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Cod Stock Discrimination Using ICPMS Elemental Assays of Otoliths

Steven E. Campana and Jacques A. Gagné

ABSTRACT: Atlantic cod (*Gadus morhua*) otoliths were collected from seven spawning grounds in the northwest Atlantic and tested for stock-specific differences in elemental and isotopic composition (the elemental "fingerprint"). A total of 34 isotopes, representing 27 elements, were detected using inductively-coupled plasma mass spectroscopy (ICPMS). Most elements were present at concentrations well below the detection limit of the electron microprobe, and all differed significantly among sample sites. Several isotope ratios, such as $^{87}\text{Sr}:$ ^{88}Sr , also differed among sites. Discriminant analyses of the otolith weight-detrended elemental fingerprints indicated that the sample sites could be differentiated with 83-94% accuracy, depending on the otolith type (sagitta or lapillus) analyzed. 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 for cod.

Introduction

Studies of the population dynamics of marine fishes are heavily dependent upon the ability to differentiate among adjacent or mixed stocks. Measures of growth, survival, and reproductive success all assume that a single population is being monitored. Where such measures are confounded by the presence of multiple or mixed populations, studies of fish biology, population dynamics, and much of the basis of modern fisheries management can be invalidated. Unfortunately, there appear to be few (if any) markers, whether genetic or morphological, that can be used to reliably differentiate among all populations of any marine fish species.

The otolith would appear to be an ideal natural marker for fish populations. 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). Therefore, otoliths remain unaffected by the short-term changes in fish condition, such as starvation and mode of preservation, which can confound stock identification studies based on body morphometrics. Since the calcium carbonate and trace elements that make up 90% of the otolith are derived primarily from the water (Simkiss 1974), the chemical composition of the otolith reflects that of the water in which the fish lives, although not necessarily in a simplistic fashion (Kalish 1989).

And since the chemical composition of sea water varies spatially, the chemical composition of the otolith should also vary with the home range of the population. As is the case with morphometrics, meristics, and most other stock identification procedures, the use of otolith composition to infer stock identity does not distinguish between environmental and genetic differences. Nevertheless, as long as such differences exist, otolith composition should vary among populations as long as they remain segregated during some part of their life-history.

A handful of previous studies have provided encouraging signs that the elemental composition of the otolith (elemental fingerprint) may prove to be the most powerful means yet developed of distinguishing among fish stocks. While these earlier studies used instrumentation capable of detecting only one to six elements (Gauldie et al. 1980; Papadopoulou et al. 1978, 1980; Kalish 1990; Gunn et al. 1992), all reported varying degrees of success in distinguishing between fish from different populations. Recent studies by Edmonds et al. (1989, 1991, 1992) and Sie and Thresher (1992) indicate that a much broader suite of elements exists in the otolith, but at concentrations well below that detectable with the X-ray microanalysis (electron microprobe) used so frequently in elemental composition studies.

Inductively-coupled plasma mass spectroscopy (ICPMS) is the preferred instrument for detecting and quantifying the concentration of multiple trace elements (Beauchemin et al. 1987; Date 1991). Its advantages over other approaches include the ability to simultaneously assay numerous elements (and isotopes of elements) quickly and inexpensively, with a sensitivity that matches or exceeds that of X-ray microanalysis, the proton microprobe, neutron activation analysis, atomic absorption spectroscopy, inductively-coupled plasma atomic emission spectroscopy, and X-ray fluorescence. While it has been successfully applied to assays of various fish tissues (Ishii et al. 1991; Hellou et al. 1992; Lobel et al. 1992), only Edmonds et al. (1992) have tested its value on otoliths.

The objective of the current study was to provide a large-scale test of ICPMS-based elemental fingerprints for distinguishing among fish stocks. Atlantic cod (*Gadus morhua*), collected from spawning grounds throughout the northwest Atlantic, served as the test species. Most of the samples represented stocks that do not intermix at any time of the year. Additional factors evaluated in this study were age, size, and sex, all of which could have confounded the interpretation of apparent stock differences. Finally, analytical reproducibility and comparability between sagittae and lapilli from the same fish were assessed.

Materials and Methods

OTOLITH COLLECTION

The sampling program was designed to catch adult fish of known stock through capture on the spawning ground in spawning condition. It is generally assumed that 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 possible exception of the 4Vs sample, which was collected several months after spawning (Table 1).

Table 1. Sample collection information. Each sample consisted of 50 fish. L = lapillus pair; S = sagitta pair.

Sample #	Area	NAFO Division	Collection Date	Mean Length (range) cm	Mean Age (range) yr	Otolith Type
1	Cheticamp	4T	26 May 1986	50.5 (45-63)	5.9 (5-8)	L
2	Fundyrip	4X	6 Mar 1986	54.5 (45-74)	3.1 (2-4)	L
3	Georges Bank	5Zj	16 Apr 1986	62.1 (48-77)	3.9 (2-6)	L
4	Iceland	-	6 May 1986	70.1 (62-75)	6.8 (5-10)	L
5	Newfoundland	30de	26-28 May 1986	62.4 (52-69)	6.9 (5-9)	L
6	N. Gulf of St. Lawrence	4RS	14-25 May 1991	51.8 (45-68)	6.7 (5-12)	S
7	E. Scotian Shelf	4Vs	25 July 1983	-	-	L,S

Cod were either collected at sea aboard research vessels using otter trawl gear, or sampled from commercial catches where the fishing location could be accurately determined. All but the 4Vs sample 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 50 fish from each of seven sites were selected for otolith elemental assays (Table 1; Fig. 1). While not shown in Fig. 1, the Iceland sample was collected several miles off the western coast of Iceland. All samples were collected in 1986, with the exception of two sites (4Vs in 1983; 4RS in 1991). Sampling dates varied across several months, because of the tendency for cold-water stocks to spawn later than warm-water 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 vials until they could be examined further. Details of lapillus and asteriscus removal are presented in Campana and Casselman (1993). Broken and crystalline otoliths were discarded (< 1% of the sample).

For five of the samples (Georges, Fundyrip, Cheticamp, Newfoundland, and Iceland), one lapillus per fish was prepared for elemental analysis. The matching sagittae were sectioned and aged as per established procedures (Campana and Casselman 1993). Lapilli were not collected from the 4RS sample, so one sagitta from each fish was aged and the other saved for assay. In the case of the 4Vs sample, both pairs of lapilli and sagittae were analyzed to provide a measure of elemental differences between otolith types, as well as quantify analytical precision within fish. Accordingly, ages were not available for the 4Vs sample.

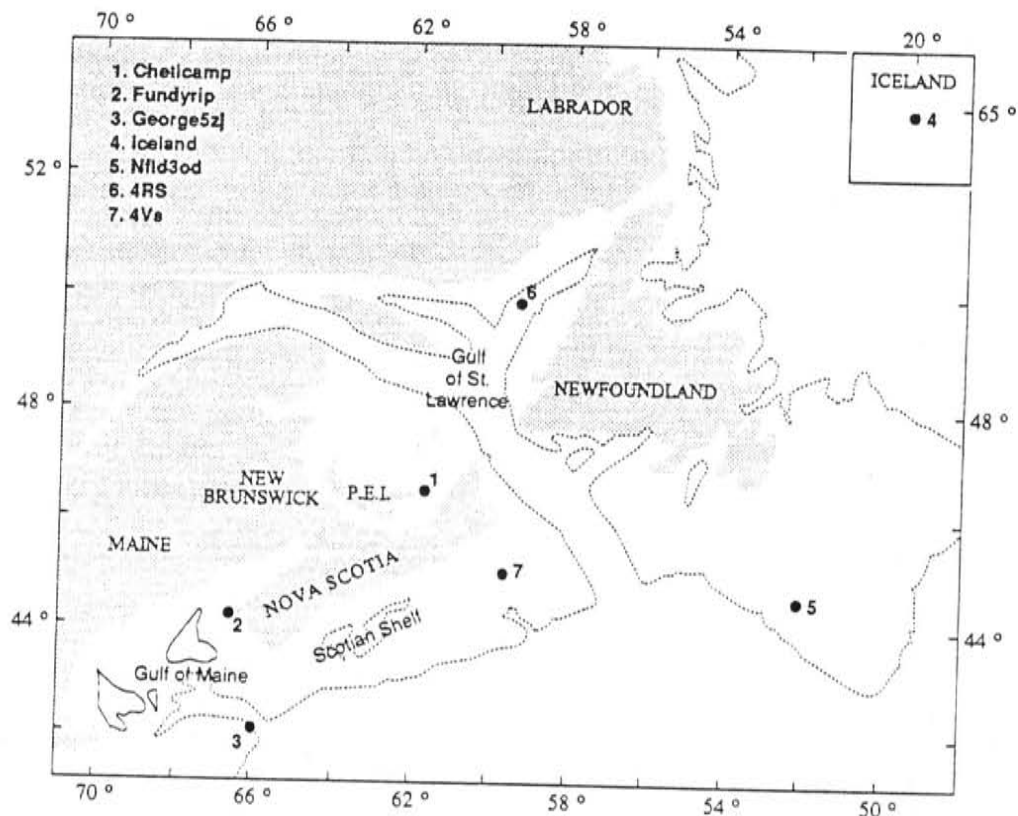


Fig. 1. Map of the study area and sampling sites. The sample from Iceland was collected several miles off the western coast of Iceland. The 200-m contour is shown.

OTOLITH PREPARATION

All otoliths were carefully treated before elemental analysis to minimize the possibility of contamination. Contamination is of particular concern with ICPMS, given the extremely low detection limits of the technique. Accordingly, otoliths were handled at all times after collection with either acid-washed Teflon-coated forceps or acid-washed glass rods; at times other than weighing, they were kept under a positive-pressure fume hood to eliminate the possibility of airborne contamination.

To remove any surface contamination, sagittae were first scrubbed with a synthetic-bristle brush to remove any remaining tissues. Both lapilli and sagittae were then rinsed in Super-Q water (water that has been deionized, further purified through reverse osmosis, and then millipore-filtered), sonified for 15 min, triple-rinsed in Super-Q water, and transferred to an acid-washed polypropylene vial. After drying, the otolith was weighed to the nearest 0.01 mg and stored capped.

Otoliths were dissolved in double-distilled Seastar nitric acid (70%): 5 ml was added to the sagittae and 0.3 ml to the lapilli. Acid additions to the acid-washed vials were made with a plastic-

tipped, air-buffered macropipette to eliminate contact between the macropipette piston and the acid. After a 30-min reaction period, 45 ml and 2.7 ml of Super-Q water was added to the sagittal and lapillar reaction vials, respectively. The vials were gently swirled and kept loosely capped until dissolution was complete. There was no evidence of evaporative loss. The vials were then tightly capped after having returned to room temperature. Blank vials were prepared in an identical manner, although no otolith was present. Sagittal vials were subsequently diluted by a factor of five to reduce calcium buildup within the ICPMS.

ELEMENTAL ANALYSIS

The otolith solutions were analyzed with a Perkin-Elmer/Sciex Elan 5000 ICPMS in normal-resolution quantitative analysis mode. All elements were referenced against Tb. Calibration was by external standardization with spikes added to a sample of bulk digestate for recovery verification. Where multiple isotopes for a given element were analyzed, each isotopic concentration was treated as an independent estimate of the concentration of the corresponding element. Therefore, the isotopic concentrations that are reported are actually elemental concentrations. To obtain the actual isotopic concentration in the sample, the results must be multiplied by the proportional natural abundance of that isotope. On the other hand, isotope ratios of a given element have been adjusted for natural abundance, and thus represent true isotope ratios.

The high calcium content of the otolith solutions caused significant buildup on the nebulizer tips, sampler, and skimmer cones, as well as on the inside of the quadropole. As a result, instrument drift was unavoidable and detection limits were increased somewhat over normal levels. Detection limits varied inversely with the concentration of the otolith solution, and thus were lower in the lapillar solutions than in the sagittal solutions. However, given the greater otolith weight/volume concentrations in the sagittal solutions, detection of trace elements tended to be better in sagittae than in lapilli. The calcium ions also formed various molecular ions that interfered directly with a number of the isotopes; those isotopes could not then be reliably measured. The ICPMS was recalibrated at frequent intervals (every 15-20 samples), but drift and carryover effects were still detectable for some elements. Prior to statistical analysis, all elemental concentrations (both in terms of solution concentration ($\mu\text{g l}^{-1}$) and in terms of parts-per-million (ppm) per otolith weight) were plotted against analysis sequence number; where sequence effects were detectable, the isotope was removed from subsequent analyses. The removal of sequence effects was important, since the order of sample analysis was not randomized. Normal distributions were confirmed for all isotope concentrations and ratios. A list of measured elements and isotopes, along with their relative freedom from isobaric interferences, is presented in Table 2.

Results

The analytical sensitivity of the ICPMS was excellent: 34 different isotopes, representing 27 elements, could be detected in the otoliths (Table 2). Of that number, 26 isotopes were relatively free of isobaric interferences (particularly from Ar, Ca, and N molecular ions), and thus could be quantified, although several were very near the detection limit. A few of the isotopes could not be

Table 2. Elements and isotopes detectable with ICPMS in acid-dissolved cod otoliths. Solution concentrations and sample sites differed between otolith types; therefore, the lapillar and sagittal concentrations listed below are illustrative only, and are not directly comparable. NQ - detectable, but not quantifiable; ID - instrument drift, quantification may not always be reliable; INT - interference from other ions, making absolute levels unreliable, but not affecting relative levels.

Element/ Isotope	Natural Abundance (%)	Median Value in lapillus (ppm)	Median Value in sagitta (ppm)	Analytical Reliability
¹¹ B	80.22	2	1	ID
²⁶ Mg	11.17	8	14	ID
²⁹ Si	4.70	NQ	NQ	INT
⁴⁶ Ca	0.186	3.5 X 10 ⁵	4.2 X 10 ⁵	ID
⁴⁸ Ca	0.180	3.7 X 10 ⁵	4.1 X 10 ⁵	ID
⁴⁵ Sc	100	NQ	NQ	INT
⁴⁹ Ti	5.51	NQ	NQ	
⁵² Cr	83.76	0.6	0.2	INT
⁵³ Cr	9.55	0.2	0.1	ID
⁵⁵ Mn	100	0.2	1.4	INT
⁵⁴ Fe	5.82	NQ	NQ	INT
⁵⁹ Co	100	0.4	NQ	INT
⁶² Ni	3.59	NQ	0.4	INT
⁶³ Cu	69.09	0.1	0.1	ID
⁶⁶ Zn	27.81	0.6	0.5	
⁶⁸ Zn	18.57	0.7	0.2	
⁷¹ Ga	39.6	0.004	0.004	
⁷⁵ As	100	0.1	0.07	
⁷⁹ Br	50.54	4	2	
⁸⁵ Rb	72.15	0.2	0.2	
⁸⁶ Sr	9.86	3175	2655	ID
⁸⁷ Sr	7.02	3145	2682	ID
⁸⁸ Sr	82.56	3204	2667	ID
⁸⁹ Y	100	0.03	0.05	
⁹⁵ Mo	15.72	NQ	NQ	
⁹⁸ Mo	23.78	NQ	NQ	
⁹⁹ Ru	12.72	NQ	0.004	
¹⁰³ Rh	100	0.15	0.17	
¹¹¹ Cd	12.75	NQ	0.004	
¹³³ Cs	100	NQ	NQ	
¹⁴⁰ Ce	88.48	NQ	0.0009	
¹³⁷ Ba	11.32	2.3	2.7	
¹³⁸ Ba	71.66	2.4	2.7	
¹⁵¹ Eu	47.82	NQ	0.0009	
²⁰⁸ Pb	52.3	NQ	0.006	

included in subsequent analyses because of instrument drift. In general, heavy elements were more easily quantified than were light elements; the ICPMS was not well suited for accurate measurements of the light elements such as Ca, S, Cl, K, and Mg known to be present at relatively high levels in the otolith (Fig. 2). However, the majority of elements detected were found to be present at the ppm or parts-per-billion (ppb) range, well below the detection limit of the electron microprobe (Fig. 2).

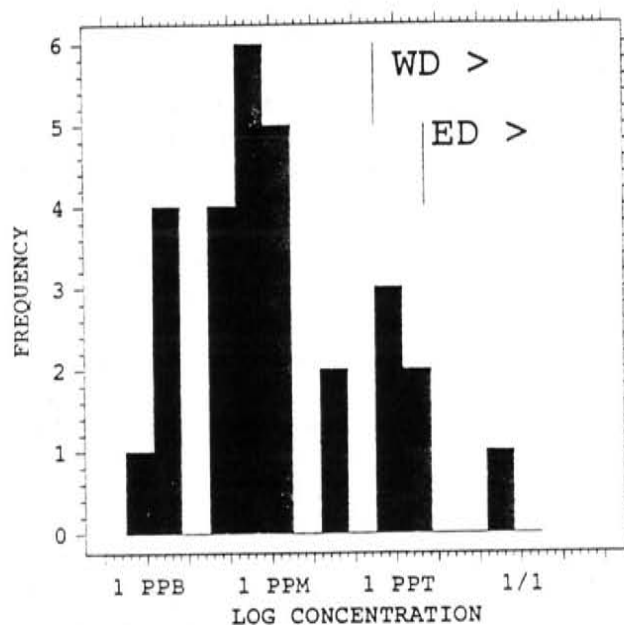


Fig. 2. Frequency distribution of elemental concentrations reported to be present in otoliths of various fish species, as drawn from this study and the literature. Most of the elements below 100 ppm could be quantified with ICPMS but were not detectable with an electron microprobe. WD = lower detection limit of a wavelength-dispersive electron microprobe; ED = lower detection limit of an energy-dispersive electron microprobe.

The analytical reproducibility of the ICPMS assays was tested through comparisons between otoliths of the same type within the same fish. In a series of nested ANOVAs of elemental concentrations by fish and sagitta (left vs right) in the 4Vs sample, the percentage of the explained variance associated with the fish was usually higher than that associated with the individual sagitta. All isotopes other than ^{111}Cd and ^{208}Pb had more than 30% of the explained variance associated with the fish, and elements such as Rb, Ba, Rh, Ca, and Sr exceeded 70%. The analytical reproducibility of the isotope ratios (e.g., ^{95}Mo : ^{98}Mo) was not nearly so good, and, with the exception of the Zn and Sr isotope ratios, was never more than 9%. Similar analyses for the lapillar pair produced comparable trends but with lower reproducibility. The greater reproducibility of the

sagittal assays compared to lapillar assays probably reflected both the difference in solution concentrations between sagittae and lapilli and the lower weighing precision possible with the lapilli (lapillus mean weight ± 1 sd = 1.45 ± 0.38 mg vs sagitta mean weight = 311.5 ± 92.5 mg).

Comparison of elemental concentrations (ppm) between sagittae and lapilli of the same fish revealed comparable patterns but at levels that were often significantly different. Concentrations of ^{66}Zn , As, Y, Ba, Sr, Ca, ^{86}Sr : ^{87}Sr , ^{87}Sr : ^{88}Sr , and ^{137}Ba : ^{138}Ba differed significantly between otolith types, while ^{68}Zn , Rb, Rh, ^{66}Zn : ^{68}Zn , and ^{86}Sr : ^{88}Sr did not. Elemental concentration differences between otolith types was generally on the order of only a few percent, although Sr levels were 30-50% higher in the lapilli than in the sagittae, and Ca levels were about 20% higher in the sagittae. The differences between sagittal and lapillar concentrations were inconsistent in the sense that no one otolith type had consistently greater elemental concentrations. It was also difficult to determine if the differences were real, reflecting different elemental fingerprints between otolith types, or an artifact of differing solution concentrations (and detection limits) and instrument baseline conditions. The latter factors probably explain why certain isotopes were quantifiable in one otolith type but not in the other (Table 2).

There were significant differences in the concentrations (ppm) of all isotopes across sample sites (ANOVA, $p < 0.05$). Despite the statistical significance, many of the intersample differences were relatively small: typically less than 50% (Tables 3 and 4). Many of the isotope ratios also differed significantly among sites.

Given the differences in fish size among sample sites, comparisons of elemental concentrations across sample sites were only valid if otolith composition was independent of fish size. Regressions of within-sample elemental concentrations (ppm) against otolith weight indicated that some elements varied significantly with fish size. Examination of the regression residuals did not reveal the curvilinearity that would be expected of differences in elemental concentration by otolith weight. However, since all samples were restricted to adult fish, it was impossible to determine if smaller fish incorporated various elements at different rates than did larger fish or, alternatively, if the high calcium concentration in the otolith solutions made the ICPMS quantifications nonlinear at low concentrations. Either cause could have induced spurious results in the intersample comparisons that follow. Accordingly, the effect of otolith weight was removed from all solution concentration data ($\mu\text{g l}^{-1}$) with an analysis of covariance (ANCOVA) approach: using sample site as a factor, the common within-group slope of otolith weight was removed from each of the elemental solution concentrations (Clayton and MacCrimmon 1987). The result was data that were completely detrended of otolith weight effects, from whatever cause.

Analysis of both the weight-detrended and the concentration per otolith weight (ppm) data demonstrated that age (year-class) and sex effects were weak, if present at all. Two-way analyses of variance of elemental concentration, isotope ratios, otolith weight, fish length, and age by sample site and sex all showed sample site to be significant ($p < 0.05$), and sex to be nonsignificant ($p > 0.05$). Both sagittae and lapilli were similar in this respect. Similar two-way ANOVAs by sample site and age revealed a declining but nonsignificant trend in sagittal ^{75}As with age, and a significant increasing trend in sagittal ^{89}Y with age. Among lapilli, both ^{85}Rb and ^{87}Sr : ^{88}Sr differed significantly among ages, but no trend was apparent. Given the number of elements and otolith types analyzed for age effects, the few significant age effects that were observed were probably spurious.

Table 3. Mean lapillar elemental concentrations (ppm in the otolith), isotope ratios, and otolith weight by sample site. Sample standard deviations are presented in brackets. Isotope values actually reflect the concentrations of the corresponding element; true isotope concentrations must be adjusted to the proportional abundance levels presented in Table 2, although isotope ratios have already been so adjusted.

Element/Isotope	Sample Site					
	Cheticamp	4Vs	Fundyrip	Georges Bank	Iceland	Newfoundland
⁶⁶ Zn	1.08 (0.82)	0.24 (0.50)	0.81 (0.34)	0.94 (0.44)	1.27 (0.87)	0.49 (0.21)
⁶⁸ Zn	1.13 (0.85)	0.26 (0.55)	0.91 (0.33)	1.02 (0.43)	1.25 (0.85)	0.54 (0.24)
⁷⁵ As	0.12 (0.03)	0.09 (0.04)	0.11 (0.04)	0.14 (0.06)	0.10 (0.03)	0.11 (0.04)
⁸⁵ Rb	0.22 (0.03)	0.14 (0.05)	0.16 (0.02)	0.20 (0.02)	0.22 (0.03)	0.21 (0.03)
⁸⁹ Y	0.027 (0.005)	0.028 (0.009)	0.019 (0.004)	0.021 (0.004)	0.028 (0.005)	0.028 (0.006)
¹⁰³ Rh	0.17 (0.02)	0.16 (0.04)	0.13 (0.02)	0.14 (0.02)	0.14 (0.02)	0.16 (0.02)
¹³⁷ Ba	2.33 (0.49)	2.13 (1.08)	3.26 (1.08)	2.41 (0.51)	2.29 (0.66)	2.72 (0.66)
¹³⁸ Ba	2.35 (0.49)	2.40 (1.09)	3.27 (1.07)	2.41 (0.50)	2.47 (0.68)	2.93 (0.69)
⁶³ Cu: ⁶⁵ Cu	0.94 (0.36)	0.48 (0.39)	1.25 (0.30)	1.24 (0.36)	1.10 (0.34)	1.48 (0.34)
⁶⁶ Zn: ⁶⁸ Zn	1.45 (0.20)	1.22 (0.19)	1.33 (0.17)	1.36 (0.15)	1.43 (0.11)	1.30 (0.14)
¹³⁷ Ba: ¹³⁸ Ba	0.156 (0.005)	0.154 (0.006)	0.157 (0.004)	0.158 (0.004)	0.155 (0.004)	0.154 (0.004)
⁸⁶ Sr: ⁸⁷ Sr	1.42 (0.02)	1.42 (0.01)	1.42 (0.02)	1.42 (0.02)	1.40 (0.01)	1.41 (0.01)
⁸⁷ Sr: ⁸⁸ Sr	0.084 (0.001)	0.084 (0.001)	0.083 (0.001)	0.084 (0.001)	0.083 (0.001)	0.084 (0.001)
Lapillus wt. (mg)	1.47 (0.26)	1.13 (0.33)	1.36 (0.32)	1.68 (0.33)	1.75 (0.26)	1.62 (0.26)

Table 4. Mean sagittal elemental concentrations (ppm in the otolith), isotope ratios and otolith weight by sample site. Sample standard deviations are presented in parentheses. Isotope values actually reflect the concentration of the corresponding element; true isotope concentrations must be adjusted to the proportional abundance levels presented in Table 2, although isotope ratios have already been so adjusted.

Element/Isotope	Sample Site	
	4RS	4Vs
^{48}Ca	4.14×10^5 (3.5×10^4)	4.13×10^5 (3.2×10^4)
^{66}Zn	0.44 (0.37)	0.59 (0.32)
^{68}Zn	0.32 (0.41)	0.23 (0.33)
^{71}Ga	0.0035 (0.0042)	0.0053 (0.0071)
^{75}As	0.049 (0.019)	0.079 (0.024)
^{79}Br	1.70 (1.67)	2.18 (0.94)
^{86}Sr	3148 (655)	2600 (270)
^{87}Sr	3160 (659)	2630 (271)
^{88}Sr	3094 (632)	2606 (262)
^{89}Y	0.055 (0.011)	0.047 (0.006)
^{85}Rb	0.21 (0.06)	0.13 (0.03)
^{99}Ru	0.0039 (0.0067)	0.0061 (0.0086)
^{103}Rh	0.19 (0.03)	0.16 (0.02)
^{111}Cd	0.0027 (0.0049)	0.0063 (0.0061)
^{137}Ba	3.14 (0.74)	2.72 (0.77)
^{138}Ba	3.16 (0.75)	2.74 (0.79)
^{140}Ce	0.00069 (0.00082)	0.00110 (0.00156)
^{151}Eu	0.0011 (0.0014)	0.0013 (0.0023)
^{208}Pb	0.014 (0.017)	0.008 (0.011)
$^{66}\text{Zn} : ^{68}\text{Zn}$	1.78 (4.99)	0.68 (11.67)
$^{86}\text{Sr} : ^{87}\text{Sr}$	1.399 (0.021)	1.389 (0.024)
$^{87}\text{Sr} : ^{88}\text{Sr}$	0.0868 (0.0010)	0.0858 (0.0011)
$^{137}\text{Ba} : ^{138}\text{Ba}$	0.157 (0.003)	0.157 (0.004)
Sagitta wt. (mg)	360.2 (96.0)	287.4 (90.0)

DISCRIMINANT ANALYSES

Stepwise discriminant analyses of the weight-detrended sagittal elemental concentration data demonstrated consistent and highly significant differences between sample sites ($p < 0.01$). The stepwise variable selection included only six of the 23 possible isotopes and isotope ratios, with ^{75}As and ^{85}Rb of primary importance (Table 5). None of the variables selected were isotope ratios. However, discriminant analyses based only on isotope ratios also produced highly significant sample separation. Analyses that excluded each of the variables in turn also produced significant results, suggesting robust discrimination. Using a cross-validation procedure (the "leave one out" procedure of SAS, akin to jackknifing) to produce an unbiased view of classification success, classification accuracy of the two samples was found to vary between 90% and 98% (Fig. 3). A frequency histogram of the individual discriminant scores confirmed the nearly complete separation of the two samples (Fig. 4).

Discriminant analysis of the six lapillus samples also resulted in highly significant sample discrimination. The stepwise variable selection included 11 of 13 possible variables, and included both elements and isotope ratios (Table 6). The list of selected variables included all those that were also selected in the analysis of the sagittae. Discriminant analyses that excluded isotope ratios were significant, as were analyses of isotope ratios alone. Cross-validated classification accuracies averaged 83%, with most of the errors associated with the discrimination of the Georges Bank and adjacent Fundyrip samples (Fig. 5). Scatterplots of the first and second discriminant function scores revealed two distinct aggregations of samples: Fundyrip, Georges, and Cheticamp in one, and Newfoundland, Iceland, and 4Vs in the other (Fig. 6). While not presented, scatterplots of the two remaining sets of discriminant function scores served to more completely separate the sample sites within each aggregation.

Table 5. Standardized function coefficients from the stepwise discriminant analysis of the weight-detrended sagittal fingerprints. Elements are listed in order of entry into the analysis.

Variable	Coefficient
^{75}As	-0.449
^{85}Rb	1.442
^{79}Br	-0.941
^{208}Pb	0.381
^{48}Ca	0.477
^{89}Y	-0.629

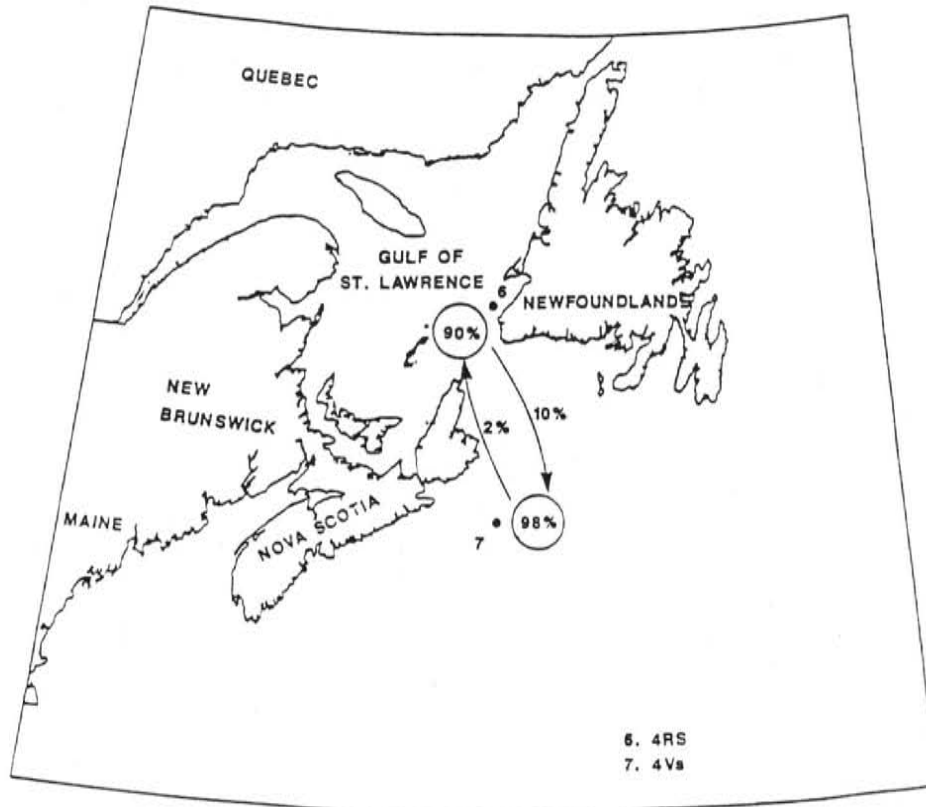


Fig. 3. Cross-validated (jackknifed) classification accuracy of the 4Vs (Scotian Shelf) and 4RS (Gulf of St. Lawrence) sagittal elemental fingerprints using the six-element discriminant function presented in Table 5. Arrows show the percentage of fish misclassified to other regions.

Discussion

Elemental fingerprinting of otoliths served not only to distinguish among cod from different geographic sites, but also to differentiate among cod populations with an accuracy that has seldom been possible in the past. 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

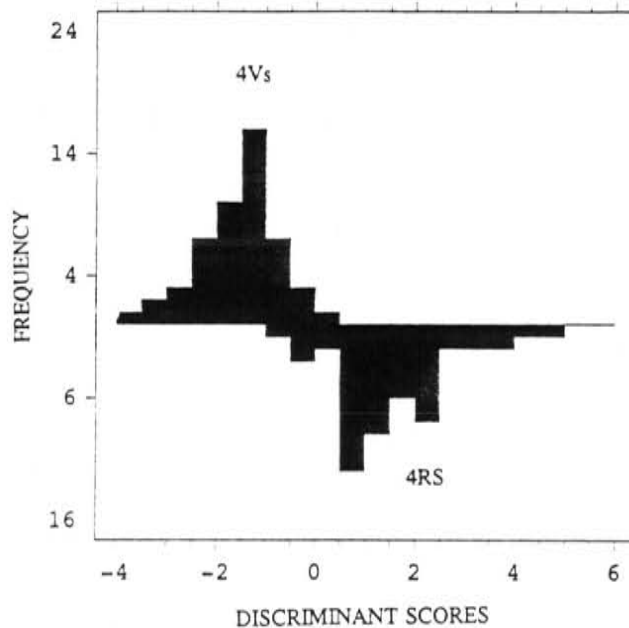


Fig. 4. Frequency histogram of the discriminant scores of the 4Vs (Scotian Shelf) and 4RS (Gulf of St. Lawrence) sagittae using the six-element discriminant function presented in Table 5.

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 suggest that otolith elemental fingerprints may provide the most rapid and accurate means of stock identification available for multiple cod stocks, at least in the case of stocks with largely discrete home ranges.

Otolith elemental fingerprinting of other species has also been successful in differentiating among samples from different sites. Edmonds et al. (1989, 1991, 1992) reported site-specific differentiation of a number of different Australian fishes. Site-specific elemental concentrations were also reported by Grady et al. (1989), Gunn et al. (1992), Secor (1992), Sie and Thresher (1992), and Mulligan et al. (1987), although the validity of the latter 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.

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

Table 6. Standardized function coefficients from the stepwise discriminant analysis of the weight-detrended lapillar elemental fingerprints. Elements are listed in order of entry into the analysis.

Variable	Discriminant Function Coefficients		
	1	2	3
$^{63}\text{Cu}:$ ^{65}Cu	0.112	0.254	-0.577
^{85}Rb	-0.111	1.005	0.469
^{103}Rh	-0.009	-0.824	-0.039
^{89}Y	0.238	-0.081	0.228
^{66}Zn	-0.173	0.374	0.395
$^{86}\text{Sr}:$ ^{87}Sr	0.005	-0.373	0.209
$^{87}\text{Sr}:$ ^{88}Sr	0.096	-0.208	0.393
^{137}Ba	-28.277	-2.453	1.520
^{138}Ba	28.042	2.565	-1.943
$^{137}\text{Ba}:$ ^{138}Ba	2.585	0.341	-0.138
^{75}As	-0.181	-0.047	-0.070
% of variance	72.2	16.2	6.9

deposition. As a result, the temporal stability of the elemental concentrations in non-otolith tissues is questionable. On the other hand, the acellular otolith is metabolically static (Campana and Neilson 1985) and is not exposed to ambient water, making it an ideal storage site for trace elements after incorporation.

ICPMS offers numerous advantages, and some disadvantages, over competing instrumentation used for developing an elemental fingerprint. The primary advantages are those of speed and the simultaneous determination of both isotopic and elemental concentrations, with a sensitivity that is currently unmatched by other techniques. Detection limits of about 5,000 ppm and 300 ppm for energy-dispersive and wavelength-dispersive electron microprobes, respectively, (Gunn et al. 1992) greatly exceed the limits possible with X-ray fluorescence (3 ppm, Kalish 1990), the proton microprobe (1 ppm, Ishikawa et al. 1987), neutron activation analysis (NAA) (200 ppb, Bortolotti and Barlow 1985), inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (100 ppb, Edmonds et al. 1991), and atomic absorption spectroscopy (AAS) (50 ppb, Grady et al. 1989). By

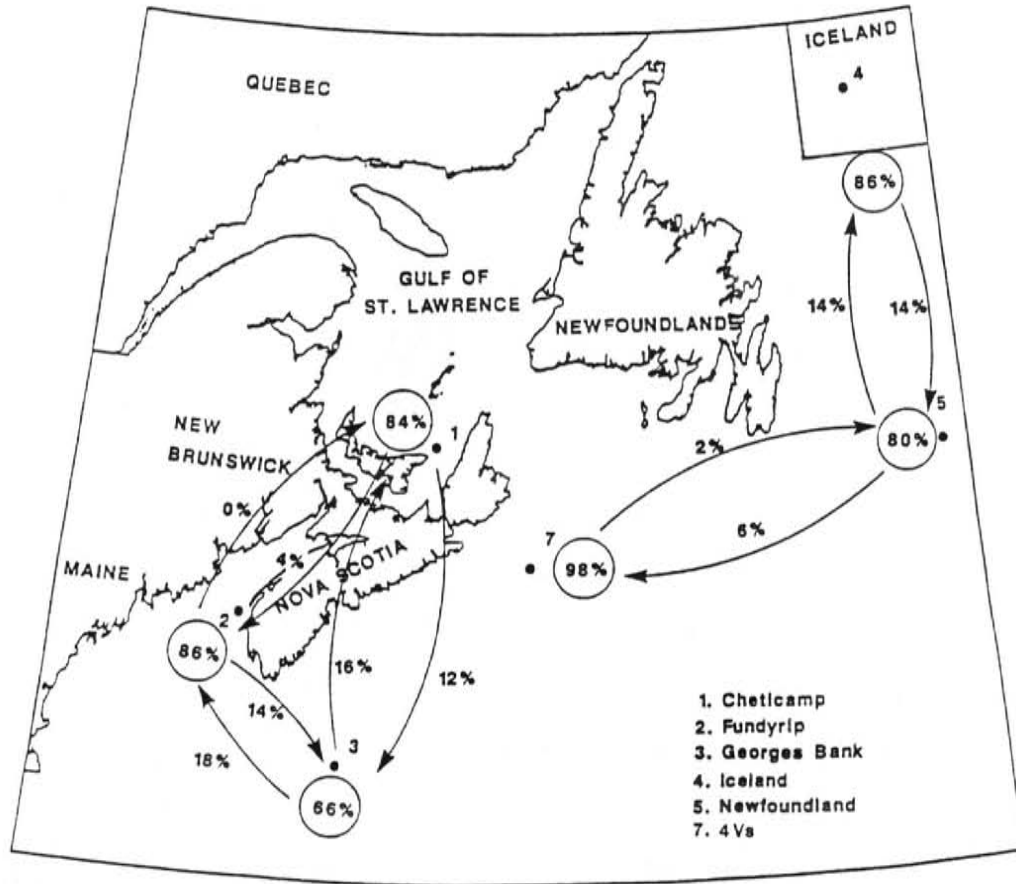


Fig. 5. Cross-validated (jackknifed) classification accuracy of lapillar elemental fingerprints from six sample sites using the discriminant functions presented in Table 6. Arrows show the percentage of fish misclassified to other regions.

contrast, the minimum detection limit of ICPMS for some elements in this study was 0.1 ppb, with limits of 0.03 ppb or less having been reported elsewhere (Houk 1986). Disadvantages of ICPMS include the inability to accurately measure the abundance of the lighter, relatively abundant elements, molecular interferences with certain isotopes, and the potential for instrument drift due to calcium buildup during sequential assays. These constraints could be overcome by conducting ICPMS assays in concert with those of a complementary technique (e.g., AAS or ICP-AES), and by ensuring that the order of sample assays is random with respect to sample site. Note that the ICPMS used in this study was not capable of spatial analysis as in an electron microprobe, although laser-ablation ICPMS units are capable of such analyses (Coutant 1990; Campana unpublished data).

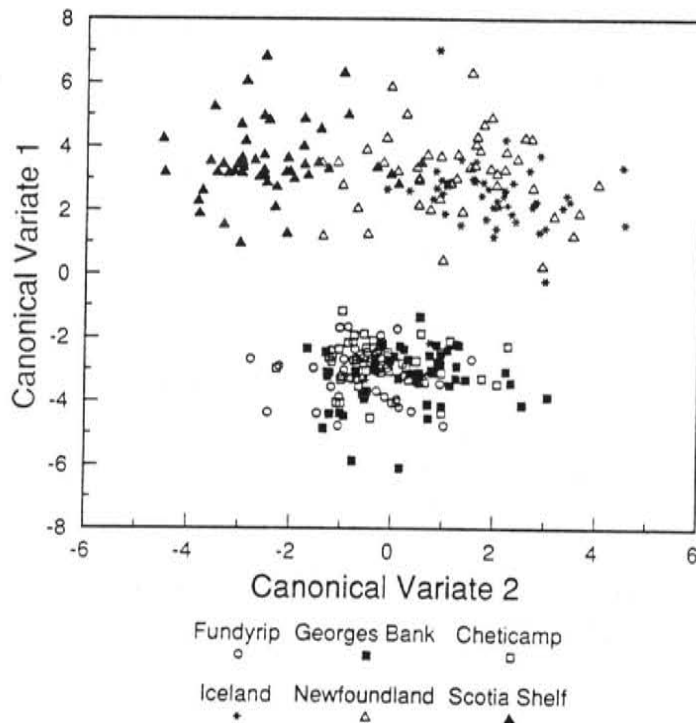


Fig. 6. Scatterplot of the discriminant scores from the six lapillar samples across the first two canonical axes.

Given the use of otolith elemental fingerprints in this study and elsewhere, it is somewhat paradoxical to note that the otolith is actually a very pure combination of protein and calcium carbonate. As demonstrated by Fig. 2, most of the elements reported to be present in otoliths are present at concentrations well below 50 ppm. However, even the otolith elements present at levels of <1 ppm are among the more common in marine waters (Johnson et al. 1992). As such, it is highly probable that numerous other elements will be detected in otoliths at levels <1 ppb as analytical techniques are further improved.

While one might expect the elemental fingerprints of lapilli to be identical to those of sagittae from the same fish, it is unlikely that the many significant differences noted between otolith types in this study were artifacts or errors. At least some of the differences were due to different solution concentrations and instrument conditions between otolith types. However, lapilli and sagittae grow at very different rates in cod, despite the fact they are of similar size at the time of hatch (Campana 1989). Further, the differential in their growth rates varies with age, such that lapillus composition better reflects larval and juvenile life than does sagittal composition, which is a better indicator of adult life. Given that incorporation of trace elements into calcium carbonate is, at least in part, a function of crystallization rate (Lowenstam and Weiner 1989), it is not surprising that the two structures record their respective histories from different perspectives. Comparisons of oxygen isotope concentrations (Meyer-Rochow et al. 1992) and shape (Campana and Casselman

1993) between sagittae and lapilli of the same fish also demonstrated differences due to the relative growth rates of the two otoliths.

One of the most interesting discoveries in this study centered on the significant differences in isotope ratios among sample sites. Isotope ratios of elements such as carbon, nitrogen, and sulfur have been used to monitor trophic pathways (Schoeninger and DeNiro 1984; Peterson et al. 1985), but are under too much physiological regulation to serve as useful stock discriminators. In contrast, isotope ratios of elements such as strontium reflect both surficial geology (Graustein 1988) and salinity (Schmitz et al. 1991), and are unlikely to be subject to physiological discrimination within the fish. As such, they are logical candidates for stock discrimination. We are not aware of any other studies that have attempted to use isotope ratios to differentiate among populations of aquatic organisms, although strontium isotope ratios have been used successfully to identify the geographic source of elephant ivory (Vogel et al. 1990). Isotope ratios of elements such as Ba, Cu, and Zn have never even been reported in animals, in part because isotope quantification is not possible with traditional analytical techniques such as AAS, NAA, X-ray fluorescence, and the various microprobes. Given that the ICPMS can be operated in an isotope ratio mode with a precision that is far greater than that reported here, and given that relatively high strontium levels occur in otoliths, we suspect that further work on strontium isotope ratios may substantially improve our ability to differentiate among stocks. This work is particularly important given the probability that instrument drift contributed, at least in part, to our sample-specific differences. Isobaric interferences from isotopes such as ^{87}Rb should also be quantified, although they would be unlikely to alter relative differences among samples.

Aside from continued research on isotope ratios, 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 unexpectedly complete separation of the 4Vs sample from the other samples was not entirely consistent with expectations based on water circulation patterns and stock structure, suggesting that other unknowns may have contributed to the discreteness of the fingerprint. Since the fish in the 4Vs sample were somewhat smaller than the others, size may have played a role, despite the statistical removal of fish-size (otolith-weight) effects. Other possibilities include the date of sample collection, although the fingerprints did not differ among ages within a sample, and the confounding of instrument drift effects with the sequence of sample analysis. In future work, we recommend that these factors be evaluated, and that care be taken to minimize extraneous effects (e.g., randomize the sequence of otolith assays so as not to confound instrument drift with site effects). In particular, we urge caution with respect to potential sources of sample contamination; otolith analytical capabilities have reached the point where airborne dust, the use of metal forceps, and the use of other than trace-metal grades of solvents can overwhelm (or artificially create) the elemental fingerprints we seek to measure.

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