Stock Identification Methods
Applications in Fishery Science

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CHAPTER 12

Otolith Elemental Composition as a Natural Marker of Fish Stocks

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I. INTRODUCTION

To the extent that groups of fish inhabit different environments, the otolith elemental composition often serves as a natural marker or tag of those groups. Two key properties of the otolith underlie the use of the otolith elemental composition as a natural marker: (1) unlike bone, the otolith is metabolically inert; therefore, newly deposited material is neither resorbed nor reworked after deposition (Campana and Neilson, 1985); and (2) trace element uptake onto the growing otolith reflects the physical and chemical environment (Fowler et al., 1995; Gallahar and Kingsford, 1996), albeit with significant physiological regulation (Kalish, 1989; Farrell and Campana, 1996). Isotopic ratios of elements such as strontium (Kennedy et al., 1997) and oxygen (Thorrold et al., 1997a) are similarly influenced by environmental availability and temperature. Such

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environmental responses, recorded permanently in the otolith, imply that the otolith concentration 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 (Fig. 12-1). As a

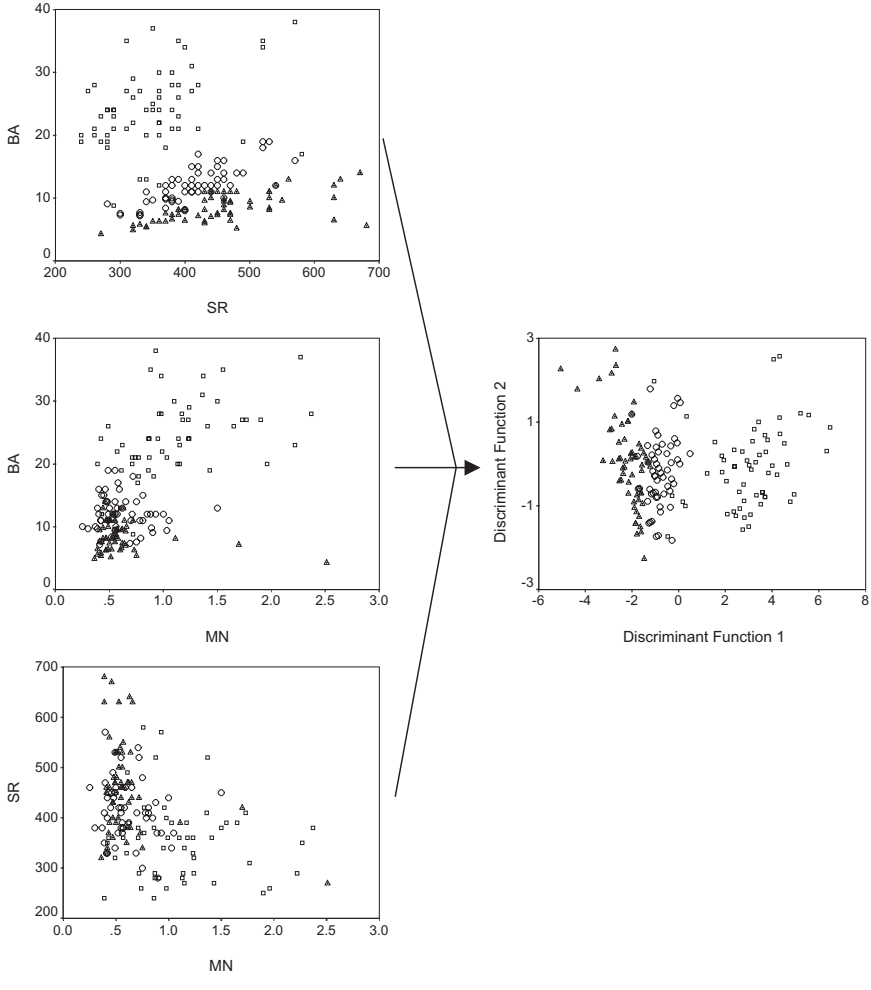

FIGURE 12-1. Example of the preparation of a multivariate otolith elemental fingerprint for shad (*Alosa sapidissima*). The concentrations of three elements (Ba, Mn, and Sr) were measured in otoliths from about 60 shad collected in each of three river systems. When one element is plotted against another (left panels), there are varying degrees of differences among the three rivers (each river is represented by a different symbol). The differences in elemental composition among rivers become more evident when the individual elements are pooled into a multivariate fingerprint (right panel). Full details of the shad study are available in Thorrold et al. (1998).

result, the elemental fingerprint appears to be an excellent biological tracer of groups of fish, both in freshwater (Kalish, 1990; Northcote et al., 1992; Bronte et al., 1996; Kennedy et al., 1997, 2000, 2002; Limburg, 1998; Thorrold et al., 1998a) and saltwater environments (Edmonds et al., 1989, 1991, 1992, 1995; Gunn et al., 1992; Sie and Thresher, 1992; Campana et al., 1994, 1995, 1999; Campana and Gagné, 1995; Thresher et al., 1994; Proctor et al., 1995; Severin et al., 1995; Dove et al., 1996; Gillanders and Kingsford, 1996; Milton et al., 1997; Thorrold et al., 1997b, 1998b, 2001; Begg et al., 1998; Dufour et al., 1998; Newman et al., 2000; Volk et al., 2000; Gillanders, 2001, 2002; Secor et al., 2001).

The presence of geographic variations in water temperature and chemistry, both of which can result in different otolith composition, suggests that otolith elemental fingerprints should discriminate well among fish that have grown up in different environments. However, 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 (Campana et al., 2000; Thorrold et al., 1998a). 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 (Edmonds et al., 1989; Hoff and Fuiman, 1993; Campana et al., 1995, 2000; Begg et al., 1998; Begg and Weidman, 2001). 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. Nevertheless, the presence of different fingerprints among groups of fish of similar age necessarily implies different environmental histories. To the extent that populations or stocks of fish inhabit different environments, otolith elemental composition can then serve as an indicator of stock identity. 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 the effect of size-related effects on the fingerprint have been statistically removed. The assumption of long-term stability in the fingerprint is probably met in some, but not all, stocks.

In principle, the otolith elemental composition can be used to identify and track any groups of fish of different environmental history. In practice, there are three practical limitations to their use. The first limitation is that many of the most easily measured elements are under strict physiological regulation, and thus unsuitable for use as environmental indicators. This list includes the major elements calcium, oxygen, and carbon (which make up the calcium carbonate matrix), as well as the minor (>100 ppm) elements Na, K, S, P, and Cl, although it excludes Sr (Thresher et al., 1994; Proctor et al., 1995; Schwarcz et al., 1998). Nevertheless, even physiologically regulated elements can prove useful as biological tracers of a group of fish, as long as the otolith concentrations of those elements vary significantly among groups. The second limitation concerns the analysis of the less abundant trace (<100 ppm) 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 (Hanson and Zdanowicz, 1999). Both temperature and growth rate are known to be at least as influential as ambient concentration in modifying otolith elemental composition. With these caveats in mind, the elements most likely to serve as environmentally influenced stock markers include Sr, Ba, Mn, Fe, and Pb (and perhaps Li, Mg, Cu, and Ni), in which both ambient element:Ca concentrations and/or temperature produce significant effects on otolith composition (Fowler et al., 1995; Farrell and Campana, 1996; Dove, 1997; Geffen et al., 1998; Campana, 1999; Bath et al., 2000; Milton and Chenery, 2001). The isotopes most useful as natural tags are Sr, S, Pb, and O (Kennedy et al., 1997, 2000; Spencer et al., 2000; Thorrold et al., 2001; Weber et al., 2002).

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 that have experienced different overall environmental exposures. The fingerprint thus serves as a natural tag of these groups of fish if they were to mix with other groups shortly after characterization of their fingerprints. In contrast, analysis of the otolith core is generally intended as a more direct measure of stock or nursery origin. Both of these approaches are discussed further below.

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 predefined groups of fish over short periods of time (Campana et al., 1995, 1999, 2000; Gillanders and Kingsford, 1996; Kennedy et al., 1997, 2000). Application 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. The fingerprint would not be expected to remain stable over extended periods of time (e.g., years), since interannual variation in the habits and environment of the fish would eventually produce a detectable change in the overall elemental composition (Fig. 12-2). However, short-term stability is both expected and observed, particularly with respect to differences among groups (Fig. 12-3) (Campana et al., 1995; Kennedy et al., 1997; Thorrold et al., 1998b). An appealing feature of this application is that the elemental fingerprint need not be linked to potential sources or locations in the environment.

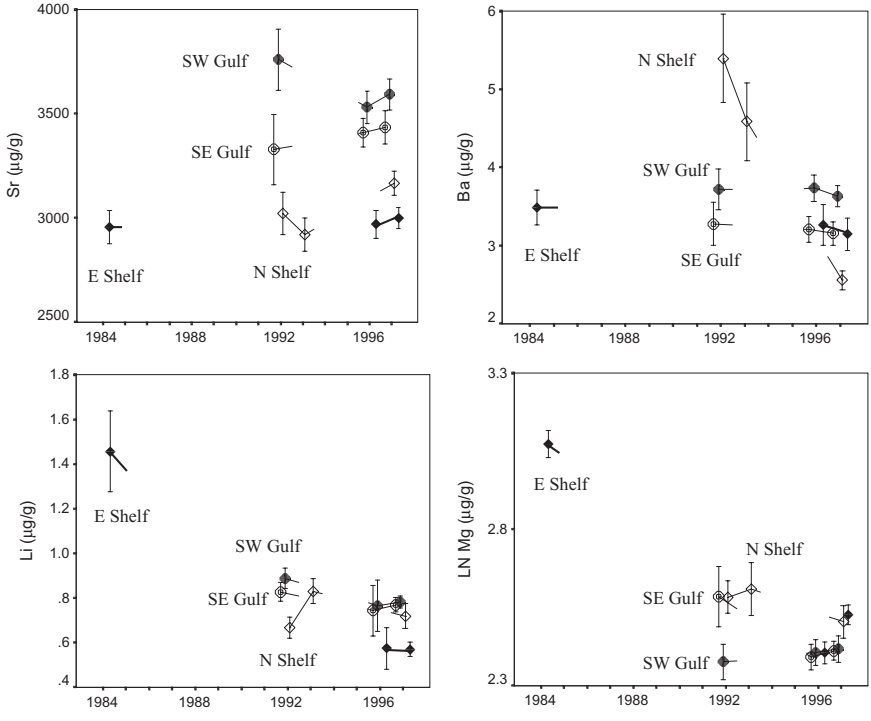

FIGURE 12-2. Long-term variation in mean (±95% CI) elemental concentration in cod otoliths at four spawning sites off eastern Canada. Long-term stability was noted for some elements at some locations, but not others, nor did all elements at a given location necessarily change in tandem. E Shelf, eastern Scotian Shelf; N Shelf, northern Scotian Shelf; SE Gulf, southeast Gulf of St. Lawrence; SW Gulf, southwest Gulf of St. Lawrence. Full details of the cod study are available in Campana et al. (2000).

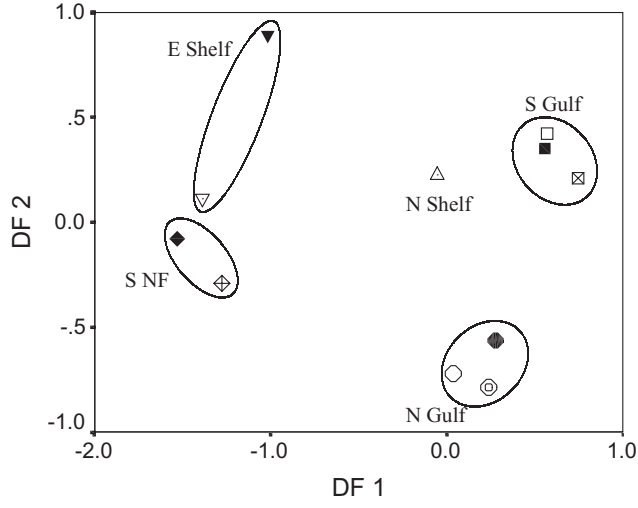

FIGURE 12-3. Short-term stability and specificity of the elemental fingerprint as a marker of cod spawning aggregations off eastern Canada. Differences among groups were highly significant, but remained relatively stable across adjacent years (1995: open symbol; 1996: filled symbol; 1997: hatched symbol). DF, discriminant function; E Shelf, eastern Scotian Shelf; N Shelf, northern Scotian Shelf; S Gulf, southern Gulf of St. Lawrence; N Gulf, northern Gulf of St. Lawrence; S NF, southern Newfoundland. Full details of the cod study are available in Campana et al. (2000).

Irrespective of the cause of the differences in elemental fingerprints among the groups, the fingerprints become the natural distinguishing feature of those groups at a given point in time. Accordingly, otolith elemental fingerprints appear to be well suited as biological tracers of groups of fish, requiring relatively few assumptions for confident application to difficult tracking or stock mixing situations.

Use of otolith elemental fingerprints as natural tags makes three central assumptions, all of which apply as much to genetic and morphometric stock mixture analyses as to otolith-based assays (Wood et al., 1989; Wirgin et al., 1997):

Assumption 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 and Gagné, 1995; Campana et al., 1995, 1999; 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 (Edmonds et al., 1989; Hoff and Fuiman, 1993), it is important that differences in elemental concentration among groups not be confounded by size dif-

ferences among groups. Statistical removal of the effect of otolith weight on elemental concentration is a ready solution to this problem (Campana et al., 2000).

Assumption 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 discrete (e.g., on the spawning or nursery grounds).

Assumption 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., 2000; Begg and Weidman, 2001). 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, 2000; Kennedy et al., 1997, 2000; Thorrold et al., 1998a). In the case of Atlantic cod (*Gadus morhua*), the interval between characterization and mixing was less than 6 months, and thus much less than the 1- to 2-yr period required for a noticeable change in the elemental fingerprint (Campana et al., 2000). 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. In general, the period of relative stability can probably be approximated as the period during which the mean otolith weight increases by <5%.

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 that is assayed, whether that is the period around hatch, the first few months of life, or some other period. The subsequent life history is not sampled and therefore is irrelevant. To the extent that spawning or nursery grounds are characterized by different temperature or chemical environments, this approach has proved effective in distinguishing among groups of fish with different origins (Kalish, 1990; Sie and Thresher, 1992; Campana et al., 1994; Thresher et al., 1994; Proctor et al., 1995; Severin et al., 1995; Dove et al., 1996; Gillanders and

Kingsford, 1996; Milton et al., 1997; Thorrold et al., 1997b, 2001; Gillanders, 2002).

II. SAMPLING AND ASSAYS

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 for use as a natural tag, since the primary question is one of overall differences between groups of fish, irrespective of the portion of the lifetime which produced the difference. 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 to detect differences between groups at some earlier life stage. 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 (Campana, 1999).

Advantages of whole-otolith assays for use as a natural tag 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) (Hoff and Fuiman, 1993), inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Edmonds et al., 1995), neutron activation analysis (Papadopoulou et al., 1980), and inductively coupled plasma mass spectrometry (ICPMS) (Edmonds et al., 1991; Dove et al., 1996) are among the techniques that have been used to analyze otoliths. However, it is ICPMS that 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. Isotope dilution ICPMS (ID-ICPMS), a variant of ICPMS often used to certify reference materials (Fassett and Paulsen, 1989), is the most accurate of the otolith analytical techniques currently available (Campana et al., 1999). 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).

Beam-based assays target a particular age or date range in the sectioned otolith and thus can be used to identify nursery areas (Thresher et al., 1994; Milton

et al., 1997; Thorrold et al., 2001; Gillanders, 2002). 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. As a result, the assay can be limited to the time scale of interest, which for stock identification purposes, would be the early life history. 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 assumption that elemental concentration is independent of the growth axis is discussed in Campana (1999).

There are a wide variety of sophisticated instruments available for probed assays of the otolith, 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 and Thresher, 1992), and laser ablation ICPMS (LA-ICPMS) (Campana et al., 1994; Thorrold and Shuttleworth, 2000). 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 generally used in stock identification studies required PIXE or LA-ICPMS. Sr was measured accurately and precisely with either WD-EM, PIXE, or LA-ICPMS.

Assays for 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 that physically remove a portion of the otolith for subsequent analysis. Computerized micromilling machines have proved effective in some studies, whereby seasonal or annual growth zones visible in otolith cross sections are milled to a discrete depth and the powder collected for assay (Wurster et al., 1999). 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 include access to bulk analytical techniques of high sensitivity and accuracy: mass spectrometry for stable isotope ratios (Schwarcz et al., 1998), and ICPMS for trace element assays (Campana et al., 1995). The disadvantage is one of limited sampling resolution, since the temporal resolution of the extracted sample is seasonal at best. It appears unlikely that microsampling or coring would introduce contaminants that would confound stable isotope assays as long as the extracted samples were treated carefully. On the other hand, there is potential for contamination from the sampling process on trace element assays, despite the fact that Dove et al. (1996) reported no artifacts due to sectioning with an Isomet saw.

A. Sample Preparation and Quality Control

With analytical sensitivity comes the potential for contamination from unwanted sources. Factors such as the the mode of fish or otolith preservation, composition of the instruments used to remove the otolith from the fish, cleaning methods, handling, and even household dust are all potentially major modifiers of the perceived trace element composition (Milton and Chenery, 1998; Thresher, 1999). Preservation in fluids such as ethanol and formalin appears to have the greatest potential for contamination, given the microchannel architecture of the otolith and the relative impurity of most preservatives. Therefore, trace element analysis of otoliths stored dry or frozen appears to be safest. Current protocols for handling and preparing otoliths are drawn from the water analysis literature and always involve isolation from skin, metallic instruments, and solutions that are of other than trace metal grade. In general, decontamination based on brushing and sonification in ultrapure water, followed by storage in acid-washed polyethylene vials, results in minimal contamination (Campana, 1999). Minor elements such as Na, K, Cl, and S appear to be affected by the water sonification stage (Proctor and 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., 2000; Secor et al., 2001), despite the fact that it is an important step in the decontamination of sediment-laden forams.

Complete protocols for handling and preparing otoliths for elemental assay are presented elsewhere (Campana et al., 2000). A simplified protocol is as follows:

1. Remove sagittal otolith pair from fish immediately after capture; alternatively, freeze fish, but do not store in liquid preservative.
2. Upon otolith removal, immediately remove all adhering tissue. Handling with metal forceps at this stage is acceptable.
3. Decontaminate otolith by sonifying in a series of distilled, deionized, reverse osmosis water baths (Super Q or Milli Q water) in acid-washed polyethylene vials. Brushing with an acid-washed nylon toothbrush under a flow of Super Q water can be used to remove any adherent tissue before the first sonification. All handling at this and subsequent stages must be with nonmetallic, acid-washed tools.
4. Dry decontaminated otoliths in a positive-flow laminar-flow fume hood (Class 100); store in dry, acid-washed polyethylene vials.

5. For assay by ICPMS, dissolve otolith in redistilled nitric acid to a concentration of no more than 0.1% w/v; isotope spikes should be added at this stage if analyzing with ID-ICPMS.
6. Randomize assay sequence.

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 instrument days. 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 (Campana and Gagné, 1995). Use of ID-ICPMS minimizes (although it may not eliminate) instrumental drift.

Differences in otolith elemental composition among groups of fish may be statistically significant, but will not necessarily be large. Artifactual but significant differences among groups of otolith elemental assays are not uncommon (Campana et al., 1997). Therefore, calibration of separate assay runs or laboratories against an otolith reference powder is highly recommended to ensure that any observed differences among runs are real rather than artifactual (Campana, 1999; Thresher, 1999; Yoshinaga et al., 2000).

B. Statistical Analysis

While elemental concentrations are generally reported in terms of microgram per gram of otolith, many studies have noted size-specific concentrations for some elements which could otherwise be confused for stock-specific differences (Fig. 12-4). To insure that differences in fish length and/or otolith weight among samples do not confound any stock-specific differences in elemental composition, it is important to remove the effect of otolith weight from the statistical analysis. In steelhead trout (*Oncorhynchus mykiss*) for example, Mg varied significantly with otolith weight, while Ba did not. Subtraction of the common within-group linear slope (derived from the ANCOVA) from the Mg data removed the trend from the element–otolith weight relationship. In instances where the element–otolith weight relationship is markedly nonlinear, alternative detrending procedures are possible (Campana et al., 2000). Detrended elemental concentrations should show no obvious residual relationship with otolith weight, but in any event, relative differences among detrended samples should be similar to those based on original data.

Within-group distributions of elemental concentrations are sometimes skewed and thus must be transformed prior to statistical analysis. Each otolith is characterized by a suite of several elements; therefore, multivariate statistics are used

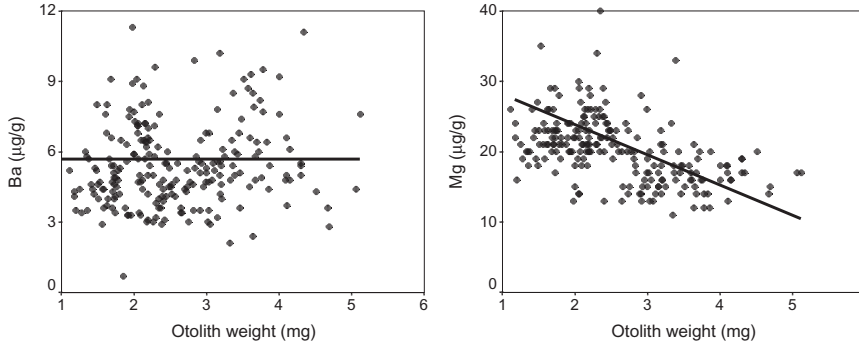

FIGURE 12-4. Examples of the relationship between elemental concentration and otolith weight in steelhead trout. A significant negative relationship was noted for Mg, but no relationship was evident for Ba. To insure that variations in otolith weight among samples do not confound the interpretation of differences among areas, the effect of otolith weight should be removed statistically from elements where the relationship exists.

to distinguish among samples. MANOVA is used to test for significant differences among samples, while discriminant analysis can be used to prepare two-factor elemental fingerprints for illustrative purposes. Discriminant analysis is not necessarily the best method to classify samples of unknown stock affinity, since it performs poorly when the stock markers are similar. In contrast, stock composition analysis using a maximum likelihood-based method provides maximal discriminatory power in mixed stock situations (Wood et al., 1987; Campana et al., 1999; Gillanders, 2002).

A simplified protocol for the statistical analysis of otolith elemental data is as follows:

1. Examine frequency histograms of the concentration of each element in each group. For elements where the distribution is nonnormal in most or all groups, transform that element appropriately (e.g., ln transform; transformation must be applied to that element in all groups). Remove clearly aberrant outliers (>5 SD away from the mean) from the transformed data if not associated with particularly small or large fish.
2. Visually and statistically assess each element within each group for a relationship with fish size or otolith weight. Where a relationship is evident in most or all groups, the effect of the relationship must be removed statistically by subtracting the common, within-group slope (obtained from the ANCOVA of the element with group as the factor and otolith weight as the covariate) from the observed value in each group. Nonlinear relationships must be removed differently, as in Campana et al. (2000).

3. Test for univariate differences in the concentration of each element across groups (e.g., through ANOVA). Error bar plots or box and whisker plots help visualize the intergroup differences.
4. Test for overall differences in the elemental fingerprint among groups using MANOVA.
5. Use stepwise discriminant function analysis to identify the elements that contribute the most to fingerprint differences among groups. Visually assess the differences among groups by plotting the first two discriminant function axes against each other. Note that classification of unknown fish using discriminant analysis can give highly inaccurate results and is not recommended.
6. Classify an unknown mixture using a maximum likelihood-based or Bayesian mixture analysis, using the known identity fish as the reference. Reference fish must be completely comparable to the unknown fish, as per the assumptions of the method discussed earlier.

III. CASE STUDIES

A. Population Mixing of Atlantic Cod

The purpose of this study was to identify the Atlantic cod (*Gadus morhua*) populations and their proportions contributing to a densely populated overwintering ground off the coast of eastern Canada (Campana et al., 1999, 2000). The overwintering ground was adjacent to four large cod populations suspected of contributing to the overwintering grounds, so the otolith elemental fingerprints of known-identity spawners were used as tracers to classify the unknown winter mixture. The initial phase of the study consisted of collecting otoliths from spawning individuals of all four populations on their respective spawning grounds for use as known-identity reference groups. Later in the same year, samples from throughout the overwintering ground were collected. All otoliths were dissolved and assayed for B, Li, Mg, Zn, Sr, Ba, and Pb using ID-ICPMS. Mn is monoisotopic; thus, it was assayed with conventional ICPMS using an internal standard. A cod otolith reference powder was used to insure analytical consistency. B, Zn, and Pb exhibited low reproducibility and/or concentrations near the limit of detection, and thus were not considered further. After statistical removal of size effects, significant differences were noted in the elemental concentration of Li, Mg, Mn, Sr, and Ba among the spawning groups; therefore this suite of five elements was used in all multivariate analyses that followed. Using the spawning stock samples as the reference (known-identity) groups, a maximum likelihood-based stock mixture analysis was used to estimate the proportion of each spawning stock present in the overwintering mixture. The

results were unequivocal and completely consistent with previous tagging studies.

B. Natal Homing of Weakfish

The purpose of this study was to estimate the extent of natal homing in weakfish (*Cynoscion regalis*), a species that spawns in estuaries along the eastern coast of North America (Thorrold et al., 1998b, 2001). By using the otolith elemental fingerprints of one year-class of juvenile weakfish (while on their nursery grounds) as reference marks, it was possible to identify and classify as to estuarine origin the same year-class of fish when they returned to the estuaries years later to spawn. Juvenile otoliths were collected from five major estuarine nursery grounds; these served as the known-identity reference groups. One sagittal otolith of each pair was assayed for the elements B, Mg, Zn, Sr, and Ba using ID-ICPMS, and Ca and Mn using ICPMS. The remaining otolith was assayed for the stable isotopes $\delta^{13}C$ and $\delta^{18}O$ using isotope ratio mass spectrometry. Due to low reproducibility and concentrations near the limit of detection, B and Zn were not considered further. Thus, the combination of the trace elements Mg, Mn, Sr, and Ba (each standardized to Ca concentration) and the stable isotopes $\delta^{13}C$ and $\delta^{18}O$ was collectively considered the elemental fingerprint, and these differed significantly among the estuaries. Significant relationships between concentration and otolith weight were noted for some elements, but since they were not consistent across all estuaries, they were assumed to be unrelated to fish size.

To compare the elemental fingerprint of the returning adult to that of the juvenile, one of the adult otoliths from each pair was sectioned to expose the core corresponding to the juvenile region, which was then assayed with a UV laser ablation system coupled to an ICPMS (LA-ICPMS). The core of the remaining otolith from each pair was microsampled for stable isotope assay. Various univariate and multivariate analyses were carried out to confirm the similarity of juvenile and adult fingerprints. A maximum likelihood-based mixture analysis program was used to estimate the proportion of the returning adults at each estuary which originated as juveniles from that same estuary. The results indicated that the degree of natal homing was very high.

C. Estuarine Contribution of Juvenile Snapper to the Adult Fishery

The purpose of this study was to estimate the proportional contribution of several estuarine nursery grounds to the adult fish caught in the fishery (Gillanders, 2002). Juvenile snapper (*Pagrus auratus*) were collected from 15 estuaries off the

southeastern coast of Australia for use as known-identity reference groups. Otoliths were sectioned through the core, then assayed for the elements Mg, Ca, Mn, Sr, and Ba using LA-ICPMS. Calcium was used as an internal standard to correct for variations in ablation yield, and a snapper otolith reference powder was used to insure analytical consistency. Otoliths from adult fish sampled from the commercial fishery were similarly sectioned; fish aged as being of the same year-class as that of the juveniles were then assayed with LA-ICPMS. Both univariate and multivariate tests indicated that there were significant fingerprint differences among estuaries, based largely on Mn, Sr, and Ba. Maximum likelihood-based estimation indicated that most adult snapper recruited from nearby estuaries, and that juvenile exchange among estuaries was limited.

D. Determination of River of Origin of Atlantic Salmon

The purpose of this study was to reconstruct the movement of individual Atlantic salmon (*Salmo salar*) among and within streams based on the strontium isotopic composition of the otoliths (Kennedy et al., 1997, 2000). Juvenile salmon were collected from 29 sites in two river systems. Sagittal otoliths were dissolved for assay of $^{87}Sr/^{86}Sr$ using a thermal ionization mass spectrometer (TIMS). There were significant differences in the isotopic composition of both vertebrae and otoliths among the various streams. Therefore, salmon from most of the streams could be clearly differentiated from one another based on Sr isotope composition. Deviations in the fish's isotopic composition from that of the surrounding water indicated that some fish had migrated into a stream from another stream of different isotopic composition; in several instances, the source stream could be identified. This work set the stage for a subsequent study which reconstructed the juvenile life history based on micromilled otoliths of sea-run adults (Kennedy et al., 2002).

IV. CONCLUSION

Specific elements and isotopes incorporated into the growing surface of the fish otolith reflect the physical and chemical characteristics of the ambient water, although not necessarily in a simplistic manner. Since fish that spend at least part of their lives in different water masses often produce otoliths of different elemental composition, the otolith elemental composition ("elemental fingerprint") can serve as an environmentally induced tag of groups of fish. These tags tend to be physically stable, reproducible, and different among stocks, but are not necessarily stable over long periods. Thus, they do not serve as a proxy for genetic

identity. However, the fingerprint is very stable over the short term, making it valuable as a seasonally stable biological tracer of predefined groups of fish. Alternatively, the fingerprint of the otolith core can be used as a marker for groups of fish hatched in different environments. Technological advancements in recent years have made otolith elemental fingerprints a viable, and sometimes preferable, means for distinguishing among fish stocks.

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