temperature-dependent Sr:Ca fractionation in otoliths is absent, it does indicate that it is not a generalized phenomenon.

Townsend et al. (1992) postulated that temperature-dependent Sr:Ca fractionation may only occur at low temperatures, perhaps because of some reduced ability to discriminate against Sr during entry into the endolymph. To test this hypothesis, the slopes of all published Sr:Ca versus temperature relationships were regressed against the median temperature of the study (Fig. 8), with the expectation that fractionation (negative slopes) would only be apparent at low temperatures. The slope in Fig. 8 is significant ( $p = 0.03$ ), although only those experiments carried out below  $10^{\circ}\text{C}$  produced temperature coefficients significantly different from zero. If real, such a relationship suggests that Sr:Ca ratios decrease with increasing temperature at low temperatures, but the ratios increase with temperature at high temperatures. As will be seen later, however, there is an alternative explanation for these results.

Fig. 8. Temperature sensitivity of the Sr:Ca ratio in otoliths as a function of mean experimental temperature. Temperature sensitivity was defined as the slope of the within-study regression of Sr:Ca on temperature, as determined from published values. The regression is significant ( $p = 0.03$ ), suggesting that temperature sensitivity declines with increasing temperature. Also shown are the temperature coefficients for inorganic aragonite (Kinsman & Holland 1969) and an aragonitic coral (Shen et al. 1996)



Given the controversy in the literature concerning the utility of otolith Sr:Ca ratios as indicators of either salinity (Kalish 1990, Halden et al. 1995, Secor et al. 1995b) or temperature (Radtke 1989, Townsend et al. 1992, 1995), there has been disagreement over which of the 2 environmental factors would be expected to be most influential in modifying otolith composition. Simple calculations, however, suggest that changes in salinity would generally be more detectable than would changes in temperature. On the basis of the observed difference in otolith Sr:Ca ratios (shown in Fig. 6) between fresh water and salt water, each 1‰ increase in salinity would be expected to produce a  $0.05 \times 10^{-3}$  increase in the otolith Sr:Ca molar ratio. Given the 30 to 35‰ difference in salinities between marine and riverine waters, corresponding to a  $1.5 \times 10^{-3}$  change (or 3-fold) in the otolith Sr:Ca ratio, it is obvious why otolith Sr:Ca is such an effective indicator of anadromy in fishes. Mean observed Sr:Ca temperature slopes of about  $-0.1 \times 10^{-3}$  per degree for *Clupea* sp. (Fig. 8) are twice those observed in corals (Shen et al. 1996) and nearly 3 times that observed in inorganic aragonite (Kinsman & Holland 1969), and are thus somewhat larger than would otherwise be expected. Even if accepted as given, a slope of  $-0.1 \times 10^{-3}$  implies that each degree of rising temperature would only result in a  $0.1 \times 10^{-3}$  decline in the otolith Sr:Ca ratio. Thus a 15°C temperature shift would be required to produce a change in otolith composition equivalent to that produced by anadromy. Using the temperature effect observed in corals, a 30°C shift would be required. Note, however, that a temperature effect has only been observed in otoliths at median temperatures of less than 10°C (Fig. 8).

#### Integrated effects on otolith Sr:Ca

Notwithstanding the controversial effects of temperature on otolith Sr:Ca, it is clear that there is a wide range of observed Sr:Ca ratios among species, habitats and studies, even within a given salinity regime. Thus any generalized hypothesis for Sr:Ca incorporation into the otolith must reconcile the following apparently unrelated observations: (1) a broad tendency towards lower Sr:Ca ratios in the faster growing individuals of a species (Sadovy & Severin 1994); (2) the apparent temperature effect in low-temperature larvae, but not in mid- and high-temperature larvae (Fig. 8); (3) the very high Sr:Ca ratio in larvae of eels, *Anguilla* species, even at high temperatures (Otake et al. 1994, Tzeng 1996); (4) the commonly observed increase in otolith Sr:Ca with fish age (Radtke & Targett 1984, Radtke 1987, Sadovy & Severin 1992, Proctor et al. 1995); and (5) annual variations in Sr:Ca which are in phase with,

but not necessarily correlated with, temperature cycles (Sadovy & Severin 1992, 1994, Fuiman & Hoff 1995). The common factor among all of these observations is variability associated with the rate of protein synthesis in relation to the crystallization rate of the otolith.

The rate of calcium carbonate crystallization would normally be considered a major source of Sr:Ca variability in fish otoliths, just as it is in calcite (Lorenz 1981), bivalves (Stecher et al. 1996), and corals (de Villiers et al. 1994). However, various studies have failed to find a relationship between Sr:Ca and otolith increment width (Kalish 1989, Gallahar & Kingsford 1992), indicating that otolith Sr:Ca is not a simple function of calcification rate. To some extent, this may be explained by the fact that otolith and endolymph composition appear to be more tightly regulated than are the same processes in other taxa. In addition, the role of water soluble, calcium binding proteins in regulating otolith growth appears to be substantial (Wheeler & Sikes 1984, Wright 1991, Asano & Mugiya 1993). Therefore, while the rate of crystallization may well be one of the factors influencing Sr:Ca incorporation into the otolith, it seems likely that the crystallization rate is controlled by the formation rate of proteinaceous matrix on the growing otolith surface. The rate of matrix formation in turn is highly correlated with the rate of somatic growth, since the latter reflects the rate of net protein synthesis.

Our current understanding of the otolith mineralization process is too scanty to allow the development of a detailed hypothesis to explain otolith Sr:Ca ratios. However, a crystallization process which is controlled by the rate of matrix protein formation, and inversely proportional to otolith Sr:Ca, is consistent with most of the observations. Since the rate of protein synthesis is often highly correlated with metabolic rate, temperature and somatic growth rate, explanations for presumed temperature (Radtke 1989, Townsend et al. 1989, 1992, 1995) and growth rate effects (Sadovy & Severin 1992, 1994) on otolith Sr:Ca become readily apparent. However, the link between temperature and protein synthesis often fails at higher temperatures, due to higher metabolic losses. Such losses would explain the absence of Sr:Ca temperature sensitivity in fish maintained at higher temperatures (Fig. 8). By corollary, both Sr:Ca ratios and their sensitivity to temperature should be maximal in otoliths with low protein synthesis (growth) rates. This prediction of the hypothesis is consistent with the elevated Sr:Ca ratios in eel *leptocephalus* otoliths, which exhibit extremely low growth rates even at high temperatures (Otake et al. 1994, Tzeng 1996). It also explains, for the first time, the peak in the Sr:Ca ratio at the time of metamorphosis from the larval stage, since metamorphosis is a life history stage with markedly curtailed growth (Toole et

al. 1993, Otake et al. 1994, Tzeng 1996). Similarly, protein synthesis and otolith growth tend to decline with age in adult fish (Mulcahy et al. 1979, Schwarcz et al. 1998), which is consistent with the observed long-term increase in Sr:Ca (Radtke & Targett 1984, Radtke 1987, Sadovy & Severin 1992, Proctor et al. 1995). Even otolith checks, which are regions of reduced otolith growth (Campana & Neilson 1985), are characterized by elevated Sr:Ca levels (Kalish 1992). Thus it appears that variations in the rate of otolith matrix formation are at least partially responsible for the observed range of Sr:Ca ratios across species within a given salinity regime, with temperature and/or growth being occasional, and often frequent, correlates. Of course, Sr incorporation into the accreting otolith is also a function of the Sr:Ca ratio in the endolymph (Kalish 1989). Since the latter varies with the salinity of the ambient water, variation in otolith Sr:Ca due to protein-regulated crystallization would overlay, not replace, variation due to salinity effects.

The key to testing the hypothesis linking otolith Sr:Ca with the rate of proteinaceous matrix formation would appear to lie with experiments in which otolith Sr:Ca, matrix formation and calcification rate are simultaneously monitored. In light of the 'uncoupling' of fish and otolith growth noted in many studies, it is quite possible that the rate of matrix formation provides threshold limits for calcification rate, but that the effect of temperature on calcification rate is additive within those limits (Mosegaard et al. 1988). If such a mechanism exists, it is not at all obvious how otolith Sr:Ca might respond. Further research in this area is clearly required.

### STABLE ISOTOPES

Stable isotopes are neutral, non-radioactive variants of an element whose relative uptake can be modified by the environment or biological activity due to their slightly different atomic mass. Processes that enhance the uptake of 1 isotope over another thus result in an isotopic ratio which differs from that of the source. Accordingly, stable isotope ratios have a long history of use as geological and biological tracers, recorders of temperature, salinity and pH, and indicators of feeding history, trophic level and metabolic rate (Peterson et al. 1985, Hesslein et al. 1991). Carbon and oxygen are the 2 elements with the most extensive history of interpretation, although stable isotope ratios of nitrogen, sulfur, lead, strontium and boron are increasingly being used (Vogel et al. 1990, Spivak et al. 1993, Gannes et al. 1997). In otoliths, stable isotope ratios have been used to reconstruct temperature history (Devereux 1967, Mulcahy et al. 1979, Kalish 1991b, Patterson et

al. 1993, Thorrold et al. 1997b), differentiate among groups of fish (Edmonds & Fletcher 1997, Kennedy et al. 1997, Dufour et al. 1998, Thorrold et al. 1998b), infer metabolic history (Radtke et al. 1987, Kalish 1991c, Gauldie 1996a, Schwarcz et al. 1998), and reconstruct migration history (Northcote et al. 1992).

The source of stable isotopes incorporated into the otolith varies with the element. Elements such as oxygen and strontium appear to be incorporated into the otolith with isotopic ratios which are nearly identical to that expected of crystallization from the ambient water (Kalish 1991c, Kennedy et al. 1997, Thorrold et al. 1997b), suggesting that the water is their primary source. In contrast, approximately 10 to 30% of otolith carbon may be derived from metabolic sources (Kalish 1991c, Schwarcz et al. 1998), suggesting a dietary origin. The remainder of the otolith carbon would presumably come from dissolved inorganic carbon (DIC) in the water.

The chemical processes that lead to isotopic enrichment or depletion in biogenic carbonates have been carefully described elsewhere (Leder et al. 1996, Swart et al. 1996), and do not bear repeating here. In brief, thermodynamic principles (e.g. solution concentration and temperature) regulate the degree of isotopic fractionation in inorganic aragonite; where these principles are the only ones influencing fractionation in a biological system, the system is said to be in equilibrium. The effect of these purely physical factors is easily predicted when the temperature and the isotopic composition of the precipitating solution (e.g. ambient water) is known. Thus carbonates precipitated under equilibrium conditions are often used to estimate past temperatures and/or water composition (Kim & O'Neil 1997). However, many biogenic carbonates undergo additional fractionation due to 'vital effects', which includes both kinetic and metabolic effects (Kalish 1991c, Thorrold et al. 1997b). These effects have been carefully studied in corals and bivalves, but recent studies have indicated that fractionation in corals and otoliths differ in some key respects. For example, corals are often isotopically depleted (typically -3 to -5‰) in  $\delta^{18}\text{O}$  relative to marine fish otoliths (-2 to +4‰) from comparable habitats. For this reason, corals are probably a poor model for isotope fractionation in otoliths.

Oxygen isotopes in otolith aragonite are deposited in, or very near to, equilibrium with the ambient water (Kalish 1991b,c, Patterson et al. 1993, Radtke et al. 1996, Thorrold et al. 1997b), suggesting similar behaviour to that of inorganic aragonite. Inorganic aragonite fractionation has not been studied at more than 1 temperature, but the recent study of Kim & O'Neil (1997) rigorously quantified the temperature dependence of the oxygen isotope fractionation factor in calcite. Since a 0.6‰ enrichment in aragonite rela-

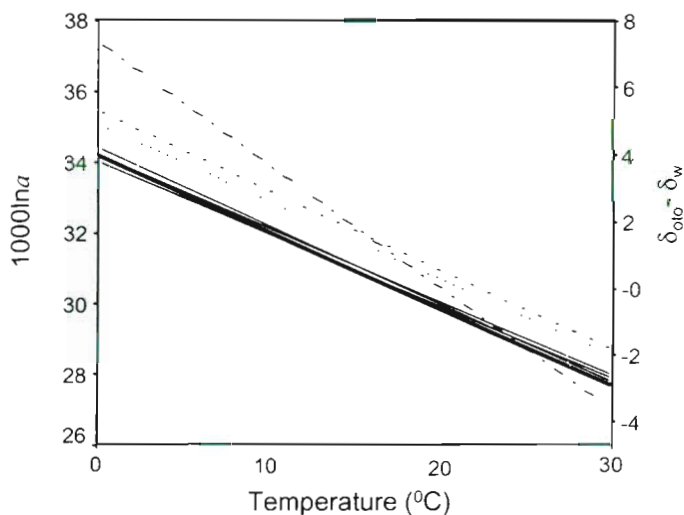


Fig. 9. Relationship between the estimated oxygen isotope fractionation factor ( $1000 \ln \alpha$ ) and temperature for inorganic aragonite (heavy solid line = calcite of Kim & O'Neil 1997, enriched by 0.6‰ for aragonite). Also shown are the published otolith relationships of Kalish (1991b) (— · — · —) and Thorrold et al. (1997b) (- - - -); the otolith-based relationships of Patterson et al. (1983), Kalish (1991c) and Radtke et al. (1996) are also shown, but are indistinguishable from that of inorganic aragonite. The overall relationship of aragonitic forams and molluscs studied by Grossman & Ku (1986) (· · · · ·) has been commonly used for otoliths in the past, but no longer seems appropriate

tive to calcite has been well documented (Tarutani et al. 1969, Grossman & Ku 1986, Kim & O'Neil 1997), the fractionation equation for aragonite can be calculated. A comparison between the resulting inorganic aragonite fractionation relationship and that estimated in several otolith studies shows a striking resemblance (Fig. 9). Furthermore, there is no evidence of a relationship between precipitation rate and fractionation (Thorrold et al. 1997b). Deviations among studies in the intercept of the fractionation relationship shown in Fig. 9 are unlikely to be biologically significant, since some studies inferred, rather than measured, the isotopic composition of the water (Radtke 1984, Kalish 1991b). An additional report of non-equilibrium deposition was probably the result of a calculation error (Radtke et al. 1996). Most of the remaining studies show good agreement with the equation for inorganic aragonite (Kalish 1991c [across all species]; Patterson et al. 1993, Radtke et al. 1996), but differ slightly from that based on aragonitic foraminifera and molluscs, which was previously applied to otoliths (Grossman & Ku 1986). In light of the close similarity of the recent experimental results on otoliths with the theoretical expectation, there is no reason to continue to use the Grossman & Ku (1986) equation with otoliths. Therefore, the relationship between temperature and the oxygen isotopic ratio is best described by that of inorganic aragonite

(calcite equation from Kim & O'Neil [1997] applying their revised acid fractionation factor, enriched by 0.6‰ for calcite-aragonite):

$$1000 \ln \alpha = 18.03 (1000 T K^{-1}) - 31.82$$

where  $\alpha$  = the fractionation factor  
 $= (\delta_{oto} + 1000) / (\delta_w + 1000)$   
 or approximately:

$$\delta_{oto} - \delta_w = 3.71 - 0.206 T ^\circ C$$

Normally, the temperature term would refer to the temperature of the ambient water, but in thermo-regulating fishes such as tuna, it must be referred to the internal temperature of the fish (Radtke et al. 1987). As is clearly evident from both of the above equations, it is not possible to estimate temperature from otolith oxygen isotopic ratios unless the isotopic composition of the water at the time of otolith formation is known (or can be estimated). Broad correlations between the isotopic composition of water and salinity exist, but these often vary substantially with water depth, latitude and other factors (Tan et al. 1983). As a result, interpretation of otolith oxygen isotopic ratios can be dangerous in the absence of independent information on the isotopic composition of the water (Thorrold et al. 1998b).

There is broad agreement that carbon isotopes are deposited in otoliths under non-equilibrium conditions, probably due to metabolic generation of the carbonate ion incorporated into the endolymph from the blood plasma (Kalish 1991c, Gaudie 1996a, Thorrold et al. 1997b, Schwarcz et al. 1998). Reported values for otolith  $\delta^{13}C$  range from  $-9$  to  $+1$ , and are typically 5‰ more depleted than inorganic aragonite (Romanek et al. 1992). In contrast, blood and other fish tissues are highly depleted in  $\delta^{13}C$  due to their metabolic origin, with values of about  $-17$ ‰ (Fry 1983). The large discrepancy in the  $\delta^{13}C$  composition between otolith and blood is attributable to the fact that only 10 to 30% of the otolith carbon is derived from metabolic sources (Kalish 1991c, Schwarcz et al. 1998); the remainder probably comes from DIC which is typically about 1‰ in marine surface waters (Schwarcz et al. 1998). Overlying all of these effects is a weak positive relationship between otolith precipitation rate and  $\delta^{13}C$ , which is further evidence of a metabolic influence on fractionation (Thorrold et al. 1997b).

Interpretation of the otolith  $\delta^{13}C$  signal is complicated by more than just metabolic fractionation. As noted by Schwarcz et al. (1998), the metabolic component of otolith  $\delta^{13}C$  is in turn influenced by the  $\delta^{13}C$  of the diet and the metabolic rate of the fish. In general, the  $\delta^{13}C$  of the diet can be expected to increase with trophic level, whereas the metabolic rate (and meta-

bolic contribution) would be expected to decrease with age. These 2 processes are probably responsible for the oft-observed increase in otolith  $\delta^{13}\text{C}$  until the age of maturity (Mulcahy et al. 1979, Kalish 1991c, Gauldie 1996a, Schwarcz et al. 1998), since both would result in an increase in otolith  $\delta^{13}\text{C}$  with age. Temperature effects on otolith  $\delta^{13}\text{C}$  appear to be small, resulting in a decline of 0.2‰ for each degree of warming (Kalish 1991c, Thorrold et al. 1997b). A previous report of no temperature effect appears to be based on an error in calculation (Radtke et al. 1996). Variations in the  $\delta^{13}\text{C}$  of DIC due to water depth are slightly larger, declining by about 2‰ over the upper 300 m of the water column (Schwarcz et al. 1998).

## RADIOISOTOPES

Radiochemical dating of calcified structures has a long history in corals and molluscs (Moore & Krishnaswami 1972, Druffel & Linick 1978, Turekian et al. 1982, Cochran & Landman 1984). The same underlying concepts apply to fish otoliths, and are based on well established physical principles governing radioactive decay. Radioisotopes are incorporated into fish otoliths in exactly the same way as are stable isotopes of any given element. Once incorporated into the otolith, the radioisotopes decay into radioactive daughter products, which are themselves retained within the acellular crystalline structure. Since the half-lives of the parent and daughter isotopes are known (and fixed), the ratio between them is an index of elapsed time since incorporation of the parent isotope into the otolith. In the case of man-made radioisotopes (e.g. those from nuclear explosions), an additional application is possible; the presence of the parent isotope can be used as a dated marker in the calcified structure, taking advantage of independent knowledge of the year of introduction of the radionuclide into the fish's environment. Since natural radioisotopes are generally interpreted in terms of the extent of radioactive decay, while radioisotope products of nuclear explosions are used as dated marks, they will be treated separately in the following discussion.

### Natural radioisotopes

Given their extremely low concentrations in both the environment and the fish, only a handful of natural radioisotopes have been detected in otoliths (e.g.  $^{14}\text{C}$ ,  $^{226}\text{Ra}$  and

$^{228}\text{Ra}$ ,  $^{210}\text{Pb}$ ,  $^{210}\text{Po}$ ,  $^{228}\text{Th}$ ,  $^{238}\text{U}$ ). Other radioisotopes such as  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  have been detected in fish tissues (Beddington et al. 1989, Forseth et al. 1991), suggesting that they may also be incorporated into the otolith. Nevertheless, the only attention which has been given to otolith radioisotopes has been in the context of age determination and validation, and virtually all of that attention has been focused on only 2 isotope pairs:  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  (Bennett et al. 1982, Campana et al. 1990, Fenton et al. 1990, 1991, Kestelle et al. 1994, Milton et al. 1995) and  $^{228}\text{Th}$ : $^{228}\text{Ra}$  (Smith et al. 1991, Campana et al. 1993). A third isotope pair,  $^{210}\text{Po}$ : $^{210}\text{Pb}$ , has been used to age molluscs (Cochran & Landman 1984, Fabry & Delaney 1989), but has not yet seen use in otoliths.

All radioisotopes have characteristic half-lives which reflect their exponential decay rates and the rates at which they approach secular equilibrium (when the rate of loss [through decay] of the daughter comes to equal the rate of loss of the parent). The radiometric equations describing their decay and interpretation in otoliths have been described elsewhere (Campana et al. 1993, Kimura & Kestelle 1995). It is the rate at which secular equilibrium is approached which determines the preferred age range for any given isotope pair. For example,  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  activity ratios, where the half-lives of  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  are 22.3 and 1600 yr respectively, approach a secular equilibrium of 1 over a period of more than 100 yr (Fig. 10). Yet the activity ratio changes more and more slowly as the equilibrium is approached, limiting the useful range of  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  to about 0 to 50 yr. For example, a 0.1 uncertainty in

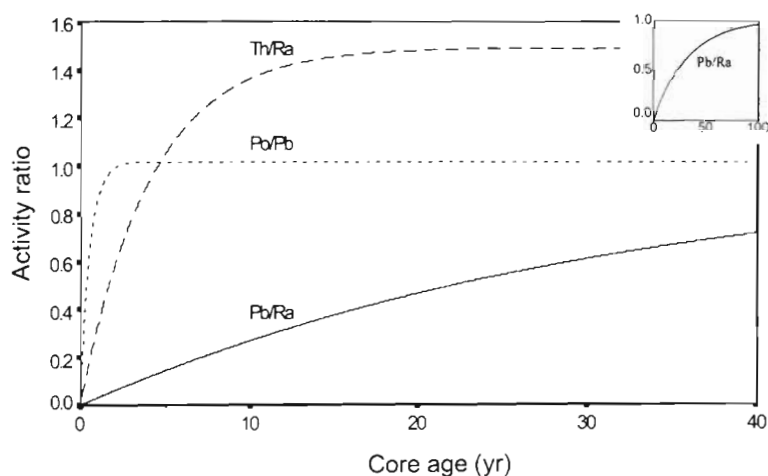


Fig. 10. Relationship between the activity ratio of  $^{228}\text{Th}$ : $^{228}\text{Ra}$ ,  $^{210}\text{Po}$ : $^{210}\text{Pb}$  and  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  as a function of fish age when the radiochemical assay is restricted to an otolith core (age 0) with an initial activity ratio of 0. Since age categories become increasingly difficult to separate as the activity ratio approaches secular equilibrium, Po:Pb, Th:Ra and Pb:Ra ratios are best suited for age determinations of 0 to 1, 0 to 8 and 0 to 50 yr, respectively. Insert shows full age range for the  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  activity ratio

the activity ratio may correspond to an uncertainty of just over 3 yr in a young otolith core, but would correspond to 20 yr in a core from a 50 yr old fish. Similar principles control the useful age range of both the  $^{210}\text{Po}$ : $^{210}\text{Pb}$  (0 to 1 yr) and the  $^{228}\text{Th}$ : $^{228}\text{Ra}$  (0 to 8 yr) isotope pairs (Fig. 10).

Unlike many other aspects of otolith science, the assumptions central to radiochemical dating in otoliths have been given careful attention by a number of authors (Fenton et al. 1990, Fenton & Short 1992, Campana et al. 1993, Kastelle et al. 1994, West & Gauldie 1994, Francis 1995, Kimura & Kastelle 1995). Some problematic assumptions must be made to interpret radioisotope ratios in whole otoliths. However, since the otolith core was formed when the fish was very young, the age of the extracted otolith core is very similar to the age of the fish (Campana et al. 1990, Smith et al. 1991, Kimura & Kastelle 1995). Thus interpretation of the otolith core avoids problematic assumptions, and is widely acknowledged to provide more reliable results than would the whole otolith (Kimura & Kastelle 1995). The assumptions for dating otolith cores are as follows:

(1) *The otolith constitutes a closed chemical system, such that radionuclides are neither gained nor lost after incorporation, except through decay. Furthermore, there is no appreciable migration of radionuclides within the otolith (e.g. into or out of the core region).* Clear violations of this assumption have been observed in shark vertebrae (Weldon et al. 1987), in which remobilization of calcified material distorted the expected daughter gradient between the older centre and the younger edge. However, such remobilizations do not occur in otoliths, since they are both acellular and metabolically inert (Campana & Neilson 1985). The possibility of loss of  $^{222}\text{Rn}$ , the gaseous intermediate of the  $^{226}\text{Ra} \rightarrow ^{210}\text{Pb}$  decay chain, through the channel architecture of the otolith has been vigorously debated in the literature (Fenton et al. 1990, 1991, Gauldie et al. 1992, Campana et al. 1993, West & Gauldie 1994), although neither side has presented experimental evidence in support of their position. Nevertheless, there is compelling empirical evidence in support of a closed system: if  $^{222}\text{Rn}$  is lost during decay, age estimates based on daughter:parent isotope ratios should be skewed towards lower ratios, and hence younger ages. Yet all published  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  ratios to date have resulted in extremely old age estimates, some of which have exceeded 100 yr (Campana et al. 1990, Fenton et al. 1991, Francis 1995). Therefore, if Rn emanation is occurring in these otoliths, it must be occurring at very low levels.

(2) *Uptake of daughter isotopes from external sources (allogenic) is small compared to that of the parent. Where this uptake is non-zero, it must be known*

*or measured.* An otolith core can be dated even if allogenic uptake of the radioisotopic daughter is large during the period of core formation, as long as the initial daughter:parent ratio is known. Moderate uncertainty (e.g. 10%) around some mean value of the ratio will have little influence on the predicted decay curve if the allogenic daughter:parent ratio is small (e.g. <0.2). However, the same relative uncertainty can introduce serious error in age estimation if the mean ratio is high, since the ability to discriminate among adjacent age groups decreases as secular equilibrium is approached (Fig. 10). The initial value of the daughter:parent ratio can be inferred using otoliths of young fish (of the same age as the core) from several year-classes from an appropriate location. Where these measurements have been made,  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  ratios have generally been less than 0.2 (Bennett et al. 1982, Campana et al. 1990, Fenton et al. 1990, 1991, Kastelle et al. 1994, Milton et al. 1995). However, large variations in the first few years of life have been noted (Milton et al. 1995), suggesting that significant uptake of allogenic Pb or  $^{210}\text{Po}$  (the intermediate daughter) is quite possible (Fabry & Delaney 1989, Fenton et al. 1990).

Radiochemical dating of whole otoliths makes the same 2 assumptions as listed above, although the assumption dealing with negligible internal migration of radioisotopes within the otolith does not apply. However, assays of whole otoliths make the following 2 additional assumptions, both of which can be troublesome:

(3) *The rate of uptake of both the parent and daughter isotopes remains in constant proportion to the mass accumulation rate of the otolith.* As was the case with the otolith cores, the initial daughter:parent ratio must either be low or known. The additional constraint with the interpretation of whole otoliths is that this ratio must remain constant and in fixed proportion with calcium deposition as the otolith grows. Otherwise, it is impossible to know the proportion of decay which occurred in each year of growth. Only Fenton et al. (1990, 1991) have tested this assumption, but they documented age-dependent trends in the  $^{226}\text{Ra}$ :Ca ratio, thus invalidating the assumption. In addition, Fowler et al. (1995a) demonstrated that the rate of Pb incorporation into the otolith increased with decreasing growth rate, suggesting that Pb incorporation probably increases with fish age.

The assumption associated with a fixed rate of radioisotope uptake can be relaxed if stable, more easily measured proxies can be found for the parent and daughter radioisotope (Fenton & Short 1992). The use of stable Pb as a proxy for  $^{210}\text{Pb}$  seems reasonable, since there is little reason to expect age-related fractionation between  $^{210}\text{Pb}$  and the far more abundant stable isotopes of Pb. However, the application of Ba as



an analog of Ra assumes that the chemical behaviour of Ba in the water column is similar to that of Ra. There is no evidence to suggest that this is the case (Bruland 1983). Re-analysis of the data of Milton et al. (1995) showed no significant correlation between Ba and Ra concentrations, whether within otolith cores or the whole otolith. Therefore it is premature to consider Ba as a stable equivalent of  $^{226}\text{Ra}$ .

(4) *The mass growth rate of the otolith is either constant or its rate of change is known.* Since the otolith core decays at a fixed and known rate over a length of time almost equivalent to the age of the fish, it is independent of any subsequent otolith growth. However, when the entire otolith is analyzed, the outer layers of the otolith will have been more recently deposited and hence will have experienced less radioactive decay than the inner growth layers. Modification of the decay equations to accommodate variable otolith growth rates are straightforward and uncontested as long as the relative otolith growth rate is known (Bennett et al. 1982, Campana et al. 1993, Francis 1995, Kimura & Kestelle 1995). However, it is circular reasoning to use annulus counts to calculate the growth rate required of the decay equation, which is then used to validate the annulus counts (West & Gauldie 1994, Francis 1995, Kimura & Kestelle 1995). In a simulation study of the effect of ageing error on the calculated radiochemical age, Kimura & Kestelle (1995) demonstrated that incorrect annulus-based ages readily translated into incorrect radiochemical ages, with each supporting the 'accuracy' of the other. Francis (1995) also recognized this problem, and proposed the use of 2 alternative approaches which provide most-probable and/or minimum age estimates, thus avoiding the need for annulus-based ages. While a notable improvement over previous whole-otolith equations, these alternative approaches still assume 2-stage linear otolith growth, an assumption whose validity remains to be tested.

### Radioisotopes from nuclear explosions

The widespread atmospheric testing of atomic bombs in the 1950s and 1960s released more than a dozen radioactive isotopes into the environment. Isotopes such as  $^{90}\text{Sr}$  and  $^{239}\text{Pu}$  tended to be associated with particulates (nuclear 'fallout'), and thus were most concentrated in the vicinity of the bomb site. On the other hand, radiocarbon ( $^{14}\text{C}$  or carbon-14) was introduced as carbon dioxide gas, spread rapidly throughout the atmosphere, and soon resulted in a doubling of background atmospheric radiocarbon levels (Nydal & Lovseth 1983). Rain and atmosphere-ocean gas exchange quickly introduced the radiocarbon into the surface layer of the world's oceans in a manner which

has been well described at large spatial scales (Broecker et al. 1985, Duffy et al. 1995). Through analysis of annular growth rings in coral, various workers demonstrated that bomb radiocarbon was incorporated into the accreting coralline skeleton in concentrations proportional to those present in the water column at the time (Druffel & Linick 1978, Nozaki et al. 1978). Thus the time series of bomb radiocarbon in the coral was shown to reflect that present in the marine environment, which increased by about 20% between 1950 and 1970. During the same time period, radionuclides such as  $^{90}\text{Sr}$  suddenly appeared in bones, antlers and other calcified structures (Beddington et al. 1989, Schonhofer et al. 1994). In the case of both radiocarbon and the other bomb-produced radionuclides, the period corresponding to their sudden increase in the environment serves as a dated marker for those calcified tissues in which they were incorporated.

Kalish (1993) was the first to demonstrate that fish otoliths also incorporated bomb  $^{14}\text{C}$ , and that the time series of radiocarbon reconstructed from presumed otolith annuli was similar to that present in nearby corals. Thus he was able to infer that the otolith annuli had been interpreted and aged correctly, because systematic under- or over-ageing would have resulted in a phase shift between the otolith  $\Delta^{14}\text{C}$  and the coral  $\Delta^{14}\text{C}$  time series. Subsequent work (Kalish 1995a,b, Kalish et al. 1996, 1997, Campana 1997, Campana & Jones 1998) has confirmed the value of the bomb radiocarbon technique for solving problems of age validation in a variety of fish species. A similar approach has been used by workers in other disciplines to infer age and the frequency of growth ring formation in both bivalves (Turekian et al. 1982, Peck & Brey 1996) and mammals (Bada et al. 1990). Note that this approach is unlike traditional radiocarbon dating, since there is no appreciable radiocarbon decay over periods of less than 100 yr.

The synchrony in the appearance of the bomb  $\Delta^{14}\text{C}$  signal in surface waters throughout the global ocean is striking, particularly since it has been observed in a diverse array of organisms (Fig. 11). Corals, bivalves and fish otoliths from the North Atlantic (Druffel 1989, Weidman & Jones 1993, Campana 1997) through to the South Pacific (Kalish 1993, Guilderson & Schrag 1998, Peng et al. 1998) all recorded a sharp increase in  $\Delta^{14}\text{C}$  between the late 1950s and early 1970s. Such large-scale synchronicity implies that the  $\Delta^{14}\text{C}$  time series reconstructed from the otolith cores of old fish can safely be compared (and validated) against a baseline chronology from any other species in the region from a comparable habitat. As will be discussed later, subtle differences in the phase of the various  $\Delta^{14}\text{C}$  chronologies may exist, attributable primarily to depth (Fig. 11) and water source (Kalish 1995b, Campana & Jones

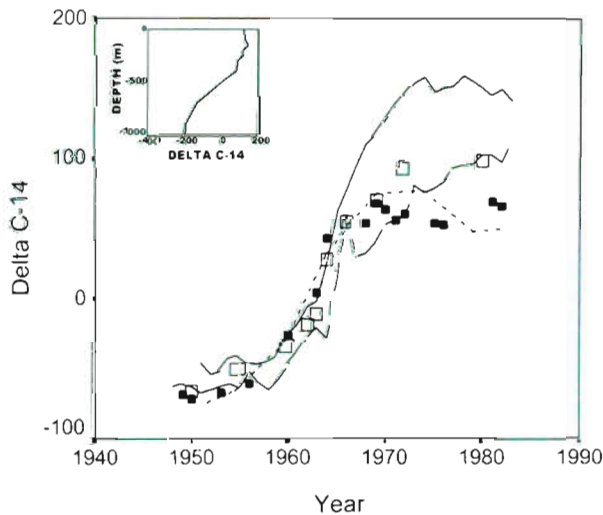


Fig. 11. Time series of bomb  $\Delta^{14}\text{C}$  uptake into Bermuda coral (—), South Pacific coral (---), and Georges Bank bivalves (· · ·), as well as known-age *Melanogrammus aeglefinus* (■) otoliths from the NW Atlantic and *Pagrus auratus* (□) otoliths from the South Pacific. Note the synchrony in the timing of the bomb  $\Delta^{14}\text{C}$  signal increase among the diverse taxa and locations, but the differences in pre- and post-bomb levels due to differences in water residence times. Insert shows typical relationship between depth and  $\Delta^{14}\text{C}$  (data drawn from Peng et al. 1998). The Bermuda and South Pacific coral chronologies are drawn from Druffel (1989) and Guilderson et al. (1998), respectively, while the bivalve time series is from Weidman & Jones (1993). The *Melanogrammus* and *Pagrus* data are from Campana (1997) and Kalish (1993), respectively

1998). However, the most substantial differences are associated with the post-bomb  $\Delta^{14}\text{C}$  history, which is strongly influenced by water mixing times (Weidman & Jones 1993, Guilderson & Schrag 1998). For this reason, the post-bomb  $\Delta^{14}\text{C}$  level requires more careful interpretation than does the period of increase, although it can be valuable when used as a tracer of upwelling and circulation (Kalish 1995b). In contrast, the phase (rather than the magnitude) of the chronology is of greater importance for age validation.

Three assumptions underlie the use of the  $\Delta^{14}\text{C}$  chronology for the validation of otolith annuli: (1) the extracted otolith core must not be contaminated with material of more recent origin; (2) annulus interpretations and any associated errors must be made consistently across all ages examined; and (3) the period of increase in the  $\Delta^{14}\text{C}$  'reference' chronologies must be synchronous with that of the fish species under study. The first assumption has been both modelled (Kalish et al. 1996) and tested (Campana 1997). In the latter study, intact age 1 otoliths and the extracted otolith core of older fish from the same year-class were similar but not identical. Since the cores from the older fish contained somewhat more recent  $\Delta^{14}\text{C}$  values,

there may have been limited contamination of the cores with more recently deposited otolith (and  $\Delta^{14}\text{C}$ ) material. The second assumption is implicit in all ageing studies, since it implies that a given growth increment is interpreted in the same way, whether observed in a young or an old fish. However, the third assumption, that of synchronicity among the  $\Delta^{14}\text{C}$  chronologies, is more interesting. This assumption has been tested and confirmed in all reported bomb radiocarbon otolith ageing studies on marine fishes living in the surface mixed layer, whether in the south Pacific (Kalish 1993, 1995a) or the north Atlantic (Campana 1997). However, lack of synchrony has been reported in both estuarine (Campana & Jones 1998) and deep-sea (Kalish et al. 1997) fishes. In the case of the estuarine fish, the  $\Delta^{14}\text{C}$  chronology reconstructed from otolith cores was phase shifted 2 to 4 yr towards earlier years, consistent with an atmospheric rather than a marine input. Because estuaries are shallow, well-mixed areas with strong riverine input, there is a rapid and relatively complete exchange of radiocarbon between the atmosphere and the water. As a result, the  $\Delta^{14}\text{C}$  chronology of an estuary is a much closer reflection of the atmospheric chronology (which started to increase in the mid-1950s) than of the marine chronology (Erlenkeuser 1976, Spiker 1980, Tanaka et al. 1986). The opposite is true of deep-sea waters, which were much slower to mix with surface waters containing the bomb  $\Delta^{14}\text{C}$  signal, and thus were characterized by a delayed  $\Delta^{14}\text{C}$  chronology. As a result, interpretation of the  $\Delta^{14}\text{C}$  signal in the otolith core of a deep-sea fish can be problematic, and must be carefully interpreted according to depth unless the fish's juvenile phase (corresponding to the otolith core) was spent in near-surface waters (Kalish 1995b, Kalish et al. 1997).

Interpretation of an otolith-based chronology will not generally be influenced by the magnitude of the pre- and post-bomb  $\Delta^{14}\text{C}$  levels, since it is the period of increase that is of interest for age determination. However, calibration of individual  $\Delta^{14}\text{C}$  values against the year of formation is also possible as long as additional information is available. Post-bomb, and to a lesser extent pre-bomb,  $\Delta^{14}\text{C}$  values vary significantly and substantially with latitude, upwelling, circulation, depth and any other factor influencing the mixing of sub-surface,  $^{14}\text{C}$ -poor waters with surface waters (Druffel 1989, Toggweiler et al. 1991, Peng et al. 1998). The variation typically associated with water depth is shown in the insert of Fig. 11. In the absence of information on the fish's habitat at the time of otolith annulus formation, it would be difficult to assign a year of formation to an individual  $\Delta^{14}\text{C}$  value. Additional variability due to diet might be expected, in light of the fact that about one-third of otolith carbon is metabolically

derived (Kalish 1991c, Schwarcz et al. 1998). However, diet is unlikely to be a significant modifier as long as the prey live in a water mass comparable to that of the fish. Examples of habitats where diet might be expected to be a confounding issue would include deep-sea habitats, where fish could feed on dead prey sinking from surface waters above, and estuarine habitats, where otolith composition could reflect a combination of terrigenous food sources (which tend to be enriched in  $^{14}\text{C}$ , similar to the atmosphere) and  $^{14}\text{C}$ -depleted marine sources. In such environments, the  $\Delta^{14}\text{C}$  signal would be expected to be more variable than in a more homogeneous environment like the open ocean (Campana & Jones 1998).

### Sampling and analyses

A distinctive and powerful feature of the field of otolith chemistry is that the assays can either be restricted to some portion of the fish's life history or integrated across the entire lifetime of the fish. In other words, the scale of sampling can be modified to address the hypothesis being tested, through analysis of either the entire otolith or through a targeted assay of a specific region. In general, analyses of whole otoliths are best suited to questions of stock discrimination, since the primary question is one of overall differences between groups of fish, integrated across their lifetimes. In contrast, microsampled or beam-based assays can target a particular range of ages or dates, and thus take advantage of the chronological growth sequence recorded in the otolith. Currently, bulk and/or solution-based elemental assays are capable of better accuracy, precision and/or sensitivity than are most beam-based assay techniques, a factor that

must be considered given the exceedingly low concentrations of many otolith trace elements.

Assays of whole dissolved otoliths have become popular for differentiating among stocks and tracking migrations, since the elemental composition can be used as a natural tag even without knowledge of the original source of the component elements (Edmonds et al. 1989, 1995, Campana et al. 1995, 1999b, Gillanders & Kingsford 1996). Advantages of whole-otolith assays include ease of preparation, absence of error associated with sampling or identifying growth increments, and the availability of accurate and precise assay protocols. The major disadvantage is associated with the inability to take advantage of the chronological growth sequence recorded in the otolith. Atomic absorption spectrometry (AAS) (Grady et al. 1989, Hoff & Fuiman 1995), inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Edmonds et al. 1995), neutron activation analysis (Papadopoulou et al. 1980), Raman spectroscopy (Gauldie et al. 1994) and inductively coupled plasma mass spectrometry (ICPMS) (Edmonds et al. 1991, Dove et al. 1996) are among the techniques which have been used to analyze otoliths. However, it is ICPMS which has emerged as the instrument of choice for such assays, due largely to its capability for rapid and accurate isotopic and elemental assays over a wide range of elements and concentrations (Table 3). Isotope dilution ICPMS (ID-ICPMS), a variant of ICPMS often used to certify reference materials (Fassett & Paulsen 1989), is the most accurate of the otolith analytical techniques currently available (Campana et al. 1995). Sample sizes required for most of the above assays are on the order of 5 to 10 mg of otolith material, although ICPMS units outfitted with high efficiency nebulizers are capable of handling otolith weights as low as 0.3 mg (Thorrold et al. 1998a).

Table 3. Summary of the most frequently measured otolith elements which can be detected and quantified by each of the most popular analytical instruments. Mean limits of detection (LOD in  $\mu\text{g g}^{-1}$ ) are listed where available, as drawn from unpublished data and the literature. Values in brackets represent elements with a record for both reliable and unreliable quantification, while blank entries are either unreliable or unmeasured. A = reliable quantification, but with an unknown LOD. Elements are listed in declining order of relative abundance. Since there is no established equation for ED-EM LODs, the listed LODs for ED-EM are approximate only

Type of assay	Instrument	Beam diameter ( $\mu\text{m}$ )	Element																	
			Ca	Na	Sr	K	S	Cl	P	Mg	Zn	B	Fe	Mn	Ba	Ni	Cu	Li	Pb	
Whole otolith	AAS	-	0.5	0.2	2	2				0.1										
	ICP-AES	-	A	50	1	150	90		100	20		(7)		(5)		(5)				
	ICPMS	-	4.3	(1)	0.3					0.06	0.05	A	(4)	0.002	0.005	0.006	0.03	0.007	0.009	
Beam-based	ED-EM	2-20	A (12000) (6000) (2000)																	
	WD-EM	2-20	525	240	300	220	200	200	600	(300)										
	PIXE	3-20			0.8						0.5		5	(8)		1.5	1.3		(5)	
	LA-ICPMS <sup>a</sup>	5-30	A		1.3					0.1	0.2	(A)			0.04	(0.3)			(A)	

<sup>a</sup>LODs based on Ar gas blank, with no attendant laser pulse

With analytical sensitivity comes the potential for contamination. Factors such as the mode of fish or otolith preservation, composition of the instruments used to remove the otolith from the fish, cleaning methods, handling and even dust are all potentially major modifiers of the perceived trace element composition. Preservation fluids such as ethanol and formalin appear to have the greatest potential for contamination, given the microchannel architecture of the otolith and the relative impurity of most preservatives (Milton & Chenery 1998, Proctor & Thresher 1998). Small but significant effects due to freezing have been observed in one study (Milton & Chenery 1998), but not in another (Proctor & Thresher 1998). Therefore, trace element analysis of otoliths removed from fish shortly after capture and stored dry appears to be safest. Current protocols for handling and preparing otoliths are drawn from the water analysis literature (Benoit et al. 1997), and always involve isolation from skin, metallic instruments, and solutions which are of other than trace metal grade. In general, decontamination based on brushing and sonification in a series of distilled, deionized, reverse osmosis water baths (Super-Q or Milli-Q water) in a positive flow, laminar flow fume hood (Class 100), followed by storage in dry, acid-washed polyethylene vials, results in minimal contamination. Minor elements such as Na, K, Cl, and S are likely to be affected by the water sonification stage (Proctor & Thresher 1998), perhaps because these elements are incorporated by occlusion and are not lattice bound. However, it is equally probable that such poorly bound elements would be severely affected by exposure to any fluid, including the endolymph if it shifts its composition during the death of the fish. As a result, such elements would probably not be well suited for use as stable biological tracers. Acid washing of otoliths does not appear to be necessary for elements such as Ba, Mg, Sr, and Li (Campana et al. 1999a), despite the fact that it is an important step in the decontamination of sediment-laden forams (Boyle 1981).

Of particular relevance to ICPMS, but applicable to all analytical techniques, is the likelihood of instrument drift (change in sensitivity) during the analysis of large numbers of samples or between analysis sessions. Since the estimated elemental concentration can be significantly affected by this drift despite the analysis of analytical standards, it is important that the analysis sequence be blocked and randomized so that the order of analysis for any one sample group is spread over the entire analysis sequence. Use of ID-ICPMS minimizes (although it may not eliminate) the effects of instrumental drift.

Assays for bomb radiocarbon and stable isotope ratios are examples of applications where a particular

age or date range in the otolith is required, but beam-based assay techniques are either inappropriate or insufficiently sensitive. For these applications, the best alternative often involves microsampling or coring techniques which physically remove a portion of the otolith for subsequent analysis. Computerized micro-milling machines have proven effective in stable isotope studies, whereby seasonal or annual growth zones visible in otolith cross-sections are milled to a discrete depth and the powder collected for assay (Prezbindowski 1980, Patterson et al. 1993, Schwarcz et al. 1998). Earlier studies used hand-held dental drills for the same purpose (Meyer-Rochow et al. 1992). Where coarser sampling is adequate, otolith cores (e.g. first year of growth) can be extracted using either a stepwise grinding (Campana et al. 1990), drilling (Campana 1997) or sculpturing (Kalish 1995a) approach. Controlled acid dissolution of overlying material has also been reported, although the acid apparently leached some material from the core (Dove et al. 1996). The advantages of microsampling or coring include access to bulk analytical techniques of high sensitivity and accuracy: accelerator mass spectrometry (AMS) in the case of  $^{14}\text{C}$  (Kalish 1993), mass spectrometry for  $^{18}\text{O}:^{16}\text{O}$  and  $^{13}\text{C}:^{12}\text{C}$  ratios (Patterson et al. 1993), and ICPMS for trace element assays (Campana & Gagné 1995). The disadvantage is one of limited sampling resolution, since the temporal resolution of the extracted sample is seasonal at best (Campana et al. 1990, Patterson et al. 1993). This limited resolution is in part a constraint of the physical sampling, since it is extremely difficult to trace the 3-dimensional, non-linear form of otolith growth zones. However, a more telling limitation is that of the sample size required for the assays: current limitations for stable oxygen isotope and radiocarbon assays are about 30  $\mu\text{g}$  (Schwarcz et al. 1998) and 5 mg (Kalish 1995b), respectively. It appears unlikely that microsampling or coring would introduce contaminants which would confound stable isotope or radiocarbon assays, as long as the extracted samples were treated carefully. On the other hand, there is potential for contamination from the sampling process associated with trace element assays, despite the fact that Dove et al. (1996) reported no artifacts due to sectioning with an Isomet saw.

Beam-based assays target a particular age or date range in the sectioned otolith, and thus take advantage of the chronological growth sequence recorded in the otolith. These types of targeted assays have been used to reconstruct migration histories (Secor et al. 1995b, Thorrold et al. 1997a), identify nursery areas (Thresher et al. 1994, Milton et al. 1997), and determine Sr:Ca ratios in order to infer temperature history (Radtke et al. 1990, Townsend et al. 1995). The advantages of an age-structured approach are obvious, particularly

since the beam sizes of the current generation of instruments approach the width of a typical daily increment (Table 3). As a result, the assay can be limited to the time scale of interest, whether it is weekly, annual or at an intermediate scale. Disadvantages of the approach include the requirement for sectioning to expose the growth sequence, the potential for contamination from the sectioning and polishing procedure, and some degree of beam penetration into underlying growth layers. The method also makes 2 significant assumptions: firstly, that there is no ageing error, and that the probe assays have been correctly aligned with the appropriate growth increment; and secondly, that the estimated elemental concentration in any given growth increment is independent of the growth axis that is sampled. This latter assumption is an interesting one, since it implies that the rate of elemental incorporation is independent of the rate of calcification, contrary to our current understanding of trace element incorporation. The few studies that have examined these assumptions in otoliths reported varying levels of consistency in minor element concentrations along a variety of transects within an otolith, or between paired otoliths (Gallahar & Kingsford 1992, Gunn et al. 1992, Secor 1992, Thresher et al. 1994). It was not clear from these studies whether the variation was real, or if it merely reflected difficulties in aligning the microprobe beam to the appropriate increment. Trace element concentrations, in which an effect of calcification rate might be expected to be more pronounced, have not yet been examined in this manner. However, there are reports of extension rate along a given growth layer influencing trace element concentrations in coral (de Villiers et al. 1994). Until this issue is examined in further detail, the selection of standardized otolith growth axes for probe assays appears wise.

There are a wide variety of sophisticated instruments available for probed assays of the otolith (Coutant 1990, Jackson et al. 1993, Gauldie et al. 1994, Jambers et al. 1995), but the most frequently used include the energy-dispersive (ED-EM) and wavelength-dispersive (WD-EM) electron microprobes (Gunn et al. 1992), proton-induced X-ray emission (PIXE) (Sie & Thresher 1992), and laser ablation ICPMS (LA-ICPMS) (Campana et al. 1994). In a detailed experimental comparison among the above instruments, Campana et al. (1997) noted that no one instrument type was sensitive to each element, nor was any one instrument preferred for use in all assays. In general, however, the minor elements such as Na and K could only be measured accurately with an electron microprobe, while the trace elements required PIXE or LA-ICPMS. Sr was measured accurately and precisely with either WD-EM, PIXE or LA-ICPMS. An overview of the capabilities of the most popular instrumentation is presented in

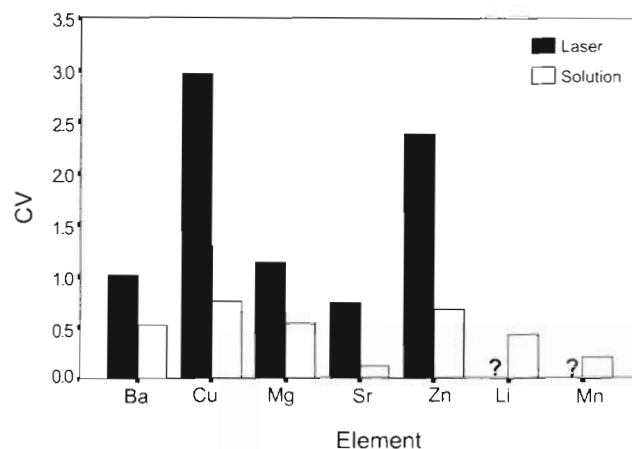


Fig. 12. Superior sensitivity and precision (CV) of solution-based over laser-based ICPMS assays in a matched comparison of otolith pairs reared under a constant environment. LA-ICPMS assays for Li and Mn were not significantly above the limit of detection. All precision values include variability among fish, and thus are not measures of analytical precision. Data are drawn from Fowler et al. (1995a, b)

Table 3. Also evident from Table 3 is the reduced range or sensitivity of most beam-based assays compared to their solution-based counterparts, in part due to the much lower sample weights being analyzed. This difference becomes even more apparent in a matched comparison of otolith pairs analyzed with 2 complementary techniques: solution-based ICPMS and LA-ICPMS (Fig. 12). Not only did the sensitivity of the solution-based assays extend over a broader range of elements, but the precision of those assays was significantly better than those of the laser-based assays. Of course, such a comparison of precision is not strictly valid, since the probed assays incorporate within-otolith heterogeneity which is not present in the solution-based assays. However, such a caveat does not apply to the comparison of sensitivity.

The absence of otolith reference material has undoubtedly hindered progress in otolith trace element analyses, both solution-based and probe-based. While analytical standards are used routinely with all instrument types (Campana et al. 1997), they lack the aragonite-dominated matrix of an otolith, and thus do not provide the matrix matching so important to quantitative assays. The result is often an assay which is accurate relative to other samples being analyzed at the same time, but quantitatively in error (Campana et al. 1997). Pressed carbonate pellets (Perkins et al. 1991, van Heuzen & Morsink 1991, Pearce et al. 1992) and otolith powder fused into glass beads (Campana et al. 1997) have both been used for beam-based assays, but neither completely addresses the requirements for matrix matching or surface smoothness. Homogeneous otolith powders have been developed to ensure com-

parability among solution-based assays (Campana et al. 1999a), but are not available in quantities suitable for broad distribution. Until a certified otolith reference material, analogous to those used by analytical chemists in other fields (Beauchemin et al. 1988), is developed, comparability among published otolith trace element studies is likely to be suspect.

## Applications

### Stock identification

To the extent that groups of fish inhabit different environments, the otolith elemental composition should serve as a natural marker or tag of those groups. Two assumptions underlie the use of the otolith elemental composition as a marker: (1) material deposited on the otolith is metabolically inert after deposition, and is not susceptible to resorption; and (2) the physical and chemical environment influences the rate of trace element incorporation into the growing otolith surface. Elements under strong physiological regulation (e.g. Na, K, S, P, Cl) probably do not meet the second assumption, and are thus of limited value for tracking or stock identification studies (Thresher et al. 1994, Proctor et al. 1995). However, both assumptions appear to be met with respect to elements such as Sr, Ba, Mn, Fe and Pb (and perhaps Li, Mg, Cu and Ni), in which both ambient element:Ca concentrations and temperature produce significant effects on otolith composition (Fowler et al. 1995a, Farrell & Campana 1996, Dove 1997). Similar effects of environmental availability and temperature apply to isotopic ratios of elements such as strontium (Kennedy et al. 1997) and oxygen (Thorrold et al. 1997b). Environmental responses such as these, recorded permanently in the otolith, imply that the otolith concentrations of selected elements and isotopes (the 'elemental fingerprint') can be used as a biological tag to discriminate among groups of fish which have spent at least part of their lives in different environments. While the application of otolith composition as a natural tag appears to be relatively well founded, use as a stock or population marker requires considerably more assumptions (Campana et al. 1999a).

It is probably inappropriate to refer to the use of elemental fingerprints as stock discriminators, since genetic differences are not implied and spatial heterogeneity in the stock environment can result in different fingerprints for different stock components (Thorrold et al. 1998b, Campana et al. 1999a). Perhaps more importantly, ontogenetic effects and age-related differences in exposure history can result in very different fingerprints for fish of different size-classes from the same population (Papadopoulou et al. 1980,

Edmonds et al. 1989, Grady et al. 1989, Hoff & Fuiman 1993, Campana et al. 1995, 1999a, Begg et al. 1998). Since the elemental fingerprint reflects the exposure of the individual fish to both the environment and its own physiology, it would be expected to differ among any groups of fish which have experienced different histories, whether or not the groups come from the same population. Logically, the presence of different fingerprints could not be used to infer the length of time that the groups of fish remained separate, since even occasional residency in a different environment would have the potential to introduce a detectable difference in the elemental composition. By corollary, the absence of differences would not necessarily imply that the groups of fish are of common origin. As a result, it is fair to categorize otolith elemental fingerprints as powerful discriminators of groups when differences exist, but of negligible value when differences cannot be detected. Where differences are detected, additional information would be required to determine if the groups actually corresponded to stocks or populations.

Two types of elemental fingerprinting are in general use: one based on whole dissolved otoliths, and the other based on analysis of the otolith core. Since the otolith grows continually throughout the life of the fish, the whole-otolith fingerprint integrates across the entire lifetime, and thus serves as a marker for groups of fish which have experienced different overall environmental exposures (Edmonds et al. 1989, 1991, 1992, 1995, Northcote et al. 1992, Campana & Gagné 1995, Campana et al. 1995, 1999a,b, Bronte et al. 1996, Gillanders & Kingsford 1996, Edmonds & Fletcher 1997, Kennedy et al. 1997, Begg et al. 1998, Dove & Kingsford 1998, Secor & Zdanowicz 1998, Thorrold et al. 1998a). While the elemental fingerprint of a group of fish may or may not be characteristic of a stock or population, use of the fingerprint as a long-term stock discriminator may be justified in instances where environmental differences among stock areas are larger than those within areas or across year-classes, and where differences in the fingerprint due to fish size have been statistically removed. The assumption of long-term stability in the fingerprint is probably met in some, but not all, stocks (Campana et al. 1999a).

Analysis of the otolith core is generally intended as a more direct measure of stock origin than is the analysis of the whole otolith. As is the case with whole-otolith elemental fingerprints, the presence of fingerprint differences implies differences in the history of environmental exposure which may or may not correspond to genetic differences. In this application, however, the environmental exposure is limited to the period of growth represented by the otolith material which is assayed, whether that is the period around hatch, the first few months of life, or some other period. The sub-

sequent life history is not sampled, and is therefore irrelevant. To the extent that spawning grounds are characterized by different temperature or chemical environments, this approach has proven effective in distinguishing among groups of fish with different origins (Kalish 1990, Sie & Thresher 1992, Campana et al. 1994, Thresher et al. 1994, Proctor et al. 1995, Severin et al. 1995, Dove et al. 1996, Gillanders & Kingsford 1996, Milton et al. 1997, Thorrold et al. 1997a).

The most robust application of whole-otolith fingerprints is one which is targeted at questions of stock mixing or for tracking stock migrations, in which the fingerprints are used as natural tags of pre-defined groups of fish (Campana et al. 1995, 1999a, b, Gillanders & Kingsford 1996, Kennedy et al. 1997). Use of an elemental fingerprint as a natural tag takes advantage of the fact that otolith size and composition cannot change appreciably over a brief time period. Once the elemental fingerprint of all potential source groups has been determined, fish should remain identifiable as to their source group, despite any mixing with other groups, until the elemental composition of later otolith growth has significantly altered overall elemental composition. An appealing feature of this application is that the elemental fingerprint need not be linked to potential sources or locations in the environment.

Use of otolith elemental fingerprints as natural tags makes 3 central assumptions: (1) *There are characteristic and reproducible markers for each group.* If the elemental fingerprints of the groups of interest do not differ significantly, little more can be accomplished. However, group-specific variation in elemental composition appears to be common (Edmonds et al. 1989, 1991, 1992, 1995, Northcote et al. 1992, Campana & Gagné 1995, Campana et al. 1995, 1999a, Bronte et al. 1996, Thorrold et al. 1998a). Since smaller fish seem far more likely to contain elevated (or depressed) concentrations of any given element than larger fish (Papaopoulou et al. 1980, Edmonds et al. 1989, Grady et al. 1989, Hoff & Fuiman 1993), it is important that differences in elemental concentration among groups are not confounded by size differences among groups. Statistical removal of the effect of otolith weight on elemental concentration is a ready solution to this problem (Campana et al. 1999a); (2) *All possible groups contributing to the group mixture have been characterized.* This assumption applies as much to genetic studies as it does to otolith elemental fingerprints, with the implication being that uncharacterized groups of fish present in the mixture could be mistakenly interpreted as one or more of the reference groups (Wood et al. 1987, 1989, Wirgin et al. 1997). Careful selection of reference groups can help minimize this problem, particularly if they are sampled at a time when the groups are known to be discreet (e.g. on the spawning or nursery grounds);

(3) *The marker remains stable over the interval between characterization and mixing.* Long-term stability of an environmentally induced marker would not be expected, nor has it been observed (Edmonds et al. 1995, Campana et al. 1999a). However, short-term stability over the interval between characterization (e.g. spawning group) and mixing is both expected and observed, particularly with respect to differences among groups (Campana et al. 1995, 1999a, Kennedy et al. 1997, Thorrold et al. 1998a, but see Edmonds et al. 1995). In the case of the Atlantic cod *Gadus morhua*, this interval was less than 6 mo, and thus much less than the period required for a noticeable change in the elemental fingerprint (Campana et al. 1999b). Longer intervals may be possible in some instances, but the potential for drift in elemental composition becomes greater as the interval length is extended (Edmonds et al. 1995). By corollary, shorter interval lengths would presumably be required in analyses of young fish, in which the proportional annual change in otolith weight (and, potentially, composition) would be more marked.

#### Migration history

Reconstruction of migration histories using the elemental or isotopic composition along an otolith growth axis is similar in many respects to stock discrimination based on analysis of the otolith core, and shares many of the same assumptions. However, migration analysis expands the scope of the interpretation, by linking a series of elemental assays along an otolith transect to the growth chronology recorded in the otolith, thus allowing the reconstruction of migration pathways structured by age or date. An additional assumption of this application is that there is negligible ageing error and that growth increments have been accurately linked to assay locations. In principle, migration analysis is one of the most powerful applications of otolith microchemistry, although its successes to date have been largely limited to the detection of anadromy (Kalish 1990, Meyer-Rochow et al. 1992, Secor 1992, Halden et al. 1995, 1996, Limburg 1995, Secor et al. 1995b, Tzeng et al. 1997, Otake & Uchida 1998, Tsukamoto et al. 1998). Subtler migration patterns are now being detected with instrumentation such as PIXE and LA-ICPMS (Proctor et al. 1995, Blaber et al. 1996, Halden et al. 1996, Thorrold et al. 1997a), which are more sensitive to non-physiologically regulated trace elements (Campana et al. 1997). Past difficulties in interpreting migration histories may have been due to the use of the electron microprobe, which is more suited to assays of physiologically regulated elements. Several forms of migration analysis have been reported. One of the most successful has been the com-

parison of elemental trajectories among fish in search of a common history, or, alternatively, the determination of the age or date at divergence (Thresher et al. 1994, Limburg 1995, Proctor et al. 1995, Thorrold et al. 1997a). This is a particularly robust application, since it requires no knowledge of the fish's past environment, and is relatively insensitive to any ontogenetic shifts in otolith elemental composition which may have occurred. In many respects, this comparative approach is analogous to the use of elemental fingerprints as biological tracers, as described earlier. A potentially more powerful approach is one in which the otolith elemental trajectory is linked to shifts in temperature and water chemistry along possible migratory routes. Such an approach is the basis for the detection of anadromy using Sr:Ca ratios, in which the shift between freshwater and saltwater environments is clearly evident in the otolith (Kalish 1990, Secor 1992, Halden et al. 1995, 1996, Limburg 1995, Secor et al. 1995b, Tzeng et al. 1997, Otake & Uchida 1998, Tsukamoto et al. 1998). Migration through a more homogeneous environment should also be detectable. However, interpretation of such subtle shifts in composition is complicated by the assumption that a given elemental concentration reflects the same environmental condition at different points along an otolith transect. In other words, ontogenetic changes in otolith elemental composition are assumed not to exist. Yet such changes have been clearly documented, even in fish held under constant environmental conditions (Gallahar & Kingsford 1992, Fowler et al. 1995b, Fuiman & Hoff 1995, Hoff & Fuiman 1995). As a result, observed trends in concentration across an otolith could reflect either a shift in the fish's environment, an age-related change in incorporation rate independent of the environment, or both. With further experimentation, it may be possible to factor out ontogenetic shifts in elemental composition, thus simplifying the interpretation of possible migration pathways. To date, however, it is not always clear from published reports whether reconstructed distributions have been adjusted for ontogenetic effects (Townsend et al. 1992, 1995, Blaber et al. 1996).

#### Age determination

Age determination studies based on elemental or isotopic concentrations in the otolith are motivated by the ongoing requirement for accurate age-structured information in support of many fish studies. The objectivity of elemental assays is appealing in light of the subjectivity that can confound or invalidate the interpretation of annuli in otoliths or other structures. As a result, all chemically based age determination studies to date have focused on the yearly scale, and virtually

all have been used to validate more traditional, less expensive methods of age determination.

The incorporation of at least some isotopes and elements into the otolith is likely to vary with a yearly periodicity, but studies to date have provided mixed results. Annual Sr:Ca ratios in particular might be expected based on yearly cycles in temperature, migration patterns, distribution, sexual maturation, growth rate or other physiological variables. Indeed, clear periodicity in Sr:Ca ratios, inferred to be annual, have been reported in several studies (Radtke & Targett 1984, Gauldie et al. 1991, 1992, Sadovy & Severin 1992, Secor 1992, Toole et al. 1993, Halden et al. 1995). However, other studies reported no consistent correlation between the Sr:Ca value and the annuli visible in the otolith (Fuiman & Hoff 1995, Gauldie et al. 1995). Oxygen isotope cycles are evident in both freshwater and saltwater otoliths, due to annual patterns in temperature and/or distribution (Iacumin et al. 1992, Patterson et al. 1993). Annual cycles in elements such as Na, K and S, out of phase with temperature cycles and perhaps due to seasonal reproductive activity, have also been reported (Kalish 1989, Fuiman & Hoff 1995). While annual isotopic and elemental cycles undoubtedly occur in some species, the environmental (and therefore transient) basis for their formation suggests that their use for age validation is no more firmly grounded than are the annuli which are visible in otoliths.

Radiochemical dating of otoliths is based on the radioactive decay of naturally occurring radioisotopes which are incorporated into the otolith during its growth. As discussed above under 'Natural radioisotopes', assays of the extracted otolith core appear to provide objective, accurate age estimates (Bennett et al. 1982, Campana et al. 1990, 1993, Fenton et al. 1990, 1991, Smith et al. 1991, Kestelle et al. 1994, Milton et al. 1995). Nevertheless, the isotopic concentrations requiring measurement are exceedingly low, resulting in assay precisions which are often less than optimal. Current discriminatory power is on the order of 5 yr for  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  and 1 to 2 yr for  $^{228}\text{Th}$ : $^{228}\text{Ra}$ , over age ranges of 0 to 50 and 0 to 3 yr, respectively. Therefore, this approach is best suited to species where the candidate age interpretations are widely divergent, such as in *Sebastes* or *Hoplostethus* (Campana et al. 1990, Fenton et al. 1991).

Age validation on the basis of radiocarbon derived from nuclear testing is currently one of the best age validation approaches available (Kalish 1993, 1995a,b, Kalish et al. 1996, 1997, Campana 1997, Campana & Jones 1998). As described above under 'Radioisotopes from nuclear explosions', marine waters with  $\Delta^{14}\text{C}$  values greater than 0‰ did not generally exist prior to the onset of nuclear testing in the late 1950s. Even otolith contamination with material of more recent origin



could only increase the  $\Delta^{14}\text{C}$  value, not decrease it. Thus the otolith  $\Delta^{14}\text{C}$  value sets a minimum age to the sample, and the years 1958 to 1965 become the most sensitive years for  $\Delta^{14}\text{C}$ -based ageing. For fish born during this time period, bomb radiocarbon can be used to confirm the accuracy of more traditional ageing approaches with an accuracy of at least  $\pm 1$  to 3 yr; the discriminatory power of samples collected before or after this period is more than an order of magnitude lower. Since the  $^{14}\text{C}$  signal recorded in deep-sea and freshwater environments is different from that of surface marine waters (deep-sea = delayed; freshwater = advanced), reference chronologies appropriate to the environment experienced during the period of otolith core formation must be used (Kalish 1995b, Campana & Jones 1998).

#### Reconstruction of environmental history

Otolith-based reconstruction of individual temperature histories is now possible, albeit with some caveats. Despite the initial promise, otolith Sr:Ca ratios do not appear to reflect temperature in a consistent manner. As noted in the section 'Temperature', temperature reconstructions based on Sr:Ca have performed well in only a handful of coldwater examples (Townsend et al. 1989, 1992, 1995, Radtke et al. 1990), and are readily confounded by changes in salinity. However, oxygen isotope ratios do not appear to suffer the same constraints, and their relationship to temperature has been experimentally validated in several fish species (Kalish 1991c, Thorrold et al. 1997b). Temperature reconstructions based on oxygen isotope ratios have been reported for both freshwater (Patterson et al. 1993) and saltwater (Mulcahy et al. 1979, Kalish 1991b, Iacumin et al. 1992) fishes, and have been based both on whole otoliths (to derive a mean temperature) and microsamples taken along an otolith transect (to derive a temperature history). These successful applications are in keeping with the extensive literature on the use of oxygen isotopes to reconstruct the temperature history of corals, bivalves and other calcified poikilothermic aquatic organisms (Weidman & Jones 1994, Leder et al. 1996). However, oxygen isotopes in the otoliths of temperature-regulating fish such as tuna appear to reflect body temperature more than that of the ambient water (Radtke et al. 1987).

The accuracy of oxygen isotope-based temperature reconstructions can be limited by insufficient information concerning the  $\delta^{18}\text{O}$  of the water. Since otolith  $\delta^{18}\text{O}$  is deposited in equilibrium with that of the water, variations in the latter must be accounted for before the temperature at deposition can be calculated. Where the composition of the water is unknown but is

believed to have been constant, the difference between 2 isotopic assays can be used to estimate the temperature differential, but not the absolute temperature (Thorrold et al. 1997b). Temperature reconstructions made without any knowledge of water composition necessarily assume some given value (Edmonds & Fletcher 1997), although variations in salinity are sometimes used as a proxy for variations in  $\delta^{18}\text{O}$  (Tan et al. 1983).

Otolith-based reconstructions of recent salinity history have generally been restricted to anadromous migrations on the basis of Sr:Ca ratios (Kalish 1990, Secor 1992, Halden et al. 1995, 1996, Limburg 1995, Secor et al. 1995b, Tzeng et al. 1997, Otake & Uchida 1998) or stable oxygen isotopes (Meyer-Rochow et al. 1992, Northcote et al. 1992), although Dufour et al. (1998) were able to distinguish between hypersaline lagoonal water and that of the open ocean using stable oxygen and carbon isotopes. Detection of the fine-scale salinity differences evident in coralline Sr:Ca ratios (de Villiers et al. 1994) are likely to be more difficult in otoliths given the other factors that are known to influence Sr:Ca deposition in otoliths. Strontium isotope ratios have been used to reconstruct salinity history in fish bones and other taxa (Schmitz et al. 1991, Holmden et al. 1997), but have not yet been tested for this purpose in otoliths.

Paleoclimate reconstructions take advantage of the same suite of temperature and salinity indicators noted above, but require additional assumptions concerning the  $\delta^{18}\text{O}$  and trace element composition of the water at the time. Nevertheless, there is a long history in reconstructing past temperature regimes on the basis of calcified invertebrate tests (Rye & Sommer 1980, Weidman & Jones 1994), and initial results on fossil otoliths promise similar conclusions (Devereux 1967, Elder et al. 1996). Reconstruction of paleosalinity on the basis of strontium isotopes has not yet been attempted with otoliths, despite reports of their use in other structures (Schmitz et al. 1991, Holmden et al. 1997). However, Sr:Ca ratios in fossil otoliths have been used to reconstruct flooding events in inland lakes (J. Kalish, Australia National University, Canberra ACT 0200, Australia, pers. comm.). Of course, attention must always be given to possible diagenetic shifts in fossil composition, although Gaudie et al. (1991) noted that such changes were often visually obvious.

#### Pollution

Trace elements are often considered to be excellent pollution indicators, given their relatively high concentrations in industrial effluent (Phillips & Rainbow 1993). Accordingly, the trace element composition of

soft tissues of aquatic organisms is used routinely to monitor pollution (Cutshall et al. 1978, Settle & Patterson 1980). Calcified tissues in organisms such as bivalves and corals preserve a longer, less refractory record of exposure, and are thus preferred for use where available (Scott 1990, Brown & Luoma 1995). In fish, there is a similar precedent for use of calcified structures such as scales and bones (Moreau et al. 1983, Johnson 1989). Yet initial reports suggest that the otolith is not an obvious indicator of pollution (Geffen et al. 1998, Hanson & Zdanowicz 1999, but see Dove & Kingsford 1998). Since the partition coefficients for most pollution-related metals into the otolith are much lower than those into other calcified tissues, the otolith is probably less than ideal for use as a proxy for pollution.

#### Physiological indicators

Although there are several physiologically important life history events that can influence elemental or isotopic uptake into the otolith, not all can be interpreted as such in the absence of supporting information. The most robust of the physiological indicators is the generalized increase in otolith  $\delta^{13}\text{C}$  with declining metabolic rate (Mulcahy et al. 1979, Kalish 1991c, Gauldie 1996a, Schwarcz et al. 1998). This pattern has been observed in a variety of taxa across a range of habitats and appears to be indicative of a reduced contribution of metabolic carbon associated with increased age and declining growth rate (Kalish 1991c, Schwarcz et al. 1998). Peak or asymptotic  $\delta^{13}\text{C}$  levels appear to be indicative of the onset of sexual maturity (Gauldie 1996a, Schwarcz et al. 1998). While diet and ambient  $\delta^{13}\text{C}$  may also influence otolith  $\delta^{13}\text{C}$ , the relationship with metabolic rate appears to be sufficiently strong to provide a relatively clear signal over the lifetime of the individual.

Metamorphosis of an eel *leptocephalus* to a juvenile glass eel also leaves a clear signal in the otolith, in this case in the form of an elevated Sr:Ca ratio (Otake et al. 1994, 1997, Tzeng et al. 1997). However, metamorphosis in other taxa will not necessarily leave an elemental mark: Toole et al. (1993) could find no elemental evidence of metamorphosis in 5 flatfish otoliths, and flatfish settlement was often associated with depressed, rather than elevated, Sr:Ca ratios. Further studies on other species are clearly required, but the unambiguous Sr:Ca peak deposited in the eel otolith may in part reflect the unusual and extreme nature of metamorphosis in this group of fishes.

Other physiological processes which may leave a chemical mark on the otolith include stressful events (Kalish 1992) and reproductive activity (Kalish 1989), both in the form of elevated Sr:Ca ratios. In light of the

other factors which can result in increased Sr:Ca ratios, it would probably be difficult to attribute a particular Sr:Ca peak to a stressful event without prior knowledge of the event. In principle, reproductive events should be easier to reconstruct, since the increase in calcium-binding proteins (and concomitant decrease in free calcium) associated with spawning would be expected to occur over the entire spawning season (Kalish 1989). In practice, however, the generalized increase in the Sr:Ca ratio might be difficult to disentangle from background variability. For example, Friedland et al. (1998) were not able to discriminate between mature and immature sea-run Atlantic salmon *Salmo salar* based on otolith Sr:Ca ratios.

#### Chemical marking

Chemicals incorporated into otoliths have been used as dated markers or as batch markers of groups of fish for at least 30 yr (Kobayashi et al. 1964, Meunier & Boivin 1978). When chemically tagged individuals are recaptured, time at liberty can be compared with the number of growth increments deposited distal to the chemical check (Casselmann 1983). This approach to age validation has proved effective at both yearly (Fowler 1990, McFarlane & Beamish 1995) and daily (Wild & Foreman 1980, Campana & Neilson 1982) levels. The objective of batch or mass marking is completely different, in that it is intended as a rapid, cost-effective means of tagging large numbers of fish for subsequent identification (Secor & Houde 1995, Tsukamoto 1985). Batch markers are generally applied through immersion, although incorporation into the diet has also proven successful. In many ways, this method of marking is analogous to thermal marking of otoliths, although thermal marking leaves a visual, rather than a chemical, mark (Mosegaard et al. 1987, Hagen et al. 1995). An advantage of chemical marks is that they can be applied at any life history stage, including that of the embryo (Geffen 1992).

Fluorescent calcium-binding chemicals are among the most popular of the chemical markers, since they are easily introduced into the fish and produce clear fluorescent marks in the otolith and other calcified structures when viewed with ultraviolet light. Oxytetracycline hydrochloride has the longest history of use with otoliths, and has been administered through injection (Wild & Foreman 1980, Campana & Neilson 1982, Geffen 1992, Lang & Buxton 1993), feeding (Pedersen & Carlsen 1991, Thomas et al. 1995) and immersion (Hettler 1984, Schmitt 1984, Tsukamoto 1985, Muth et al. 1988, Secor et al. 1991, Nagiec et al. 1995). Alizarin red and alizarin complexone have a shorter history of use, but are generally considered to produce

a mark superior to that of oxytetracycline when applied through immersion (Tsukamoto 1988, Blom et al. 1994, Nagiec et al. 1995, Secor & Houde 1995, Szedlmayer & Howe 1995, Thomas et al. 1995, Beckman & Schulz 1996). Alizarin incorporated into feed tended to produce a less visible mark (Lang & Buxton 1993, Takahashi 1994, Thomas et al. 1995), while injected alizarin resulted in poor marking success (Thomas et al. 1995, but see Geffen et al. 1998). In contrast, the mark quality of injected calcein is reported to be very good (Monaghan 1993, Thomas et al. 1995), although calcein application through immersion has also been successful (Wilson et al. 1987, Alcobendas et al. 1992). Irrespective of the mode of application, there have been no reports of mark loss from the otolith through the lifetime of the fish, nor would any be expected based on the chemical stability of the otolith.

Elemental supplements incorporated into the otolith have also been used as chemical tags or as dated markers, although detection is necessarily limited to analytical methods. Immersion of fish in strontium-enhanced solutions has been used successfully in several studies, either to leave a dated mark on the otolith detectable with an electron microprobe (Proctor et al. 1995, Schroder et al. 1995) or as a batch marker of groups of fish (Brown & Harris 1995). Strontium-enhanced checks may even form in wild fish in response to stress (Kalish 1992), presumably in response to abruptly lower calcification rates. Indeed, Mugiya & Muramatsu (1982) used the calcium antagonist acetazolamide to produce a visual check on the otolith, although there was no report of a matching elemental mark having been formed. However, a dated mark detectable with autoradiography was produced after fish were exposed to a radioactive isotope of calcium (Yoklavich & Boehlert 1987). Mass marking with other elements is also possible (Ennevor & Beames 1993), although given the selectivity of the otolith to specific elements, certain elements might be expected to be incorporated more easily than others.

### Future directions

Many of the applications described in the previous sections have not yet reached their full potential. For example, age-structured assays which take advantage of the otolith growth sequence have not developed as quickly as anticipated, in part due to methodological limitations. This in turn has slowed research on problems such as reconstruction of migration pathways, which requires trace elements and isotopes other than those accessible to the commonly used electron microprobe. Progress has been made in integrating trace element and stable isotope assays for stock identifica-

tion purposes (Thorrold et al. 1998b) and further developments are expected. Nevertheless, there remain applications of otolith chemistry which have received little or no attention from otolith researchers to date, and some of these areas are described below.

Methodological advances will continue to play a pivotal role in this field in light of the continued requirement for improvements in assay accuracy, sensitivity and spatial resolution. High-resolution magnetic sector ICPMS systems already offer more precise isotopic ratios and significant improvements in assays of elements subject to isobaric interference (e.g. Fe, Sn, As) (Moens & Jakubowski 1998). When coupled with UV lasers, or, alternatively, using the new generation of ion microprobes, beam-based assays of a broader suite of elements and more precise isotope ratios in otolith growth increments are likely (Christensen et al. 1995). Initial demonstrations of laser-based  $^{13}\text{C}:^{12}\text{C}$  assays (Murnick & Peer 1994), laser-based Sr isotope assays (Christensen et al. 1995) and ion microprobe-based  $^{18}\text{O}:^{16}\text{O}$  assays (Saxton et al. 1995) in minerals and bivalves have already been reported, suggesting that age-structured metabolism, salinity and temperature reconstructions at the weekly or even daily level may soon be possible. In the interim, computer-controlled microsamplers have been developed for fine-scale collection of otolith material for stable isotope and radioisotope assays (Dettman & Lohmann 1995).

The interpretation of isotopic ratios other than C and O provides many useful insights in geochemical studies, but is largely unknown to fisheries science. Examples of potentially valuable otolith applications include assays of Sr isotopic ratios as indicators of proximity to land, use of U isotopes as salinity indicators, and interpretation of Pb isotopes as proximity indicators for pollution. The isotopes of many elements are fractionated on the basis of temperature: can the isotopes of the predominant element in otoliths, Ca, be used as a temperature indicator which is insensitive to salinity variations? What about temperature reconstructions based on otolith oxygen isotopes using an independent salinity indicator as a proxy for the oxygen isotopic composition of the water? These and other applications remain to be developed.

Our current understanding of the factors influencing the composition of the otolith is constraining our progress in several areas. For example, the influence of otolith proteins on trace element incorporation has not yet been studied, but may explain the affinity (or lack thereof) for certain elements. Similarly, a better understanding of the uptake dynamics of many elements is required to determine which might serve best as environmental indicators, and which serve only to confuse our interpretations.

As with any ecological methodology, applications based on otolith chemistry are often used to the greatest advantage when combined with other, complementary tools. Examples of such a multidisciplinary approach might include the use of both otolith elemental fingerprinting and microsatellite DNA for stock identification, or use of both externally attached tags and laser-based otolith assays for reconstruction of migration routes. Here as in other fields, multiple independent approaches to a problem will often yield the best results, and are to be encouraged.

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