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Otolith elemental fingerprints as biological tracers of fish stocks

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Abstract

Specific trace elements 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 which 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. On the basis of isotope dilution ICPMS (ID-ICPMS) assays of nearly 2500 dissolved adult cod (*Gadus morhua*) otoliths, it has become clear that cod otolith elemental fingerprints based on the elements Li, Mg, Mn, Sr and Ba are physically stable, reproducible and consistent between left and right otoliths. Highly significant differences existed among the fingerprints of all of the spawning aggregations, resulting in a characteristic marker for each aggregation. Long-term stability (4–13 years) of the fingerprints for a given spawning group was not evident, indicating that the fingerprint was not a proxy for genetic identity. However, the fingerprint was very stable over the short-term (up to 1 year), suggesting that it could serve as a seasonally stable biological tracer, or natural tag, of pre-defined groups of fish, even during situations of extensive stock mixing. As an illustration of the tracer approach, a maximum likelihood-based stock mixture analysis was applied to feeding (summer) and over-wintering stock distributions, using the fingerprints of the spring spawning aggregations as known-stock reference samples. The results of the summer stock mixture analyses suggested that the mixture analysis was accurate within 1%, while the stock mixture analysis of the over-wintering schools produced stock-specific distributions which would have been difficult to obtain using alternative approaches. While the use of elemental fingerprints as natural tags is not suited to all stock mixing situations, suitability can probably be determined beforehand on the basis of existing environmental and biological information.

Keywords: Otolith; Trace element; Composition; Stock identification; Tag; Cod; *Gadus morhua*

1. Introduction

Recent years have seen a rapid growth in interest in the elemental composition of fish otoliths as a means for distinguishing among populations or stocks of fish (Edmonds et al., 1989; Kalish, 1990; Thresher et al., 1994; Campana and Gagné, 1995; Severin et al., 1995). The potential is based in large part on two key properties of the otolith: (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)

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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). Similar effects of environmental availability and temperature apply to isotopic ratios of elements such as strontium (Kennedy et al., 1997) and oxygen (Thorrold et al., 1997). Such environmental responses, recorded permanently in the otolith, imply that the concentration of a suite of selected elements and isotopes (the elemental fingerprint) can be used to discriminate among groups of fish which have spent at least part of their lives in different environments. And while there are probe techniques which can be used to analyze discrete regions of the otolith (Campana et al., 1997b), dissolution and analysis of the whole otolith is preferable when using the elemental fingerprint as a group marker, due to the availability of sensitive and accurate assay protocols (Catterick et al., 1995).

There is no a priori reason why the elemental fingerprint should serve as a proxy for population identity, since genetic differences are not implied. Nor there is any reason to expect the whole otolith fingerprint of a fish to remain stable over a long period of time, any more than the environment at a given location would remain stable. However, since the otolith grows continuously throughout a fish's life, the elemental fingerprint of the whole dissolved otolith integrates across the entire lifetime of the fish, and thus can be used to distinguish among fish which have experienced different overall environmental exposures (Edmonds et al., 1989, 1991, 1992, 1995; Northcote et al., 1992; Campana and Gagné, 1995; Campana et al., 1995; Bronte et al., 1996; Gillanders and Kingsford, 1996; Edmonds and Fletcher, 1997; Kennedy et al., 1997; Thorrold et al., 1998). To the extent that populations or stocks of fish inhabit different environments, otolith elemental composition might then serve as an indicator of stock identity. However, a more robust application of whole otolith fingerprints might be one which is targeted at questions of stock mixing or for tracking stock migrations, in which the fingerprints are used as natural tags, or biological tracers, of pre-defined groups of fish over short periods of time (Campana et al., 1995; Gillanders and Kingsford, 1996; Kennedy et al., 1997). Application of an elemental fingerprint as a natural tag would take advantage of the fact that newly added otolith material makes up only a small proportion of the total otolith mass. As a result, otolith growth and environmental shifts during the (short) period of mixing or migration could be expected to have a negligible effect on the fingerprint. In principle, as long as all potential source groups are characterized shortly before the time of mixing/migration, fish should remain identifiable as to their source group until the elemental composition of later otolith growth has significantly altered overall elemental composition.

In this paper, we demonstrate that the knowledge of otolith elemental composition and its associated technology has matured to the point where elemental fingerprints can be confidently applied as biological tracers, or natural tags, in a variety of stock mixing and migration situations. We begin by presenting the evidence in support of the assumptions underlying the stability and stock-specificity of otolith elemental fingerprints. We conclude by illustrating the biological tracer approach in two reconstructions of Atlantic cod (*Gadus morhua*) stock movements across seasons, including a stock mixture analysis of mixed cod stocks which would have been difficult to carry out using more traditional methods.

2. Materials and methods

2.1. Sample collection

To characterize the otolith elemental fingerprint of each of the major cod stocks in and around the Gulf of St. Lawrence, samples of adult cod (35–85 cm) in spawning or near-spawning condition were collected in Spring 1996 and 1997 from each of the known spawning grounds: the southern Gulf of St. Lawrence (NAFO Division 4T), both in the southwest (Shediac Valley) and southeast (off Cheticamp); the northern Gulf of St. Lawrence (NAFO Division 3Pn4RS), primarily near the mouth of St. George's Bay; southern Newfoundland (NF) (NAFO Division 3Ps), both offshore on St. Pierre Bank (in 1996), and inshore in Fortune and Placentia Bay (in 1997), and the Scotian Shelf (NAFO Division 4Vs), both to the east (the Gully) and to the north (Banquereau Bank) (Table 1; Fig. 1). Each spawning ground was sampled independently at least twice in any given year, and most

Table 1
Summary of cod samples collected by area and season[a]

Area	Season	Date	*N*	Length (cm)		Otolith (g)	
				Mean	S.E.	Mean	S.E.
North Gulf	Fall 1995	10 October–2 November	113	50.6	0.9	0.34	0.01
	Spring 1996	13–30 April	155	56.5	0.9	0.44	0.01
	Spring 1997	6–30 April	134	52.9	0.9	0.39	0.01
S. NF-offshore	Spring 1996	27–29 April	70	61.4	1.0	0.45	0.01
S. NF-inshore (Fortune)	Spring 1997	19 June	42	64.6	2.4	0.57	0.03
S. NF-inshore (Placentia)	Spring 1997	26 June	61	57.3	0.8	0.42	0.01
South Gulf	Fall 1995	15 September	217	43.8	0.3	0.26	0.00
	Spring 1996	13–15 June	200	52.1	0.6	0.39	0.01
	Spring 1997	11–13 June	200	53.4	0.4	0.41	0.01
Eastern Shelf (Gully)	Spring 1996	1 May	99	53.0	0.6	0.38	0.01
	Spring 1997	2 May	81	41.3	0.5	0.22	0.01
North Shelf (Banquereau)	Spring 1997	1 May	69	42.8	0.9	0.24	0.01
Cabot Strait	Winter 1996	3–25 January	781	46.0	0.3	0.28	0.01

[a] Each area was sampled independently at least twice in any given season, and most areas were sampled in more than 1 year. All samples but those taken in Fall 1995 and Winter 1996 were collected from spawning grounds around the time of spawning. Sampling locations are shown in Fig. 1.

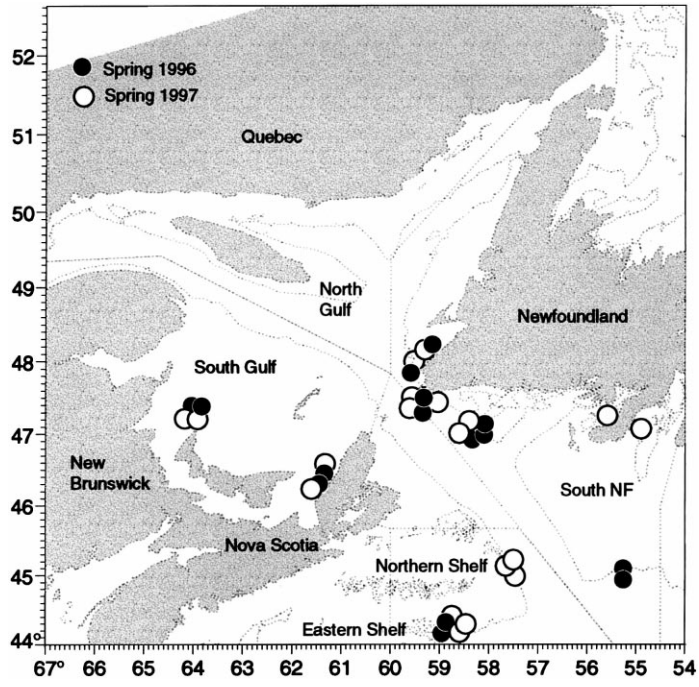

Fig. 1. Map of the study area showing the spawning areas sampled during the spring spawning season in 1996 and 1997. Cod from these samples comprised the reference collection, since they were of known stock affinity. Each circle represents a sample of about 50 adult cod (details in Table 1). The 200 m contour is also shown.

spawning grounds were sampled in both 1996 and 1997 (Fig. 1). A minimum of 100 cod was generally collected from each stock, for a total of 1111 fish. The sagittal otolith pair was removed from each fish immediately after capture. The otolith elemental fingerprints of these samples comprised our reference collection, since the stock identity of each of these fish was known with some confidence.

A second set of samples (n=420) was collected in Fall 1995 prior to any migration out of the Gulf of St. Lawrence (Table 1). These samples, restricted to the same size range as the spring samples, were collected so as to broadly represent the stock area of the northern Gulf and southern Gulf cod stocks (Fig. 1). The fall-collected fish were used both to verify the stability of the elemental fingerprints as a stock-specific marker across seasons (through comparison with the spring-collected samples), and to assess within-stock geographic variability in the elemental fingerprint.

A grid sampling design was used to survey the winter distribution of cod in and around the approaches to the Gulf and Cabot Strait area between 3 January and 25 January 1996. A total of 138 successful sets were made, of which 60 sets were sampled for otoliths (n=754) and other tissues. Unsampled sets were assumed to contain the same stock proportions as the nearest neighboring sets along a similar depth contour. After estimation of the set-by-set stock proportions (described later), stock-specific abundance was mapped and contoured with Delauney triangles using spatial analysis software (ACON).

2.2. Elemental analysis

Prior to elemental analysis, all otoliths were decontaminated using a modification of the protocol described in Fowler et al. (1995). Briefly, this involved a 5 min sonification in an acid-washed 50 ml polypropylene vial containing Super Q water (distilled, millipore-filtered, reverse osmosis water), followed by a 1 min scrubbing with an acid-washed toothbrush, triple rinsing in Super Q water, a 3 min sonification in Super Q, and a final triple rinse in Super Q. Decontaminated otoliths were air dried in acid-washed polypropylene caps under a Class 100 laminar flow, positive pressure fume hood for 24 h before weighing to the nearest 0.1 mg. Decontaminated otoliths were subsequently stored dry in sealed, acid-washed polypropylene vials to await assay. Blank vials were similarly prepared for blank corrections and calculations of limits of detection. At all stages of the decontamination process, otoliths were exposed only to acid-washed plastic materials, and all steps other than sonification were carried out in the laminar flow fume hood.

Decontaminated otoliths were dissolved in subboiling, redistilled nitric acid and brought to 0.1% (w/v) with Super Q water. A suite of six trace elements (Li, Mg, Mn, Sr, Ba, Zn) was assayed with inductively coupled plasma mass spectrometry (ICPMS), of which five were suitable for quantification by isotope dilution. Isotope dilution was the preferred method of quantification due to superior accuracy and precision in otolith assays (Campana et al., 1995). The enriched isotopes used in the spiking procedure were ^{6}Li, ^{25}Mg, ^{67}Zn, ^{86}Sr, and ^{135}Ba, while the remaining more abundant isotopes were used for quantification. Mn was not suited for ID analysis, and thus was referenced to an internal standard for assay by conventional ICPMS. The assay sequence was systematically randomized across samples to insure that instrument drift did not artifactually inflate the assay results of one sample site over another. ID assays were also carried out for Pb and B, but they proved to be close to detection limits and influenced by sample collection protocols, respectively.

2.3. Decontamination experiment

An independent experiment was carried out to determine the sensitivity of the otolith assay results to possible sources of contamination. The experimental design was based on matched pairs, in which one sagittal otolith of each pair was decontaminated to the maximum extent possible, while the remaining otolith was decontaminated to one of four lower levels. Aside from the experimental treatment, all otolith handling was carried out as described earlier. Otoliths for the experiment were obtained from International Observer Program collections of adult cod otoliths in the Gully area of the Scotian Shelf between January 1984 and September 1984 (n=80 pairs). Decontamination treatments were as follows:

Control sonified in Super Q water, scrubbed, acid-washed for 45 s in 1% trace metal grade nitric acid (resulting in an average weight

loss of 1%), rinsed, sonified/rinsed three times, dried

U unwashed; otoliths left as is

S sonified and rinsed, repeated three times; dried

SS sonified, scrubbed and rinsed; repeated three times; dried

ACID acid-washed for 45 s in 1% trace metal grade nitric acid, rinsed, sonified/rinsed three times, dried

Experimental otoliths were subsequently randomized with respect to assay sequence and analyzed for Li, Mg, Zn, Sr and Ba by ID-ICPMS as described earlier. Statistical analysis was through a matched pair design.

2.4. Quality control

Differences in otolith elemental composition among groups of fish may be statistically significant, but they will not necessarily be large. A previous study demonstrated that artifactual but significant differences among groups of otolith elemental assays were not uncommon (Campana et al., 1997b). Therefore, three protocols were adopted to minimize the possibility for artifactual differences: (1) use of ID-ICPMS, a variant of ICPMS often used as the standard in the development of certified reference materials (Fassett and Paulsen, 1989), (2) blocked randomization of the samples in the assay sequence, so as to eliminate any bias associated with instrument drift over time (Campana and Gagné, 1995), and (3) calibration of the two assay laboratories against a cod otolith reference powder, to insure that analytical differences between laboratories did not confound comparisons among their respective assays.

The otolith reference powder proved to be critical in allowing a proper integration of the assay results of the two independent laboratories. Prepared by grinding 30 adult cod otoliths in an acid-washed agate mortar to the consistency of talcum powder, the reference powder was thoroughly mixed and stored in 50 mg aliquots in separate acid-washed polypropylene vials. Samples of the reference powder were subsequently analyzed by the two laboratories at periodic intervals during the production assays of the whole otoliths. Comparison of the laboratory results demonstrated that small but significant differences existed between the laboratories in the assay of most elements (Table 2). While all of the stock mixture analyses employed otolith assays carried out by only a single laboratory, the long-term assessments of fingerprint stability required the integration of the two sets of assays. This inter-calibration was carried out using the assay results on the reference powder.

2.5. Statistical analysis

The within-group distributions of Mg and Li concentrations were skewed and thus were ln-transformed prior to statistical analysis. Each otolith was characterized by a suite of several elements, therefore, multivariate statistics were used to distinguish among samples. MANOVA was used to test for significant differences among samples, while discriminant analysis was used to prepare two-factor elemental fingerprints for illustrative purposes. Discriminant analysis was not used to classify the samples of unknown stock affinity (those collected in the Fall and Winter), since

Table 2
Quality control assay results for cod otolith reference powder analyzed by the ERI (1995–1996 samples) and RPC (1997 samples) laboratories[a]

	Ba		Li		Mg		Mn		Pb		Sr		Zn	
	ERI	RPC	ERI	RPC	ERI	RPC	ERI	RPC	ERI	RPC	ERI	RPC	ERI	RPC
Mean (μg/g)	4.68	4.40	1.04	0.57	18.4	15.6	1.89	2.16	–	0.01	3274	3093	7.3	1.1
CV (%)	6.5	5.2	28.8	2.4	7.0	1.4	14.8	3.5	–	117	2.6	0.8	77	63
LOD (μg/g)	0.03	0.03	0.1	0.02	0.1	0.3	0.1	0.03	–	0.03	10	0.3	1	0.2

[a] The reference powder was analyzed at periodic intervals through the course of carrying out the otolith assays (n=55 and n=6 for ERI and RPC, respectively). LOD: limit of detection.

it lacked sufficient discriminatory power. Rather, the stock composition analysis was carried out using a maximum likelihood-based analysis patterned after Millar (1990), an approach which other studies have shown to provide maximal discriminatory power in mixed stock situations (Wood et al., 1987). The reference fingerprints for all stock mixture analyses were the collections of spring spawners from the corresponding year, for which stock affinity was known.

3. Results

If the otolith elemental composition is to be a robust indicator of a fish's environmental history, it should not be oversensitive to fine details in sample collection or preparation. The decontamination experiment demonstrated that most of the elements examined were relatively insensitive to contamination from residual tissue on the otolith, and that the elements of interest were probably rigidly incorporated into the otolith matrix. Of the elements examined, Ba, Li and Sr did not vary significantly between treatments (ANOVA, $p>0.2$), including between the treatment extremes of completely unwashed otoliths and those that were scrubbed, sonified and acid-washed (Fig. 2). The magnitude of the differences was less than 5% (Fig. 2). Mg and Zn concentrations did vary significantly among treatments (ANOVA, $p<0.05$), with the unwashed and sonified-only treatments containing significantly more residual Mg than the acid-washed

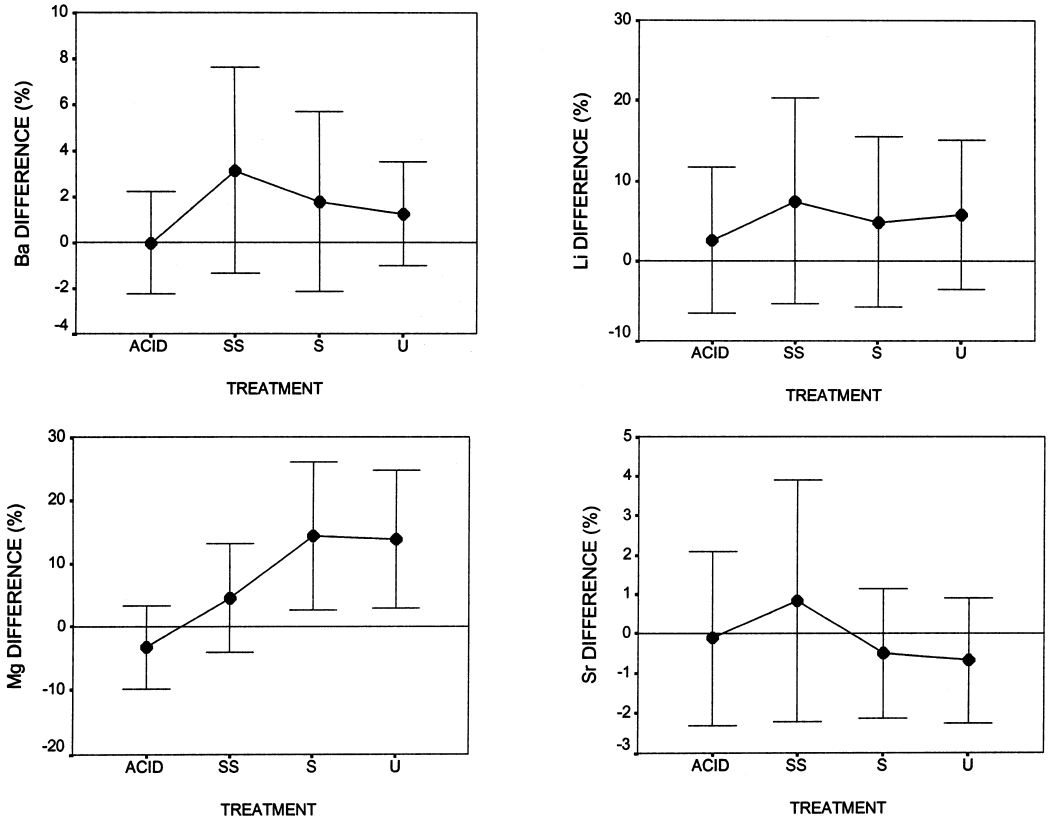

Fig. 2. Results of the decontamination experiment in which otolith pairs were split between a scrubbed, sonified, acid-washed control and a decontamination treatment of lesser degree. Data points represent the percentage difference between the control and the treatment, plus or minus the 95% confidence interval (CI). Mg was the only element of the four which differed significantly among treatments. ACID: acid-washed; SS: scrubbed and sonified; S: sonified; U: unwashed.

control. Zn concentration differed significantly among all of the treatments, and for this reason (in part), was not used subsequently in the development of the elemental fingerprints. There was no significant difference between the control and SS treatments for Li, Mg, Sr or Ba, indicating that the SS treatment applied routinely in this study did not leave significant quantities of unbound contaminants on or in the otolith.

The validity of the assumption that the otolith assay represented the fish, rather than just the individual otolith, was tested by correlating the elemental composition of a sample of matched left and right sagittae (n=60). Correlation coefficients for Ba (0.96), Li (0.95), Mg (0.71) and Sr (0.95) were all highly significant. The coefficient for Zn (0.43) was significant, but much less than that of the others. The low correlation between Zn concentrations in left and right otoliths, along with the sensitivity to decontamination protocols discussed earlier, prompted us to discontinue the analysis of Zn as a component of the otolith elemental fingerprint.

There was relatively little difference in mean fish length among the majority of samples, due largely to the fact that sampling was restricted to adults in the size range 35–80 cm (Table 1). However, the fish in the Spring 1997 samples from the Scotian Shelf were somewhat smaller than those in the other samples. While elemental concentrations were reported in terms of μg/g of otolith, both this study and previous studies have noted size-specific concentrations for some elements which could otherwise be confused for stock-specific differences (Fig. 3) (Campana et al., 1995; Edmonds et al., 1995). To insure that differences in fish length and/or otolith weight among samples did not confound any stock-specific differences in elemental composition, it was important to remove the effect of otolith weight from the statistical analysis. Only Mg, Mn and Sr varied significantly with otolith weight, with Mg and Mn varying negatively and Sr varying positively (ANCOVA, p<0.05). Subtraction of the common within-group linear slope from the ANCOVA was not appropriate in light of a curvilinear relationship for the smallest fish. However, an inverse relationship of the form [element]=b_0+(b_1/weight) fit the data rather well, whereby weight refers to otolith weight. Fitting this regression to the 1997 data for the southern Gulf (which had the broadest size range) resulted in the following parameter estimates:

$$\ln(\mathrm{Mg}): \quad b_1 = 0.0982, \qquad \mathrm{Mn}: \quad b_1 = 0.313,$$
$$\mathrm{Sr}: \quad b_1 = -137.5$$

Therefore, the effects of otolith weight were removed from all ln (Mg), Mn and Sr data by subtracting the term (b_1/otolith weight). Detrended

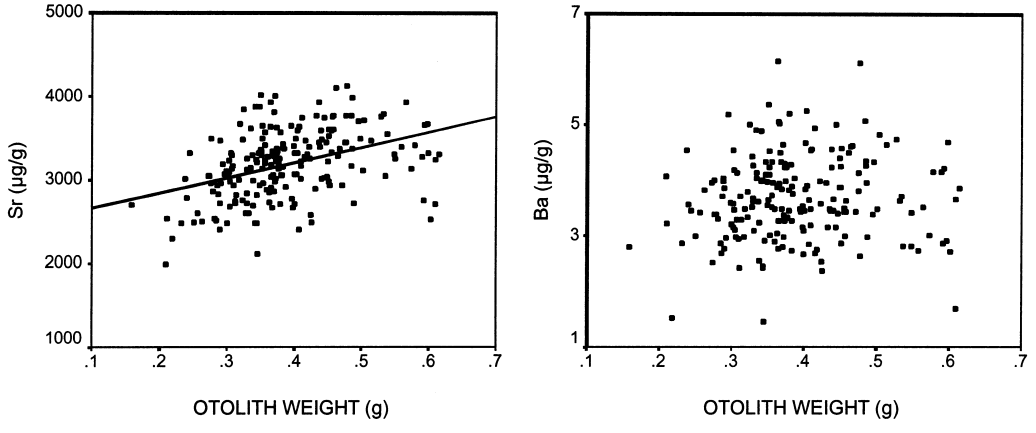

Fig. 3. Examples of the relationship between elemental concentration (μg/g) and otolith weight for Sr and Ba in cod collected from the southern Gulf in Spring 1996 and 1997. A significant positive relationship was noted for Sr, a significant negative relationship for Mn and Mg, and none for Li and Ba. To insure that variations in otolith weight among samples did not confound the interpretation of differences among areas, the effect of otolith weight was removed statistically from the Sr, Mn and Mg data.

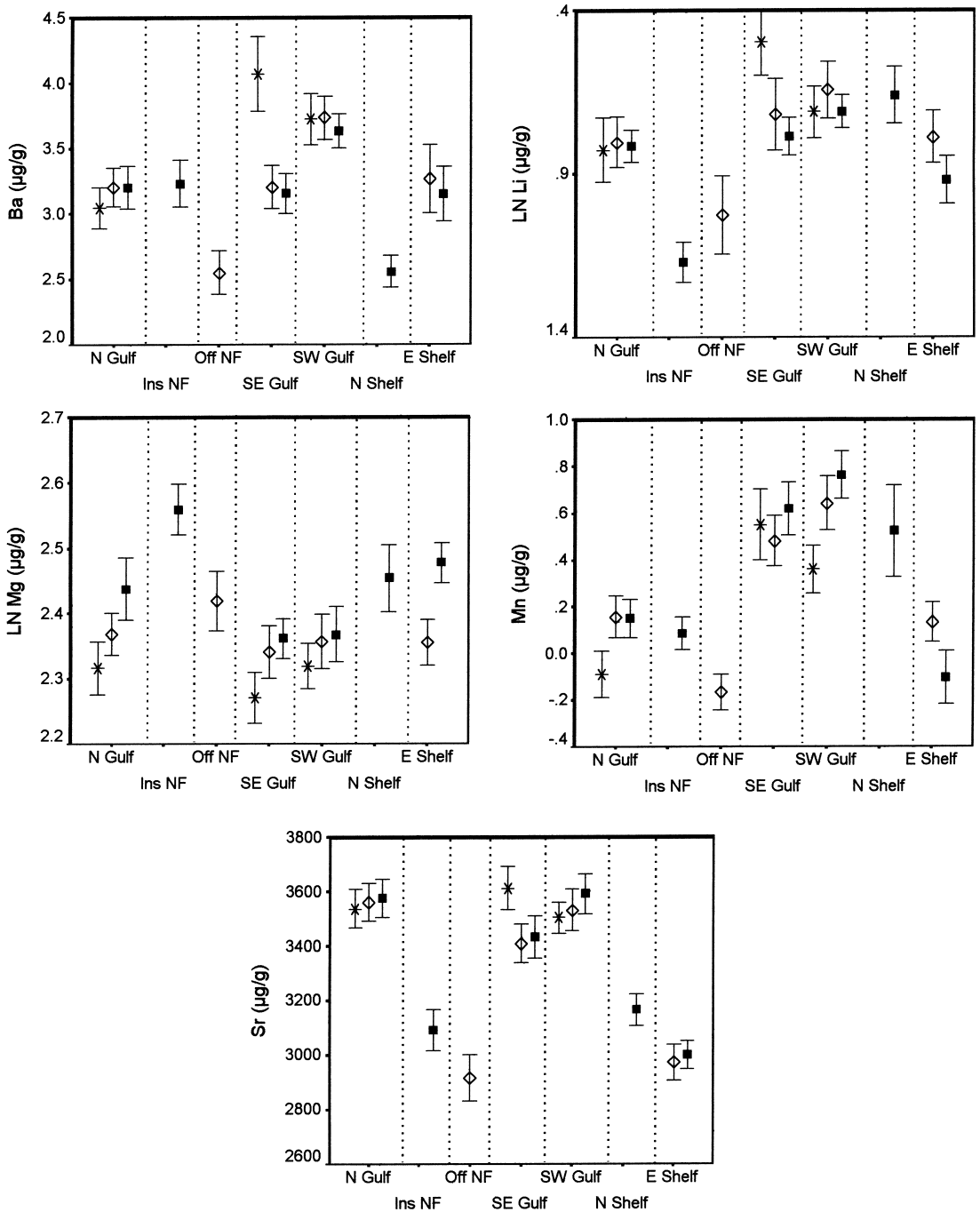

Fig. 4. Variation in mean (±95% CI) elemental concentration among locations and years. Samples collected in 1996 (open symbols) and 1997 (closed symbols) were targeted at spring spawning aggregations, while those in 1995 (star symbol) were collected over a broad geographic range in the fall. Concentrations for Mg, Mn and Sr have been weight-detrended (see text); however, the differences among locations other than the 1997 Shelf samples changed relatively little as a result of the detrending.

elemental concentrations showed no obvious residual relationship with otolith weight for most of the sample collections. In any event, relative differences among detrended samples tended to be very similar to those based on original data, with the exception of samples of very small fish.

Before an otolith elemental fingerprint can be applied as a biological tracer of stock mixing, it must be shown to differ among stocks or geographic locations. Analysis of the spring spawning aggregations indicated that the concentrations of each of the elements differed significantly and substantially among locations (Fig. 4). In general, elemental concentrations differed most between the inside and outside of the Gulf of St. Lawrence; this pattern was most apparent for elements such as Sr and Mg. Not all elements responded in parallel, however, with elements such as Ba and Mn showing marked differences between the north and south Gulf, and Li distinguishing inshore and offshore NF from other locations. Nor did spawning sites within a given cod stock necessarily share the same elemental concentration: small but significant differences in Ba concentration were observed between the southeast and southwest Gulf stock components, and most elements differed between the northern and eastern Scotian Shelf components of the NAFO Division 4VsW stock. On the other hand, elemental concentrations did not differ significantly between sites along the southwestern and western coast of NF, within the 3Pn4RS stock area.

Perhaps the most important criterion of a group-specific marker is its stability over the period of time between spawning and mixing with the other groups. With few exceptions, the concentrations of most elements remained remarkably stable over periods of 2–3 years (Fig. 4). This was particularly evident for the 1996 and 1997 samples of spring spawning aggregations. The pre-migratory Fall 1995 collections of northern and southern Gulf cod differed somewhat more for some elements, perhaps reflecting the broad geographic range of sampling. Even the eastern Shelf showed consistency between years for three of five elements, despite the large difference in fish size among years; the elements which differed most (Mn and Mg) were also those that displayed the greatest size-specific variation.

Long-term stability in elemental concentrations was generally not evident at locations resampled over periods as long as 13 years (Fig. 5). Concentrations differed relatively little over 2-year intervals. However, more substantive differences were noted for some elements and some locations after 4–13 year intervals. The length of the time interval was not necessarily an indication of the extent of the difference in elemental concentration, as demonstrated by the 13-year stability in Sr on the eastern Shelf. Nor did all elements at a given location necessarily change in tandem, e.g., Sr, Ba, and Li remained relatively constant in the SE Gulf over a 5-year interval, whereas Mg decreased significantly. Despite these changes over time, however, the relative difference in concentration between locations was often maintained for some (but not all) elements.

While it may be possible to use the concentration of any given element to distinguish between groups of fish, it is preferable to use all elements at once, e.g., a multivariate elemental fingerprint. Fig. 6 demonstrates the extent of the multivariate differences between each of the major spawning aggregations of cod, and at the same time, shows the variability among samples within any given group. The elemental fingerprint of each spawning aggregation was both discrete and significantly different from that of its neighbors, despite variability among samples within any given aggregation. Since the difference among 1996 aggregations was maintained in 1997, it is fair to characterize the elemental fingerprints as group-specific markers for those years.

The specificity of the otolith elemental fingerprint as a group-specific marker becomes more apparent when all samples from a given spawning aggregation are combined (Fig. 7). Differences among all five of the major cod spawning aggregations were highly significant ($p<0.01$), and were maintained between 1995 and 1997. Year-to-year differences in the fingerprint of the eastern Shelf component were somewhat larger than those of the other groups, but perhaps were not surprising, in light of the large difference in age and size composition between years.

As an illustration of the utility of a group-specific marker, the elemental fingerprints of the southern Gulf, northern Gulf, south NF and eastern Shelf cod spawning groups in 1996 were used as known stock reference groups in a maximum likelihood-based stock mixture analysis of pre-migratory cod distributed throughout the Gulf in Fall 1995. The stock

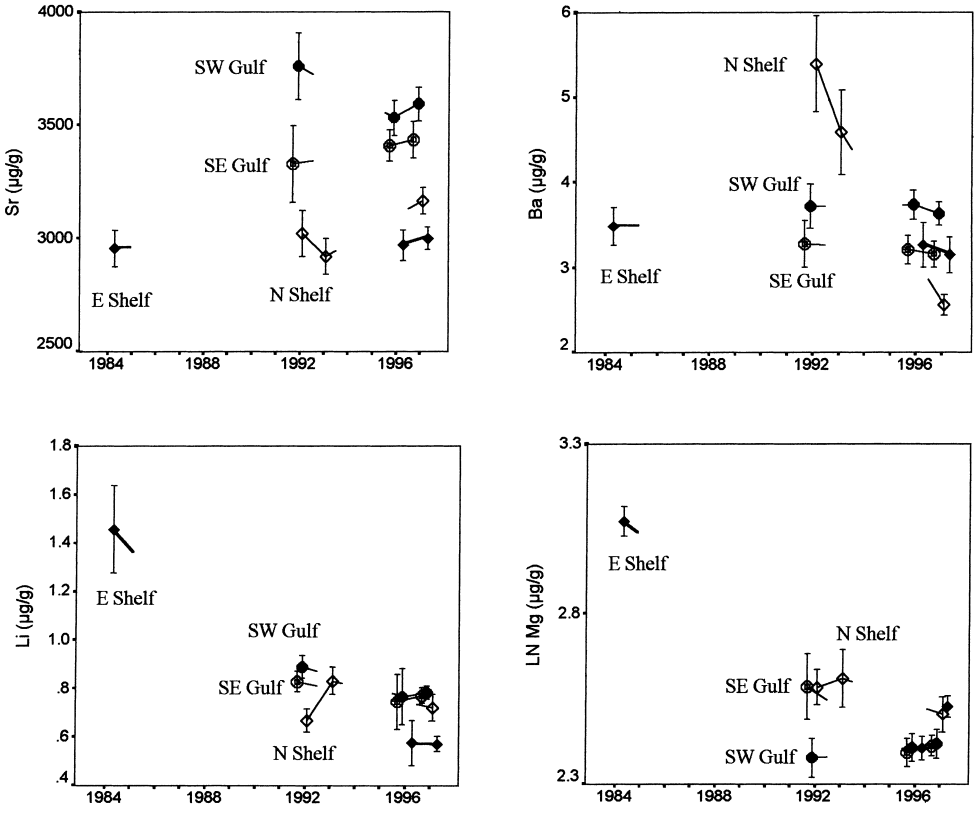

Fig. 5. Long-term variation in mean (±95% CI) elemental concentration at four locations selected to represent discrete groups of fish. Concentrations for Mg, Mn and Sr have been weight-detrended (see text).

mixture analysis indicated that 98.9% of the fall-collected cod in the southern Gulf returned the following spring to spawn in the southern Gulf, while 99.2% of those in the northern Gulf returned to the northern Gulf the next spring to spawn. Stock mixture analysis on a sample-by-sample basis indicated that southern and northern Gulf cod clearly dominated their respective stock areas, but that the distribution of northern Gulf cod extended down to the southern edge of the Laurentian Channel (Fig. 8). While the actual stock identity of the pre-migratory cod was not known definitively, it is reasonable to conclude that cod captured in their own stock area within a year of spawning were primarily residents. If true, this application serves as an independent test of the accuracy of otolith elemental fingerprints as a biological tracer.

As a second example of the biological tracer approach, the 1996 elemental fingerprints of the same four reference stocks described above were used in a stock mixture analysis of adult cod collected at the approaches to the Gulf of St. Lawrence in January 1996. Each of the 60 sets was analyzed separately, and the resulting stock-specific abundances contoured to show relative abundance (Fig. 9). The results demonstrated that southern Gulf cod dominated the stock composition on the southern side of the Laurentian Channel, while northern Gulf cod dominated the stock composition on the northern side. Densities of the remaining two stocks were relatively low, and in the case of the eastern Gulf stock, are probably attributable to minor (<1%) classification errors. The stock composition of the dense over-wintering cod aggregations in the Cabot Strait has not previously been described, in large part because it would have been difficult to determine using more traditional techniques.

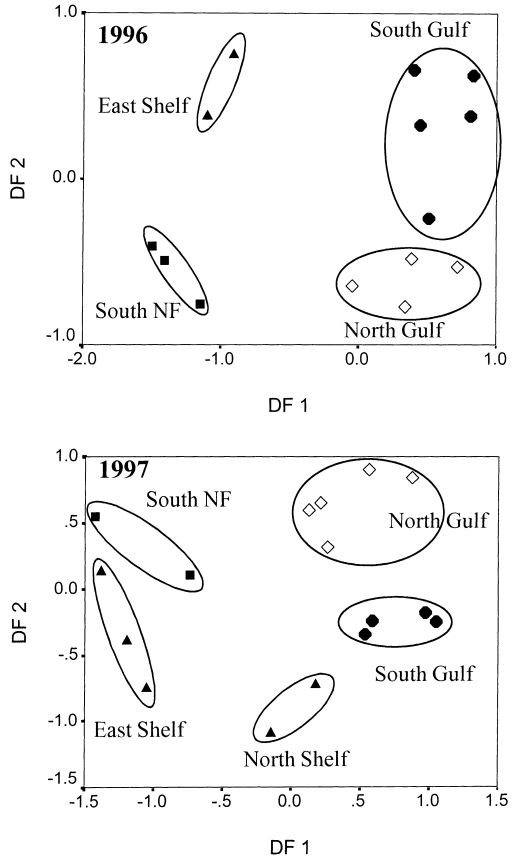

Fig. 6. The elemental fingerprint as a marker of spawning aggregations in each of 2 years, on the basis of separate discriminant functions for each year. Each data point within an aggregation represents an independent sample of that aggregation. As noted in Table 1, the offshore sites sampled in South NF in 1996 were not the same as the inshore bays in South NF sampled in 1997.

4. Discussion

Assays of whole dissolved otoliths have become increasingly popular for differentiating among fish stocks, based on the observation that chemical and physical features of the environment can induce significant changes in otolith elemental composition (Fowler et al., 1995; Farrell and Campana, 1996; Tzeng, 1996; Dove, 1997). However, the results of this and other studies have made it clear that the elemental composition is not necessarily a good indication of population identity (Campana et al., 1995; Edmonds et al., 1995). Rather the elemental fingerprint reflects the lifelong exposure of the individual fish to both the environment and its own physiology, and thus 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.

A more robust application of whole otolith fingerprints is one in which the statistical distributions of the fingerprints are used as natural tags of pre-defined groups of fish (Campana et al., 1995; Gillanders and Kingsford, 1996; Kennedy et al., 1997), based on the certainty that the otolith composition cannot change appreciably over short periods of time. An appealing feature of this application is that the elemental fingerprint need not be linked to potential sources or locations in the environment. 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, we suggest that otolith elemental fingerprints are 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: (1) there are characteristic and reproducible markers for each group, (2) all possible groups contributing to the group mixture have been characterized, and (3) the marker remains stable over the interval between characterization and mixing. While these assumptions apply as much to genetic and morphometric stock mixture analyses as to otolith-based assays (Wood et al., 1989; Wirgin et al., 1997), the following discussion applies specifically to elemental fingerprints.

Assumption 1: There are characteristic and reproducible markers for each group. This assumption is

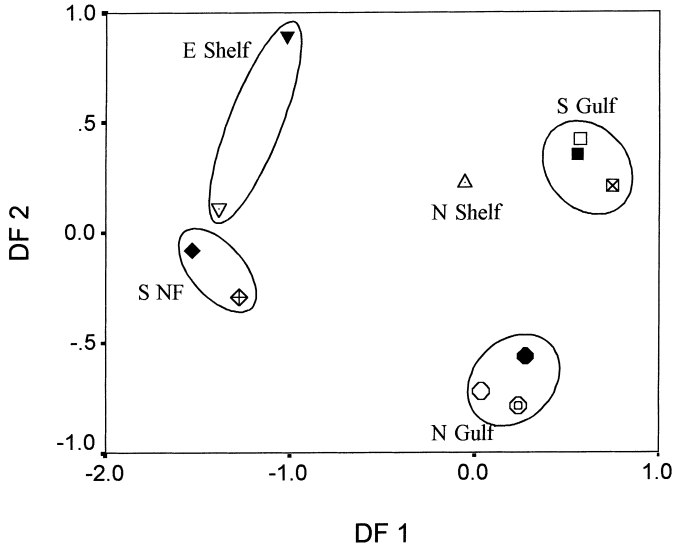

Fig. 7. Short-term stability and specificity of the elemental fingerprint as a marker of cod spawning aggregations. Differences among groups were all highly significant and relatively stable across adjacent years (1995: open symbol; 1996: filled symbol; 1997: hatched symbol). A single set of discriminant functions was fitted for all years.

easily tested, since it can be assessed empirically at an early stage of the study. If the elemental fingerprints of the groups of interest do not differ significantly, little more can be accomplished. However, the results of this and other studies indicate that group-specific variation in elemental composition is more the norm than the exception (Edmonds et al., 1989, 1991, 1992, 1995; Northcote et al., 1992; Campana and Gagné, 1995; Campana et al., 1995; Bronte et al., 1996; Thorrold et al., 1997). There is no a priori reason why the elemental fingerprint should serve as a proxy for genetic (or population) identity, nor do results to date suggest that this is the case (Campana et al., 1995; Edmonds et al., 1995). However, this does not imply that the elemental fingerprint must only be based on environmentally influenced elements. Elements under strong physiological regulation (e.g., Na, K, S, P, Cl) appear to vary relatively little among fish populations (Thresher et al., 1994; Proctor et al., 1995), and thus are probably unsuited for use in elemental fingerprints. On the other hand, even physiologically regulated elements should be appropriate for use as a group marker, as long as they varied among groups. For example, it is not clear that all of the elements used in this study are strong reflections of the environment; Li, Mn, Ba, and Sr may well reflect both water chemistry and temperature, but the rate of Mg incorporation, which is strongly physiologically regulated, is probably influenced only by otolith crystallization rate through the proxy of water temperature (Mitsuguchi et al., 1996). Whatever the ultimate cause, each of the above five elements differed significantly among groups, was relatively insensitive to decontamination protocols, and was present at comparable levels in both left and right sagittae. Thus, the selected elements satisfied most of the necessary criteria as chemically stable and reproducible group-specific markers. The last criterion, that of constancy in concentration across the size range of the fish, required the statistical removal of the effect of otolith weight, but was otherwise tractable. However, the presence of size-specific concentrations underlined the importance of insuring that the stock mixture sample contained the same size range as that in the reference samples, since smaller fish seem far more likely to contain elevated (or depressed) concentrations of any given element (Papadopoulou et al., 1980; Edmonds et al., 1989; Grady et al., 1989; Hoff and Fuiman, 1993).

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 pre-

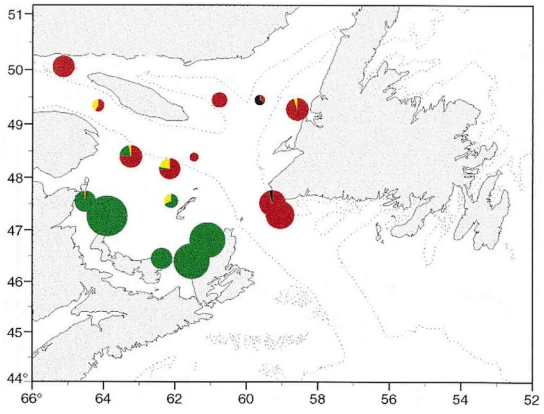

Fig. 8. Stock composition of pre-migratory adult cod collected in Fall 1995 as determined through stock mixture analysis of otolith elemental fingerprints using Spring 1996 spawners as the reference. The size of the circle represents the number of fish in the sample (total n=420), while the proportional stock composition is indicated by the colors in each pie chart. Red: northern Gulf; green: southern Gulf; yellow: eastern Shelf; blue: southern NF. While southern and northern Gulf cod clearly dominated their respective stock areas, northern Gulf cod also dominated both slopes and the center of the Laurentian Channel.

sent in the mixture could be mistakenly interpreted as one or more of the reference groups (Wood et al., 1987, 1989; Wirgin et al., 1997). Careful selection of reference groups can help minimize this problem, particularly if they are sampled at a time when the groups are known to be discreet (e.g., on the spawning or nursery grounds). Multiple samples are often required to insure that the group has been sampled randomly, and is not merely representative of a particular school or spawning wave. Depending on the hypothesis being tested, reference samples taken at a less aggregated time of year would probably require sampling over a broader geographic area (Campana et al., 1997a).

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 was it observed. However, stability over a shorter period (0.5–1 year) was observed, and is consistent with the fact that the mass

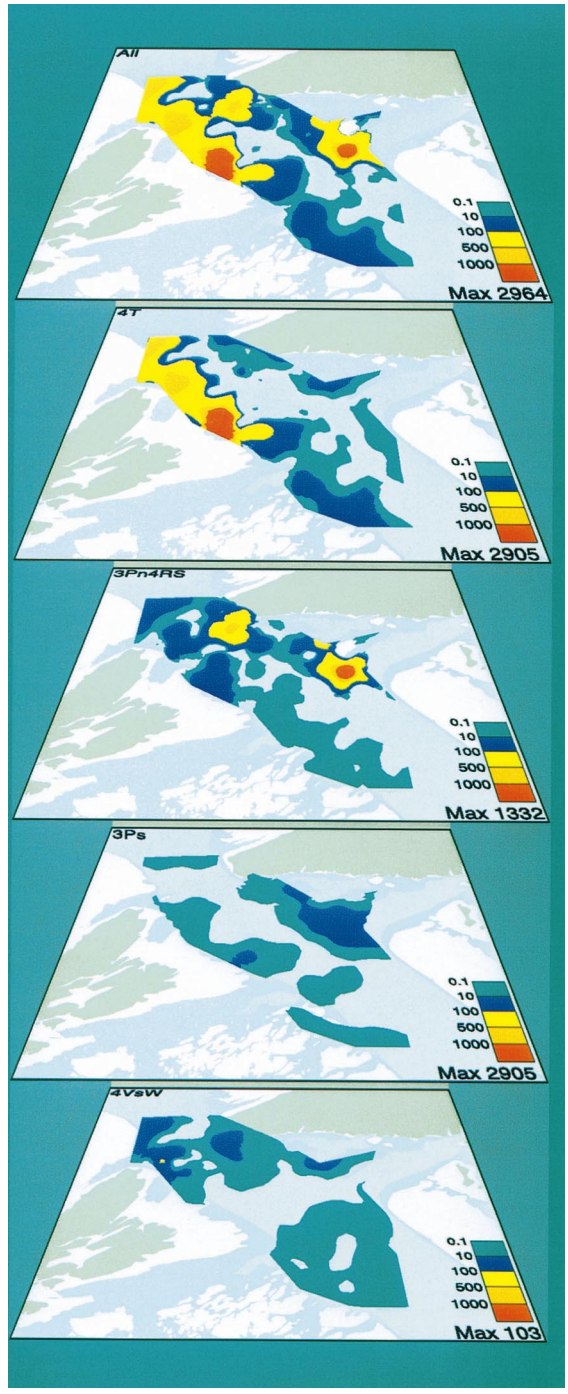

Fig. 9. Stock-specific abundance of adult cod in the Cabot Strait in January 1996 as determined through stock mixture analysis of otolith elemental fingerprints using Spring 1996 spawners as the reference. The top panel shows the overall cod abundance, while each of the following panels shows the abundance attributable to a specific reference stock. Southern Gulf: 4T; northern Gulf: 3Pn4RS; offshore southern NF: 3Ps; eastern Shelf: 4VsW.

and elemental composition of the otolith cannot change appreciably over a short period of time. Such short-term constancy is an important prerequisite of the biological tracer approach, since the group-specific marker must remain stable over the interval between characterization (e.g., spawning group) and mixing. In the case of the cod analyzed in this study, this interval was less than 6 months, and thus much less than the period required for change. 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 would be more marked.

Maximum likelihood-based stock mixture analyses based on genetic or morphometric markers have now become an established means for the accurate estimation of group proportions in a stock mixture (Wood et al., 1989; Millar, 1990; Wirgin et al., 1997). Stock mixture analyses based on otolith elemental fingerprints function in a parallel manner, since both genetic and otolith markers serve as multivariate tags which are used to estimate mixture proportions, as opposed to individual identifications. In the case of the cod stock mixtures reported here, the magnitude of the differences between the elemental fingerprints of the various source stocks was sufficiently large that stock proportions within the sample mixtures could be estimated on a sample-by-sample basis. This in turn allowed the results to be presented in either of two easily interpreted formats: expanding pie charts to show sample-by-sample stock composition, or contoured stock-specific abundance to show relative abundance. While the modes of presentation differed, both formats highlighted the potential power of elemental fingerprints for use as natural tags (Campana et al., 1999). Classification accuracy in these examples was high: based on the stock distribution of the pre-migratory fall cod, stock proportions were estimated to within $\pm 1\%$. This is not to say that elemental fingerprints are to be preferred in all situations; the conditions described earlier must be present for this approach to be effective. Nevertheless, the applications illustrated here provided mark-recapture results which would have been difficult to obtain through alternate means.

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