Otolith Elemental Fingerprinting for Stock Identification of Atlantic Cod (*Gadus morhua*) Using Laser Ablation ICPMS

Steven E. Campana and Anthony J. Fowler[1]

Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada

and Cynthia M. Jones

Applied Marine Research Laboratory, Old Dominion University, Norfolk, VA 23529, USA

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Trace element incorporation into fish otoliths varies among samples collected at different sites. If otolith elemental composition (the elemental "fingerprint") somehow reflects the characteristics of the ambient water, the elemental fingerprint of the otolith nucleus could serve as a natural marker of fish hatched at different sites. To test this hypothesis, Atlantic cod (*Gadus morhua*) otoliths collected from five spawning grounds in the northwest Atlantic were tested for differences in elemental and isotopic composition. Laser ablation – inductively coupled plasma mass spectroscopy (LA-ICPMS) was used to assay the concentration of 14 isotopes (nine elements) in otolith nuclei. The sensitivity of the laser ablation system exceeded that of the electron microprobe by 2–4 orders of magnitude, with an average CV of 21% for any given isotope. Most isotopic concentrations were consistent between left and right otoliths of a given fish, and most differed significantly among sample sites; there were no significant differences by age, sex, or fish length. Multivariate analyses of the elemental fingerprints resulted in significant discrimination among sample sites. While the mechanism underlying trace element incorporation into otoliths is still unclear, otolith elemental fingerprinting has the potential to become an effective and accurate means of stock identification.

L'incorporation d'éléments traces dans les otholites des poissons varie dans des échantillons prélevés à différents endroits. Si la composition élémentaire des otolithes reflétait de quelque manière les caractéristiques de l'eau dans laquelle évolue le poisson, cette composition (la composition élémentaire du noyau des otolithes) pourrait servir à marquer naturellement les poissons nés à différents endroits. Dans le but de vérifier cette hypothèse, des otolithes de morue franche (*Gadus morhua*) prélevés de cinq frayères dans le nord-ouest de l'Atlantique ont fait l'objet de tests visant à déceler des différences dans la composition élémentaire et isotopique. On a utilisé l'ablation par laser couplée à la spectroscopie d'émission avec plasma induit par haute fréquence pour déterminer la concentration de 14 isotopes (neuf éléments) dans des noyaux d'otolithe. La sensibilité du système d'ablation par laser était de 2 à 4 fois supérieure à celle de la sonde électronique, avec un CV moyen de 21 % pour chaque isotope. La plupart du temps, la concentration des isotopes était comparable dans les otolithes gauche et droit d'un poisson, et elle variait significativement d'un endroit échantillonné à l'autre; on n'a observé aucune différence significative en fonction de l'âge, du sexe ou de la longueur du poisson. Une analyse multivariée de la composition élémentaire a révélé des différences importantes entre les endroits d'échantillonnage. Le mécanisme responsable de l'incorporation des éléments traces dans les otolithes est encore peu connu, mais la composition élémentaire des otolithes pourrait devenir un moyen efficace et précis d'identification des stocks.

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Recent studies have suggested that the elemental composition, or "elemental fingerprint", of the fish otolith may prove to be among the most powerful means yet developed for distinguishing among fish stocks (Edmonds et al. 1989, 1991, 1992; Kalish 1990; Gunn et al. 1992; Campana and Gagné 1994). The success of the approach is based on two observations: (1) otoliths grow throughout the life of the fish and, unlike bone, are metabolically inert; once deposited, otolith material is unlikely to be resorbed or altered (Campana and Neilson 1985; Casselman 1987); and (2) the calcium carbonate and trace elements that make up 90% of the otolith appear to be mainly derived from the water (Simkiss 1974). Accordingly, the elemental composition of the otolith may reflect that of the water in which the fish lives, although not necessarily in a simplistic fashion (Kalish 1989). Since the elemental composition of seawater varies from place to place (Johnson et al. 1992), the elemental composition of the accreting otolith may vary with the water mass in which the fish is swimming. By corollary, the composition of the otolith nucleus, corresponding to the otolith around the time of hatch, would reflect the composition of the water at the hatch (spawning) site, and fish spawned at different sites would contain otolith nuclei that vary in their composition.

[1]Present address: South Australian Research and Development Institute, GPO Box 1625, Adelaide, South Australia 5001.

TABLE 1. Sample collection information. Each sample consisted of 30 fish.

Sample	Area	NAFO division	Collection date	Mean length (range), cm	Mean age (range), yr
1	Cheticamp	4T	May 26, 1986	50.5 (45–63)	5.9 (5–8)
2	Fundyrip	4X	Mar. 6, 1986	54.5 (45–74)	3.1 (2–4)
3	Georges Bank	5Zj	Apr. 16, 1986	62.1 (48–77)	3.9 (2–6)
4	Iceland	—	May 6, 1986	70.1 (62–75)	6.8 (5–10)
5	Newfoundland	30de	May 26–28, 1986	62.4 (52–69)	6.9 (5–9)

Otolith elemental composition has been used to infer stock identity both through analysis of dissolved whole otoliths and through use of beam probes. The former approach is based on the assumption that the geographic ranges of populations differ, at least on average. Given elemental differences in water composition between stock home ranges, and assuming that otolith composition reflects that of water, otolith elemental composition should also differ. Site-specific differences in elemental concentrations based on analysis of dissolved whole otoliths have been reported by a number of workers (Papadopoulou et al. 1978, 1980; Edmonds et al. 1989, 1991, 1992; Grady et al. 1989; Campana and Gagné 1994), despite the likelihood of significant ontogenetic effects (Grady et al. 1989). In general, stock discrimination capabilities have improved with the sensitivity of the instrumentation, reflecting the relative purity of the otolith (Campana and Gagné 1994). The most rapid and sensitive instrumentation for otolith analysis reported to date (Edmonds et al. 1992; Campana and Gagné 1994), and the instrument of choice for simultaneously quantifying the concentration of multiple elements and isotopes (Houk 1986; Date 1991), is inductively coupled plasma mass spectroscopy (ICPMS).

Analytical techniques that probe otolith composition in a focused beam are better suited to assaying the otolith nucleus (corresponding to the hatch site) than are whole-otolith assays, but generally suffer from poorer sensitivity. The electron microprobe is widely used for such assays and has had some success in differentiating among populations (Kalish 1990; Gunn et al. 1992), but is limited to the detection of only the six most abundant elements in the otolith (Kalish 1990). Probe techniques with slightly greater sensitivity, such as the proton microprobe, have also been used with success (Sie and Thresher 1992). However, sensitivity constraints have, until now, denied probe-based assays the same level of stock differentiation capabilities that has been available to solution-based assays. The recent development of a laser-based version of ICPMS may potentially overcome these restrictions, since it combines the beam capabilities of a high-energy laser with the analytical capabilities of ICPMS (Denoyer et al. 1991).

Conceptually, laser ablation – inductively coupled plasma mass spectroscopy (LA-ICPMS) can be envisioned as a traditional, solution-based ICPMS where sample is provided not as a solution, but as vaporized material ablated by a high-energy laser. The pulsed Nd:YAG laser (1064 nM) is focused onto a small area (nominal diameter 30–100 μm) of the sample solid, completely vaporizing the exposed surface to a depth of a few micrometres. The vaporized material is then swept by a flow of argon gas into the plasma of an ICPMS, where its relative elemental and isotopic composition can be accurately determined to at least the parts per million level. Both the sample and the ablation event can be viewed remotely in real time with 40× optics on a video camera and monitor. Electronic stepper motors attached to the sample stage allow for accurate and repeatable positioning of the sample. No sample preparation is required (once the surface of interest has been exposed), and the analysis can be done under atmospheric pressure.

The objective of this study was to provide a preliminary test of the stock differentiation capabilities of elemental fingerprints of the otolith nucleus determined with LA-ICPMS. Samples of Atlantic cod (*Gadus morhua*), collected from spawning grounds throughout the northwest Atlantic, served as the test species. Considerable attention was given to analytical sources of error and variability which could influence either the accuracy or the precision of the assays.

Materials and Methods

Otolith Collection

The sampling program was designed to catch fish of known stock through capture on the spawning ground in spawning condition. It is generally assumed that cod stock mixing is minimal at the time of spawning and that seasonal feeding migrations begin long after spawning has been completed (Templeman 1962). While not all of the cod used in this study were in spawning condition, most were preparing to spawn or had just spawned. Accordingly, we believe that our samples are reasonable representations of a number of discrete spawning stocks. With the exception of the Fundyrip–Georges Bank stock pair, all of the stocks are assumed to have remained geographically separated at all life history stages.

Cod were either collected at sea aboard research vessels using otter trawl gear or sampled from commercial catches where the fishing location was known with certainty. All samples were restricted to fish in the size range of 45–85 cm fork length to restrict the analysis to sexually mature individuals and to reduce variability caused by size-related effects. Subsamples of 30 fish from each of five sites were randomly selected for otolith elemental assays (Table 1; Fig. 1). The collection site of the Iceland sample (not shown in Fig. 1) was several miles off the western coast of Iceland. All samples were collected in 1986. Sampling dates varied across several months because of the tendency for cold-water stocks to spawn later than warmwater stocks.

Immediately after capture, fork length, head length (from the tip of the snout to the posterior end of the preoperculum), sex, and state of sexual maturity were recorded. The head was then severed, labelled, and frozen for subsequent otolith removal in the laboratory. All three otolith pairs (sagittae, lapilli, and asterisci) were removed from each fish, cleansed of adhering tissue, and stored dry in glass

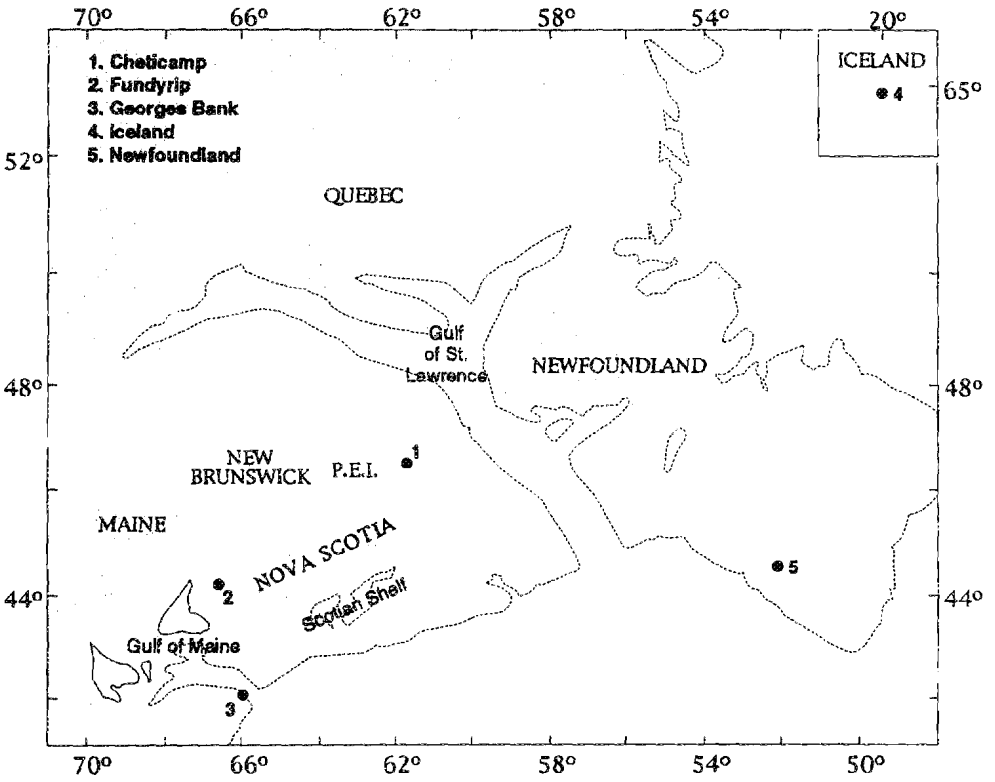

FIG. 1. Map of the study area, sampling sites, and 200-m contour.

vials until they could be examined further. Broken and crystalline otoliths were discarded (<1% of the sample).

Otolith Preparation

One lapillus per fish was prepared for solution-based elemental analysis with ICPMS, as has been reported elsewhere (Campana and Gagné 1994). The matching sagittae were embedded in polyester resin, sectioned transversely (Strong et al. 1985), and aged as per established procedures (Campana and Casselman 1993). One side of each sagittal thin section was subsequently polished with aluminum oxide lapping films as close as possible to the nucleus, using internal morphology as an index of proximity to the nuclear plane. Due to their opacity and the manner in which the otoliths had originally been sectioned, we often found it difficult to accurately locate the nucleus, particularly for the sample from Newfoundland. Accordingly, we abandoned efforts to polish to within 30 μm of the nucleus and instead targeted a plane 0–250 μm above that of the nucleus. Given otolith growth rates typical of cod off southwest Nova Scotia (Campana 1989), our final plane corresponded to a fish size of 3–22 mm and a fish age of 0–3 mo.

To remove surface contamination, sagittal sections were sonified for 5 min in Super-Q water (water that has been deionized, further purified through reverse osmosis, and then Millipore-filtered). After air drying, the sections were stored in paper envelopes and subsequently handled only with nonmetallic objects. More rigorous surface decontamination occurred later through laser-based preablation of the sample surface.

Laser Ablation

Prior to assay, otolith sections were preablated with a 6 × 6 rastered pattern of 60-μm craters in the nuclear region so as to remove any remaining surface contamination. Preablation was conducted while the sample cell was disconnected from the ICPMS to minimize unnecessary buildup of otolith material inside the ICPMS. Preablation craters were overlapped to minimize the possibility of fractionation at the crater edge (Krajnovich et al. 1993). Assays were subsequently conducted in the centre of the preablated region using 20 Q-switched energy pulses (<20 mJ) at 1-s intervals and a nominal beam diameter of 30 μm. Crater depth as measured with an atomic force microscope was about 30 μm, but the energy of the laser often shattered the surrounding region. Argon carrier flow rate was 1.5 L·min^{-1}. Isotope quantification was based on 20 channels per amu, 7-s peak integration, and a dwell time of 320 μs. Argon gas blanks were run immediately prior to each assay and subsequently subtracted from the corresponding sample values. In principle, all blanks should incorporate a laser pulse due to the possibility of shock waves dislodging minute particles in the tubing leading to the ICPMS. However, preliminary tests using LA-ICPMS to ablate Teflon caps indicated that the latter contained significant levels of Rb, Zn, and Ba but were otherwise similar to Ar blanks.

The high Ca content of the otolith material caused significant buildup on the nebulizer tips, sampler, and skimmer cones, as well as on the inside of the quadrupole. As a result, instrument drift was unavoidable, resulting in detection limits that were elevated somewhat over normal levels. The ICPMS was recalibrated periodically, but drift was evident for most elements, particularly after recalibration. Drift was most evident when relative elemental concentration was plotted as a function of order in the analysis sequence. To avoid the possibility of confounding instrument drift effects with stock differences, samples were assayed in a systematic order, e.g., sample from Stock 1, sample from Stock 2,

TABLE 2. Elements and isotopes detectable with LA-ICPMS in cod otolith nuclei. All measurements were significantly above background levels. Isotopes accepted for use in the analysis are marked with an asterisk.

Element/ isotope	Natural abundance, %	Isotope counts relative to background counts	Analytical reliability
B-10*	19.78	1.30	—
B-11	80.22	1.35	Spillover from C-12
Mg-24*	78.70	12.36	—
Mg-25*	10.13	9.89	—
Al-27	100.00	~1	Interference from Al stage
Ca-46*	0.004	7.18	—
Ca-48*	0.187	86.43	—
Fe-57*	2.19	1.18	Interference from Ar-OH?
Zn-66*	27.81	3.31	—
Br-81	49.46	~1.5	High background
Rb-85*	72.15	1.80	—
Sr-86*	9.86	284	—
Sr-87*	7.02	>1000	—
Sr-88*	82.56	>1000	—
Ag-107	51.82	~10	Probably Ar–Al (no Ag-109)
Sn-118*	24.03	40.05	—
Ba-137*	11.32	>1000	—
Ba-138*	71.66	>1000	—

sample from Stock 3, etc. The effect of instrument drift was later effectively removed as part of the statistical analysis.

Solution-based ICPMS can be calibrated by spiking bulk sample solutions with known concentrations of elemental standards. This approach is effective in spite of the potential influence of the sample matrix on detection efficiency and molecular ion interference. However, spiking of solid samples is not possible in the same manner. Many electron microprobe analyses use Ca as an internal standard against which other elemental concentrations are referenced (Kalish 1989; Gunn et al. 1992), despite the fact that the ratios are not equivalent to the ratios of their concentrations (due to interchannel differences in detection efficiency). A similar approach (using Ca-48 as the internal standard) could also have been used in this study, in light of the low interotolith Ca concentration variability found in cod otoliths (Campana and Gagné 1994). This type of standardization would not provide absolute elemental concentrations, but would remove sample to sample variability in laser energy and the weight of ablated material. However, we elected instead to adjust for the quantity of ablated Ca-48 through its incorporation into the statistical model as a covariate. As it turned out, statistical analyses of the data using both approaches revealed no appreciable differences between the two.

Analytical reproducibility of the laser ablation system was tested using replicate assays of known-composition glass (NBS 615). Ten spots were sequentially laser-sampled on each of 4 d in an ANOVA design. The isotopes assayed were the same as those assayed in the otoliths.

Statistical Analyses

All isotopic data were first examined for normality and ln-transformed where appropriate. The statistical analysis was designed to adjust for both instrument drift between ICPMS adjustments and variations in the amount of ablated material (as indexed by Ca-48). The resulting MANOVA included 14 isotopes as dependent variables, sequence block and sample site as factors, and Ca-48 as a covariate. Interaction terms were nonsignificant and therefore dropped from the model. Factor analysis, discriminant analysis and univariate ANOVA's were used to determine the isotopes most influential in the analysis. Classification success to each of the sample sites was determined through jackknifed discriminant analysis.

Results

Analytical Sensitivity and Precision

Isotopes were selected for subsequent assay after completion of a full elemental scan of both otolith material and an Ar blank. All isotopic measurements that were significantly above background levels ($p < 0.01$) were then assessed for isobaric interferences (particularly from Ar, Ca, and N molecular ions) and spillover from adjacent abundant isotopes. Only those isotopes that were free from interferences were used in subsequent analyses. A total of 14 isotopes, representing nine elements, could be measured with confidence (Table 2).

Incorporation of Ca-48 as a covariate in the statistical analysis is equivalent to using it as an internal standard. Yet, standardization of elemental concentrations against Ca concentration is only justifiable if the latter is a measure of ablated mass and is relatively invariant across otoliths. Ca-48 was highly significant ($p < 0.001$) as a covariate in the MANOVA (Table 3). In addition, univariate regressions of unstandardized isotope concentrations against Ca-48 were significant ($p < 0.05$) in all cases (except B-10), indicating that isotope concentrations increased with Ca-48 concentration. These results suggest that Ca-48 concentration is indeed a measure of ablated mass and that standardization to Ca-48 should remove sample-to-sample variation in laser energy and ablated mass. To confirm this, nested ANOVA's of each isotope, using paired otoliths (left and right from each fish), were used to partition the variance between fish effects and individual otolith and/or sample processing effects. Comparison of nested ANOVA results for each isotope

TABLE 3. Results of the MANOVA testing for effects of sequence in the analysis, ablated mass, and stock on the elemental fingerprint of the otolith nucleus.

Source of variation	Pillais value	Hypothesis df	Error df	*F*–ratio	Significance
Sequence block	1.48	52	484	5.48	0.000
Sample site	0.66	52	484	1.84	0.001
Ca-48 (covariate)	0.55	13	118	11.1	0.000

TABLE 4. Concentrations of isotopes of interest in a known-composition glass material (NBS 615) as measured with LA-ICPMS on four different days (n = 10 per day). Nominal concentrations were not available for some isotopes.

Isotope	Nominal concentration, ppm	Mean count	Within-day CV, %	Across-day CV, %
B-10	0.26	676	30	67
Mg-24	—	8 861	11	20
Mg-25	—	1 327	40	46
Ca-46	3	2 735	18	35
Ca-48	160	83 051	6	15
Fe-57	0.29	−1 392	45	144
Zn-66	—	508	40	86
Rb-85	0.62	600	20	40
Sr-86	4.52	2 254	8	19
Sr-87	3.22	1 823	6	19
Sr-88	37.81	19 228	7	21
Sn-118	—	1 344	40	126
Ba-137	—	218	13	21
Ba-138	—	1 400	8	23

TABLE 5. Nested ANOVA's of isotope concentrations in 62 paired otoliths from 31 cod. The percentage of the variation explained by the fish is a reflection of the isotope's reliability as a component of the fish's elemental fingerprint.

	Sum of squares		df		Variation %	
Isotope	Fish	Otolith	Fish	Otolith	Fish	Otolith
B-10	6.001	1.187	30	31	67.9	32.1
Mg-24	4.715	1.966	30	31	42.5	57.5
Mg-25	8.636	4.961	30	31	28.5	71.5
Ca-46	3.897	2.273	30	31	27.9	72.1
Fe-57	2.103	0.356	30	31	71.9	28.1
Zn-66	3.323	2.422	30	31	17.3	82.7
Rb-85	1.032	0.541	30	31	32.6	67.4
Sr-86	2.076	0.425	30	31	66.9	33.1
Sr-87	3.152	0.487	30	31	74.0	26.0
Sr-88	6.692	1.679	30	31	60.9	39.1
Sn-118	5.380	0.725	30	31	76.9	23.1
Ba-137	4.957	0.886	30	31	70.5	29.5
Ba-138	2.023	0.290	30	31	75.6	24.4

before and after standardization to Ca-48 indicated that the standardized contribution due to fish effects exceeded that of the unstandardized contribution for all isotopes except two (Ca-46 and Rb-85), confirming the value of standardizing isotope concentrations to Ca-48. However, the improvement was very modest, generally less than 10%.

Position in the analysis sequence had a very large effect on apparent isotopic concentrations. Plots of concentration against sequence number for each isotope revealed large, abrupt shifts in concentration associated with changes in ICPMS operating conditions. All were associated with plasma shutdown (e.g., at lunch and at the end of the day), but were not due to complete shutdown of the ICPMS. Since the Ar blanks showed similar shifts in concentration, the discontinuities were associated with ICPMS operation and not the laser. Shifts in isotope concentrations due to these effects were not parallel across isotopes, i.e., one isotope might increase and another decrease at plasma shutdown, while a third might stay constant. However, the differences tended to be both large and significant, whether analyzed individually or in the MANOVA (Table 3). It is important to note that the magnitude of the isotope concentration shifts associated with the ICPMS far exceeded the sample-to-sample or stock-to-stock variability. Failure to systemically mix samples across the analysis sequence, or to include a sequence factor in the statistical analysis, would have introduced huge variances into the data and could have introduced significant but artifactual differences among samples.

Analytical reproducibility of the laser ablation system was further tested using replicate assays (n = 10) of known-composition glass (NBS 615) on each of 4 d. Within any given day, the coefficient of variation (CV) ranged from less than 10% (for elements such as Ca, Sr, and Ba) to more than 100% (for elements such as Fe and Sn), with an overall mean of about 20% (Table 4). All isotopes showed large and highly significant ($p < 0.01$) differences across days. CV's across days were generally 2–3 times larger than those within days for any given isotope. Standardization to Ca-48 did not eliminate the significant day effect, although it did reduce across-day CV for two of the elements (Sr and Ba). These results confirm the influence of ICPMS operation (independent of laser operation) on analytical results, validate the necessity of statistically removing sequence effects, and document the necessity of systematic mixing of the

TABLE 6. Mean otolith isotopic concentrations, adjusted for Ca-48, by sample site. Variables that were ln-transformed are marked (ln). Sample size was $n = 28$ for all sites except Cheticamp, where $n = 29$. Isotopes that differed significantly ($p < 0.05$) across sites are marked with an asterisk. Where present, significant pairwise differences with another site (represented by its first letter) are indicated in parentheses.

	Sample site				
Element/isotope	Cheticamp	Fundyrip	Georges Bank	Iceland	Newfoundland
B-10 ($\times 10^4$)*	1.8	1.7 (N)	2.4	2.6	4.1 (F)
Mg-24 ($\times 10^2$)*	3.44 (G, I)	3.78	3.97 (C)	4.08 (C)	3.78
Mg-25 ($\times 10^3$)	4.87	5.27	5.09	5.49	4.95
Ca-46 ($\times 10^2$)	1.44	1.44	1.44	1.45	1.44
Fe-57 (ln)	−5.92	−5.92	−6.01	−6.09	−5.98
Zn-66 (ln)	−7.61	−7.70	−7.86	−7.49	−7.84
Rb-85 ($\times 10^5$)	5.2	3.3	4.2	4.6	5.7
Sr-86 (ln)*	−1.67 (I)	−1.72 (I)	−1.76 (I)	−1.56 (C, F, G, N)	−1.75 (I)
Sr-87 (ln)*	−1.67	−1.77 (I)	−1.74 (I)	−1.59 (F, G, N)	−1.78 (I)
Sr-88 (ln)*	0.43	0.40 (I)	0.39 (I)	0.50 (F, G, N)	0.39 (I)
Sn-118 ($\times 10^4$)	8.8	8.1	8.3	7.9	7.4
Ba-137 (ln)*	−8.13	−7.83 (I, N)	−8.21	−8.49 (F)	−8.26 (F)
Ba-138 (ln)*	−6.13	−5.83 (G, I, N)	−6.22 (F)	−6.45 (F)	−6.22 (F)

order of sample analysis. However, because of compositional differences in the sample matrix, the precision of the glass assays is not necessarily representative of that of the otolith assays.

Comparison of isotope concentrations between left and right otoliths from the same fish indicated that the elemental fingerprints were at least partially representative of the fish, and not just the laser sampling site or the otolith. Nested ANOVA's of the paired otolith data indicated that 17–77% of the partitioned variance was due to fish effects (Table 5). On the basis of these results, it is reasonable to conclude that concentrations of elements such as B, Fe, Sr, Sn, and Ba can be considered to be elemental signatures of the fish, rather than just the individual otolith. However, elements such as Ca, Zn, and Rb provided relatively weak signatures.

Reproducibility of the isotopic signatures was also assessed through comparison of multiple isotopes within any given element. Correlations of within-element isotopic concentrations were all highly significant ($p < 0.0001$) (Mg-24/Mg-25, $r = 0.78$; Sr-86/Sr-87, $r = 0.84$; Sr-86/Sr-88, $r = 0.87$; Sr-87/Sr-88, $r = 0.70$; Ba-137/Ba-138, $r = 0.96$), indicating that isotopic values were indeed reflecting true elemental abundances. A poor correlation would have suggested interference in the quantification of one or more of the isotopes. Indeed, poor correlation between the two isotopes of B (B-10 and B-11) formed part of the basis for rejecting the use of the latter.

Elemental Fingerprint Differences among Sites

The elemental fingerprints of the otolith nuclei differed significantly across sites (MANOVA, $p < 0.001$) (Table 3). Univariate F-tests indicated that the concentrations of B-10, Mg-24, Sr-86, Sr-87, Ba-137, and Ba-138 all differed significantly across sample sites ($p < 0.05$). A posteriori contrasts indicated that the Fundyrip site often differed significantly from the Iceland and Newfoundland sites and that the Iceland site often differed from Fundyrip, Georges Bank, and Newfoundland (Table 6). Despite the statistical significance, many of the intersite differences were relatively small, typically less than 50% of the mean (Table 6). None of the within-element isotope ratios differed significantly among sites.

Neither age, sex, nor fish length accounted for the intersite differences in isotope concentration. Three-way ANOVA's of each individual isotope concentration, using sequence block, site, and sex as factors, demonstrated that sex was not significant ($p > 0.10$). Since age compositions did not necessarily overlap between sites, it was not possible to carry out similar analyses for age effects. However, within-site analyses of individual isotopes demonstrated that age had no significant relationship with isotopic concentration (ANOVA, $p > 0.10$). Similarly, fish length was not a significant covariate in the analysis of intersite differences (MANOVA, $p > 0.05$).

Stepwise discriminant analyses of the elemental concentration data confirmed the significant discrimination among sample sites ($p < 0.05$). The stepwise variable selection included only three of the 18 possible isotopes and isotope ratios: Ba-138, Sr-86, and Mg-24. Pairwise differences were similar to those associated with the MANOVA contrasts, where Iceland differed significantly from all of the other sites, and Fundyrip differed from Cheticamp and Georges Bank at the $p = 0.06$ level. However, classification accuracy was relatively low, with a mean jackknifed accuracy estimate of 30%. There was no apparent tendency for misclassified samples to be classified to the nearest alternative sample site.

Discussion

Elemental fingerprinting of otolith nuclei appears to have potential for differentiating among cod populations. Cod spawn on dozens of offshore banks and in many coastal regions throughout the northwest Atlantic, with each spawning aggregation assumed to represent a distinct population (Templeman 1962). However, cod are highly migratory and many populations intermix at times other than spawning (McKenzie 1956; Templeman 1962; Wise 1963), thus confounding past attempts to distinguish among stocks. Genetic differentiation has, in general, proven unsuccessful in differentiating among multiple cod stocks (Cross and Payne

1978; Mork et al. 1985; Smith et al. 1989; but see Carr and Marshall 1991). Tagging (Wise 1963), morphometrics (Bowen 1987), meristics (Lear and Wells 1984), parasite loads (Scott and Martin 1957), and ichthyoplankton surveys (O'Boyle et al. 1984) have all been used to confirm the presence of multiple cod stocks, but none has provided a reliable measure of stock identity. The results of this study provide encouraging indications that elemental fingerprints of the otolith nucleus may prove useful for differentiating among cod stocks. While technical limitations to our ability to more completely retrieve the information locked in the otolith nucleus remain, these limitations appear to be tractable.

Various workers have reported sample-specific differences in elemental concentration in tissues other than otoliths, but the implications of their findings are somewhat unclear. Analyses of bone (Behrens Yamada et al. 1987; Hamilton and Haines 1989; Miller et al. 1992), scales (Johnson 1989), and various soft tissues (Calaprice 1971; Hellou et al. 1992) must reflect composition during growth, but also incorporate metabolic reworking after initial deposition as well as tissue depuration. As a result, the temporal stability of the elemental concentrations in nonotolith tissues is questionable. In contrast, the acellular otolith is metabolically static (Campana and Neilson 1985) and is not exposed to outside water, making it an ideal storage site for trace elements after incorporation.

Otolith elemental fingerprinting has proven successful in detecting intersite differences in a variety of fish species. Edmonds et al. (1989, 1991, 1992) reported site-specific differentiation of several Australian fishes. Site-specific elemental concentrations were also reported by Mulligan et al. (1987), Grady et al. (1989), Gunn et al. (1992), Secor (1992), Sie and Thresher (1992), and Campana and Gagné (1994), although the validity of the Mulligan et al. (1987) study now appears questionable (Kalish 1990; Gunn et al. 1992). Kalish (1990) used otolith elemental fingerprints to distinguish between anadromous and nonanadromous salmonids from the same site. In all of the above studies, it was not clear if the elemental fingerprints were environmentally driven or incorporated a genetic component. However, the validity of using stock- and site-specific fingerprints does not rest upon the mechanism underlying their formation.

Comparison of the current cod stock discrimination results with those of a sister study based on ICPMS analysis of whole dissolved lapilli (Campana and Gagné 1994) provided results that were, at least initially, counterintuitive. Many of the same isotopes differed significantly among sites in both studies. However, the elemental fingerprints of whole otoliths were far more powerful in distinguishing among the cod stocks than were laser-based assays of the same fish using the otolith nucleus. Technical reasons for the discrepancy included the greater sensitivity and enhanced precision of solution-based ICPMS compared with LA-ICPMS (van Heuzen 1991; Hall 1992; Jackson et al. 1993). In addition, the sister study failed to randomize the sample assay sequence (as was done here), which may have contributed artifactually to apparent stock discrimination success. Finally, our inability to precisely section the otolith through the nucleus undoubtedly interfered with our LA-ICPMS assay results, if only because the resulting elemental fingerprint came to represent variously the fingerprint of the egg, larva, or early juvenile, rather than just the egg. There are also theoretical explanations for the different results, not the least of which is a different underlying hypothesis. Stock differentiation based on whole-otolith fingerprints takes advantage of differences in home range (with associated differences in water composition), as well as differences in spawning site. Under Ihssen et al.'s (1981) definition of a stock as an "intra-specific group of randomly mating individuals with temporal or spatial integrity", the progeny of a single spawning aggregation could grow up in a range of different environments, with relatively little intermixing after hatch. Such stocks would likely be characterized by different whole-otolith elemental fingerprints, just as they would demonstrate other morphological differences, such as growth rate. Conversely, application of elemental fingerprinting to otolith nuclei tests only for differences in or around the hatch site; the presence or absence of differences in posthatch environment plays no part. Thus, the fingerprint of the nucleus is more closely akin to a test for genetic differences than is the whole-otolith fingerprint, despite the fact that subsequent spatial segregation would serve only to amplify the differences in otolith elemental composition.

Despite the statistically significant stock discrimination achieved with LA-ICPMS in this study, classification success was only moderate at best. This was surprising, given that all but one pair of the tested stocks (Fundyrip and Georges Bank) are believed to be completely discrete at all life history stages. More importantly, misclassified individuals were not necessarily identified as being from adjacent, or even environmentally similar, stocks. Explanations for the above are not obvious, although several levels of variability undoubtedly contributed to each reading of elemental composition:

(1) Instrumental error: the CV's documented in our experiments with the known-composition glass highlight the variability that is possible in this type of study, although matrix effects make comparisons of glass and otolith material difficult (Perkins et al. 1991).

(2) Preparation and crystal-level error: the statistical power of this study was lower than expected because of the variability introduced during the sectioning. However, the nested ANOVA's indicated that most isotopes were relatively stable components of the elemental fingerprint, as demonstrated by their parallelism between the left and right otoliths of any given fish. Therefore, the fingerprints were real, and not just instrumental artifacts.

(3) Variability among fish collected at the same site: year-to-year differences in seawater composition would be expected to induce different elemental fingerprints in fish of different age, and thus reduce our ability to differentiate among stocks. Although we did not detect any significant age effects in the analysis, that does not mean that they were not there. In addition, the incorporation of trace elements into calcium carbonate is, at least in part, a function of crystallization rate (Lowenstam and Weiner 1989) and growth rate, implying that differences in physiological condition between fish of a given stock contributed to increased variance. This hypothesis requires testing. Also possible is a violated assumption of site fidelity, wherein adults were assumed to have returned to their hatch site to spawn. This assumption is difficult to test in nonanadromous species, but is almost certainly invalid to a certain extent (Sinclair 1988). Violations of the site fidelity assumption would result in greatly reduced stock discrimination ability, since the adult fish caught at one site would actually represent a mixture

from different sites. Note that this assumption is far more critical to this type of study than to whole-otolith assays, given that fish eggs and larvae are susceptible to large-scale transport and diffusion processes.

(4) Variability among sites: this was the level of variability that was of greatest interest to us. Among-site differences are dependent on differences in the physical characteristics and composition of seawater, which may or may not come with increased geographic separation. For instance, water from the Gulf of St. Lawrence eventually feeds into the Bay of Fundy (our Fundyrip sample), linking the two widely separated sites after a lag time of several months (Smith 1983). Similarly, water from the Gulf Stream feeds both Georges Bank and Iceland, and thus may reduce the expected difference in seawater composition between the two areas. Water temperature also varies among sites, but does not necessarily decline with increasing latitude. However, there is no question that all of the sites differed in physical and chemical composition to some degree, and our results suggest that those differences were at least partially reflected in the otolith. Any physiological or genetic differences between stocks that influenced otolith elemental uptake may also have contributed to among-site differences in the elemental fingerprint.

Laser ablation ICPMS offers numerous advantages, and some disadvantages, over alternative instrumentation available for characterizing an elemental fingerprint of the otolith nucleus. The primary advantages are those of minimal sample preparation and the simultaneous determination of both isotopic and elemental concentrations, with a sensitivity that is unmatched by other probe techniques. While the sensitivity of solution-based ICPMS (<0.03 ppb, Houk 1986) greatly exceeds that possible with LA-ICPMS, the detection limits of <300 ppb observed in this study are conservative compared with those reported elsewhere for LA-ICPMS (Denoyer et al. 1991; van Heuzen 1991; van Heuzen and Morsink 1991; Hall 1992; Pearce et al. 1992) and several orders of magnitude better than the limits of about 3000 and 100 ppm possible with energy-dispersive and wavelength-dispersive electron microprobes, respectively (Denoyer et al. 1991; Gunn et al. 1992). The detection limit of the proton microprobe is of intermediate value, around 1 ppm (Ishikawa et al. 1987). However, LA-ICPMS does not suffer from the charging problems that can confound either electron or proton microprobe assays, nor does it require a smoothly polished sample surface as do the other techniques. Disadvantages of LA-ICPMS include the inability to accurately measure the abundance of some of the lighter, relatively abundant elements, interference of polyatomic ions with certain isotopes, and the potential for instrument drift due to Ca buildup during sequential assays. The latter constraint can be overcome by insuring that the order of sample assays is statistically blocked, crossed, and balanced with respect to sample site, as was done in this study. However "memory effects", due to transient particle deposition in the transfer tubes (van Heuzen 1991), are more problematic, and undoubtedly contribute to CV's that are somewhat greater than those observed with either conventional ICPMS (Jackson et al. 1993) or an electron microprobe. Spatially, the 30-μm spot size of the laser cannot compete with the 3-μm spot size of an electron microprobe (Gunn et al. 1992). As is the case with other probe techniques, it is difficult to provide quantitative estimates of elemental concentration with LA-ICPMS, although some workers have reported limited success through use of standard-spiked pressed tablets made from sample material (Perkins et al. 1991; van Heuzen and Morsink 1991; Pearce et al. 1992).

Aside from continued effort to improve the accuracy and precision of LA-ICPMS, we can identify several issues in elemental fingerprinting requiring additional research. Ontogenetic effects on otolith composition are not yet clear, nor has the source of the trace elements (water versus diet) been identified. The role of physiological regulation in controlling the uptake of trace elements into the otolith is poorly understood, even though Kalish (1989) has helped define the factors influencing the uptake of major and minor elements. Experiments to test the response of otolith trace element composition to variations in water composition are clearly required. By corollary, confirmation that otolith elemental composition reflects seawater composition at the hatch site is lacking. Although large-scale spatial variations in seawater composition exist (Johnson et al. 1992), we were not able to find any reports of large-scale spatial variation in the isotopes of interest in the northwest Atlantic. Increased interaction between chemical oceanographers and those using otolith elemental fingerprints would undoubtedly be of value.

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