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The stock identity of 4Vn cod based on an analysis of otolith elemental fingerprints

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ABSTRACT

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 otoliths grow continuously without resorption throughout the life of the fish, individuals which spend at least part of their lives in different water masses produce otoliths of different elemental composition. Thus the otolith elemental composition ("elemental fingerprint") serves as an environmentally-induced tag of fish aggregations, independent of genetic identity, and remarkably stable from season to season. We used isotope dilution ICPMS (ID-ICPMS) to determine the elemental fingerprint of 1044 adult cod, representing 5 Atlantic cod (*Gadus morhua*) stocks in and around the approaches to the Gulf of St. Lawrence. There were highly significant differences among the elemental fingerprints of all of the stocks. To confirm that these fingerprints can be used to confidently track cod during periods of migration or stock mixing, we compared the fingerprints of 3Pn4RS, 4T and 4Vn cod samples taken in the fall (prior to out-migration) and in the spring (on the spawning grounds just after in-migration), as well as various locations from within each stock area. The results indicated that the otolith elemental fingerprint was stable across seasons, and that geographic variability in the elemental fingerprints within stocks was small compared to the differences among stocks. An important exception was that the elemental fingerprint of fish collected in spawning condition in 4Vn in May was significantly different from that of the fall 4Vn fish, but essentially identical to that of the 4T spring spawners. These findings indicated that significant numbers of 4T cod were still present in the 4Vn area at a time when fisheries management assumes that they are absent.

RÉSUMÉ

Les éléments traces, contenus à l'intérieur de la surface en croissance de l'otolithe de poisson, réflètent les caractéristiques physiques et chimiques du milieu aquatique environnant mais toutefois pas d'une manière simpliste. Etant donné que les otolithes croissent de façon continue sans résorption au cours de la vie du poisson, les individus qui passent une partie de leur existence dans des masses d'eau différentes produisent des otolithes de composition élémentaire différente. Alors, la composition élémentaire de l'otolithe ("empreinte digitale" élémentaire) sert de marqueur environnemental des aggrégations de poissons indépendant de l'identité génétique et d'une stabilité remarquable d'une saison à l'autre. Utilisant le ID-ICPMS afin de maximiser l'exactitude de la procédure d'analyse des otolithes, nous avons déterminé l'empreinte élémentaire de 1044 morues adultes, représentant 5 stocks de morue atlantique (*Gadus morhua*) du golfe du Saint-Laurent et de ses approches. Des différences hautement significatives ont été observées entre les empreintes élémentaires de tous les stocks. Afin de confirmer que ces empreintes élémentaires peuvent être utilisées de façon fiable pour suivre la morue durant les périodes de migration ou de mélange des stocks, nous avons comparé les empreintes élémentaires d'échantillons de morue de 3Pn4RS, 4T et 4Vn récoltés à l'automne (avant la migration hors du golfe) et au printemps (sur les zones de fraie juste après la migration vers le golfe) ainsi qu'à plusieurs endroits à l'intérieur de chacune des unités de gestion. Les résultats ont indiqué que l'empreinte élémentaire de l'otolithe était en effet stable au cours des saisons et que la variabilité géographique des empreintes d'un même stock était faible comparée aux différences entre les stocks. D'autre part, l'empreinte élémentaire de poissons en état de fraie échantillonnés dans 4Vn en mai était significativement différente de celle de poissons de 4Vn à l'automne et, essentiellement identique à celle des frayeurs de printemps de 4T, ce qui semble indiquer qu'un nombre significatif de morue de 4T sont encore présentes dans la zone 4Vn au moment où la gestion des pêches assume qu'elles en sont absentes.

INTRODUCTION

Given its geographic location between the large 4T and 4VsW Atlantic cod (*Gadus morhua*) stocks (Fig. 1), 4Vn is widely viewed as a mixing ground for both cod and other species (D'Amours et al. 1994). 4T cod are known to migrate out of the southern Gulf through 4Vn each fall, returning to the Gulf each spring. A large portion of the 4T stock overwinters within 4Vn each winter, where it is thought to mix with the resident cod stock. An unknown amount of stock mixing also occurs during the summer months. While the assessment period was recently adjusted to May-October to exclude the initial pulse of migration of 4T cod through 4Vn, there remains the possibility that the currently defined 4Vn cod (May-Oct) assessment unit does not represent the home range of a unit stock. This possibility was highlighted by difficulties in producing an internally consistent stock assessment, and by the report of the 4Vn Cod Working Group (Campana et al. 1995a). After a thorough review of existing information, the 4Vn Cod Working Group concluded that there was strong evidence for the existence of a small and poorly defined resident stock in 4Vn, and that there was significant mixing at all times of the year with the adjacent larger stocks. Therefore, the Working Group recommended that further research be carried out to develop a means of identifying the members of the resident 4Vn stock and to develop estimates for the extent of stock mixing during the May-Oct period.

Otolith elemental fingerprints provide one means by which fish stocks can be identified and tracked during periods of stock migration or mixing. The basis for the approach is described in detail elsewhere (Campana et al. 1995b). Briefly, the approach is based on the fact that 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 (Kalish 1989). Since otoliths grow continuously without resorption throughout the life of the fish, individuals which spend at least part of their lives in different water masses produce otoliths of different elemental composition. Thus the otolith elemental composition ("elemental fingerprint") serves as an environmentally-induced tag of fish aggregations which is independent of genetic identity. While there are probe techniques which exist to analyze the elemental composition of discrete regions of the otolith (Campana et al. 1997), dissolution and analysis of the whole otolith is preferable when using the elemental fingerprint as a biological tracer, since the composition of the otolith as a whole changes very little from season to season. Thus the fingerprint remains very stable over periods of less than a year, and can be used with considerable confidence to track and identify groups of fish.

DFO's High Priority Project on Cod Stock Mixing in the Gulf of St. Lawrence and its Approaches is currently examining cod stock structure and mixing in the region based on an coordinated examination of otolith elemental fingerprints, nuclear DNA fingerprints and vertebral counts. To address the issue of stock identity and mixing in 4Vn, we present here the initial results of the otolith elemental fingerprint component of the High Priority Project. Our objectives were two-fold: 1) determine if the otolith elemental fingerprints of summer-collected cod in 4Vn were significantly different from those of the adjacent stocks, suggesting the presence of a resident stock; and 2) determine if the elemental fingerprints of cod collected in spawning condition in 4Vn in May were consistent with the presence of a resident spawning stock.

MATERIALS AND METHODS

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 cm) in spawning or near-spawning condition were collected from known spawning grounds in 3Ps, 3Pn4RS, 4T, 4Vn and 4Vs in the spring of 1996 (Table 1; Fig. 1). Each set of spawning grounds was sampled independently at least twice, and a minimum of 99 cod were collected from each stock (except 3Ps, where n=80) for a total of 623 fish. The elemental fingerprints of these samples comprised our reference collection, since the stock identity of each of these fish was known with some confidence (with the possible exception of 4Vn, as will be discussed later).

A second set of samples (n=420) was collected in the fall of 1995 prior to any migration out of the Gulf (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 3Pn4RS, 4T and 4Vn cod stocks (Fig. 1). These 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.

Prior to elemental analysis, all otoliths were decontaminated to Class 100 standards and dissolved in sub-boiling, redistilled nitric acid (Fowler et al. 1995). A suite of 5 trace elements (Li, Mg, Zn, Sr, Ba) was assayed with isotope dilution inductively-coupled plasma mass spectrometry (ID-ICPMS), which offers excellent accuracy for these types of assays (Campana et al. 1995b). Mn was assayed by conventional ICPMS. Assays were also carried out for Pb and B, but they proved to be below detection limits and problematic, respectively. Elemental concentrations were transformed where appropriate to insure normality.

While elemental concentrations were reported in terms of μg per g of otolith, previous studies have occasionally noted size-specific trends which suggested changes in the relative rate of elemental incorporation with age or size (Campana et al. 1995b). To insure that differences in fish length among samples did not confound any stock-specific differences in elemental composition, analyses of covariance were carried out for each element in a search for significant effects of otolith weight, using stock as a main effect. Only Mg, Mn and Sr varied significantly with otolith weight, with Mg and Mn varying negatively and Sr varying positively. Since the otolith weight x stock interaction was non-significant for each of these elements, the otolith weight effect was removed using the common within-group slope. However, it is important to note that the effect of otolith weight on elemental concentration was small in all cases, and that none of the conclusions that follow were modified by the correction for otolith weight.

Each otolith was characterized by a suite of 6 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. The reference data for all elemental fingerprints were the spring data, for which stock affinity was known. Discriminant functions based on the spring data were then used to calculate discriminant values for both spring- and fall-collected fish.

RESULTS AND DISCUSSION

Mean fish lengths, otolith weights and elemental concentrations by stock are presented in Table 1. There was relatively little difference in mean length among the majority of samples, due largely to the fact that sampling was restricted to the range 35-80 cm. However, the fall 4T and the spring 3Ps samples consisted of fish which were slightly smaller and larger, respectively, than the remaining stocks.

Before an otolith elemental fingerprint can be applied as a biological tracer of stock mixing, it must be shown to vary in a consistent and well-defined manner among stocks or geographic locations. Analysis of the spring spawning aggregations indicated that the elemental fingerprints differed significantly and substantially among the 3Pn4RS, 4T, 3Ps and 4Vs cod stocks (Fig. 2). These differences were representative of the stock, and not just individual schools, since multiple independent samples of each stock (some of which were collected weeks apart) were similar in elemental composition. In contrast, the elemental fingerprints of the cod collected in spawning condition in 4Vn were not significantly different from that of the 4T spawners. Indeed, the fingerprints of the 4Vn-collected fish were essentially identical to those of the 4T spawners.

The otolith elemental fingerprints were not only stock-specific, but stable across seasons (Fig. 3). Differences among all 4 of the major cod stocks were highly significant ($p<0.01$), irrespective of season. In addition, the stock-specific fingerprints for both the 3Pn4RS and 4T cod stocks differed very little across seasons, indicating that there was relatively little change in the fingerprint between fall and spring. The fingerprint of the fall-collected 4Vn cod was also stock-specific. This result is consistent with the hypothesis that the summer-fall aggregations of cod in 4Vn are comprised of resident fish, although it does not necessarily mean that they were spawned there (since the otolith does not provide a genetic marker). In contrast however, the fingerprint of the spring-collected 4Vn cod was similar to that of 4T, not of 4Vn. On the basis of this similarity, and given the very different fall 4Vn signature, it seems very likely that the cod collected in spawning condition in 4Vn in May were of 4T origin.

It is important to note that the similarity between the spring and fall elemental fingerprints is not an artifact of the discriminant analysis used to characterize the fingerprints, since the discriminant values for both the fall- and spring-collected cod were based on discriminant functions prepared from only the spring collections. In other words, the fall collections provided an independent test of the validity of the spring discriminant functions.

When the fingerprints of the fall-collected 3Pn4RS cod (pre-migration) were examined on a site-by-site basis across the entire stock area of 3Pn4RS, the site-specific fingerprints were centrally distributed around the centroid corresponding to the spring spawners (Fig. 4). The same pattern was observed in 4T. This type of within-stock geographic variability is to be expected of otolith elemental fingerprints, given the within-stock variability in the physical environment. Indeed, the two apparently anomalous sites in 4T which appear next to the 3Pn4RS fingerprints in Fig. 4 originated in or immediately adjacent to the Laurentian Channel. On the basis of the similarity of the environment of that region to that of the northern Gulf, the fingerprints of those 2 sites would be expected to be more similar to those of 3Pn4RS than the other 4T sites. Site-to-site variability in the fall 4Vn fingerprint was somewhat larger, but the fingerprints were clearly different than those of the other stocks.

While cod are known to spawn in 4Vn in May (Campana et al. 1995a), it should not be surprising that 4T cod in spawning condition are also present in 4Vn at the same time. 4T cod are known to migrate through 4Vn in the spring of each year as they return to their spawning grounds in the southern Gulf (Lambert 1993; Sinclair and Currie 1994). Furthermore, the process of sexual maturation prior to spawning takes several months, suggesting that 4T cod only one month before spawning should be in ripe condition. What is more surprising though is the fact that our sampling captured so many 4T migrants. Two independent samples of cod were collected in 4Vn in early May, and neither of these samples were from the edge of the Laurentian Channel or near the 4T-4Vn boundary, areas where late migrants might be expected to be found. Furthermore, there is no evidence of any unusual environmental conditions in the spring of 1996 which might have delayed the return migration of 4T cod; indeed, ice conditions in that year were much less severe than normal (Drinkwater 1997). Therefore, there is no reason to believe that the substantial presence of 4T cod in the 4Vn region in May is anything out of the ordinary. In support of this is Lambert's (1993) analysis of multiple years of tagging data, which showed large numbers of 4T cod in 4Vn in the month of May, and an analysis of fleet dynamics which reached the same conclusion (Sinclair and Currie 1994).

The current assessment and management unit for 4Vn cod is May-Oct. Implicit in the definition of such a unit is that cod catches during this period are largely of resident cod; significant catches of other stocks would introduce substantial error into the 4Vn cod stock assessment. However, the findings of our study and the tagging and fleet dynamics investigations conducted earlier indicate that May is a period of transition in 4Vn, and that large numbers of 4T cod can persist within 4Vn during this month. Therefore, the validity of the May-Oct assessment/management unit for 4Vn cod needs to be reexamined, and if warranted, changed.

Additional research on the 4Vn cod stock identity issue, as well as the question of cod stock identity throughout the Gulf of St. Lawrence and approaches, is ongoing under the auspices of DFO's High Priority Funding program. As additional findings from the otolith elemental fingerprint, DNA fingerprint and vertebral count studies become available, they will be used to reexamine and reassess cod stock structure throughout the Gulf and surrounding region.

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REFERENCES

Campana S, Fanning P, Fowler M, Frank K, Halliday R, Lambert T, Mohn R, Wilson S, Stobo W, Hanson M, Sinclair A (1995a) Report of the 4Vn Cod Working Group on the scientific value of a 4Vn cod (May-Oct) stock assessment. DFO Atl. Fish. Res. Doc. 95/16.

Campana SE, Gagne JA, McLaren JW (1995b) Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar. Ecol. Prog. Ser. 122:115-120.

Campana SE, Thorrold SR, Jones CM, Gunther D, Tubrett M, Longerich H, Jackson S, Halden NM, Kalish JM, Piccoli P, de Pontual H, Troadec H, Panfili J, Secor DH, Severin KP, Sie SH, Thresher R, Teesdale WJ, Campbell JL (1997) Comparison of accuracy, precision and sensitivity in elemental assays of fish otoliths using the electron microprobe, PIXE and laser ablation ICPMS. Can. J. Fish. Aquat. Sci. In press.

D'Amours D, Frank KT, Bugden G (1994) Report of the Working Group on Oceanographic Effects on Stock Migration and Mixing - reviewed by the Fisheries Oceanography Committee (FOC). DFO Atl. Fish. Res. Doc. 94/54.

Drinkwater KF, Pettipas R, Petrie L (1997) Physical oceanographic conditions on the Scotian Shelf and in the Gulf of Maine during 1996. DFO Atl. Fish. Res. Doc. 97/

Fowler AJ, Campana SE, Jones CM, Thorrold SR (1995) Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. Can. J. Fish. Aquat. Sci. 52:1421-1430.

Kalish JM (1989) Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132:151-178.

Lambert TC (1993) The timing of the winter migration of 4T cod into 4Vn. DFO Atl. Fish. Res. Doc. 93/25.

Sinclair AF, Currie L (1994) Timing of commercial fisheries based on the movement of cod into and out of the Gulf of St. Lawrence, 1986-93. DFO Atl. Fish. Res. Doc. 94/47.

Table 1. Summary of cod samples collected and otolith elements analyzed categorized by stock and season. Each stock was sampled independently at least twice each season. Trace element concentrations (μg/g) as presented are uncorrected for otolith weight, but were corrected prior to the statistical analysis.

STOCK	SEASON	DATE	N	LENGTH (cm)		OTOLITH (g)		Ba		Li		Mg		Mn		Sr		Zn	
				Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3Pn4RS	Fall	Oct 10-Nov2	113	51	1	.34	.01	3.04	.08	1.1	.0	18.4	.4	1.0	.0	3075	38	5.3	.3
	Spring	Apr 13-30	155	56	1	.43	.01	3.20	.07	1.2	.0	18.0	.3	1.0	.0	3198	38	5.0	.3
3Ps	Spring	Apr 27-29	70	61	1	.45	.01	2.55	.08	.9	.1	18.3	.4	.6	.0	2594	39	4.1	.3
4T	Fall	Sept 15	217	44	0	.26	.00	3.85	.08	1.4	.0	19.1	.3	1.7	.0	3003	26	6.3	.3
	Spring	June 13-15	200	52	1	.39	.01	3.47	.06	1.3	.0	17.8	.3	1.4	.0	3091	28	4.9	.3
4Vn	Fall	Sept 25 - Oct 5	90	50	1	.38	.01	3.48	.10	1.2	.1	17.4	.4	1.3	.1	3211	45	5.0	.3
	Spring	May 1	99	52	1	.39	.01	3.44	.11	1.2	.1	17.7	.3	1.4	.1	3072	44	4.6	.3
4Vs	Spring	May 1	99	53	1	.38	.01	3.27	.13	1.1	.0	17.9	.3	1.0	.0	2591	33	4.7	.3

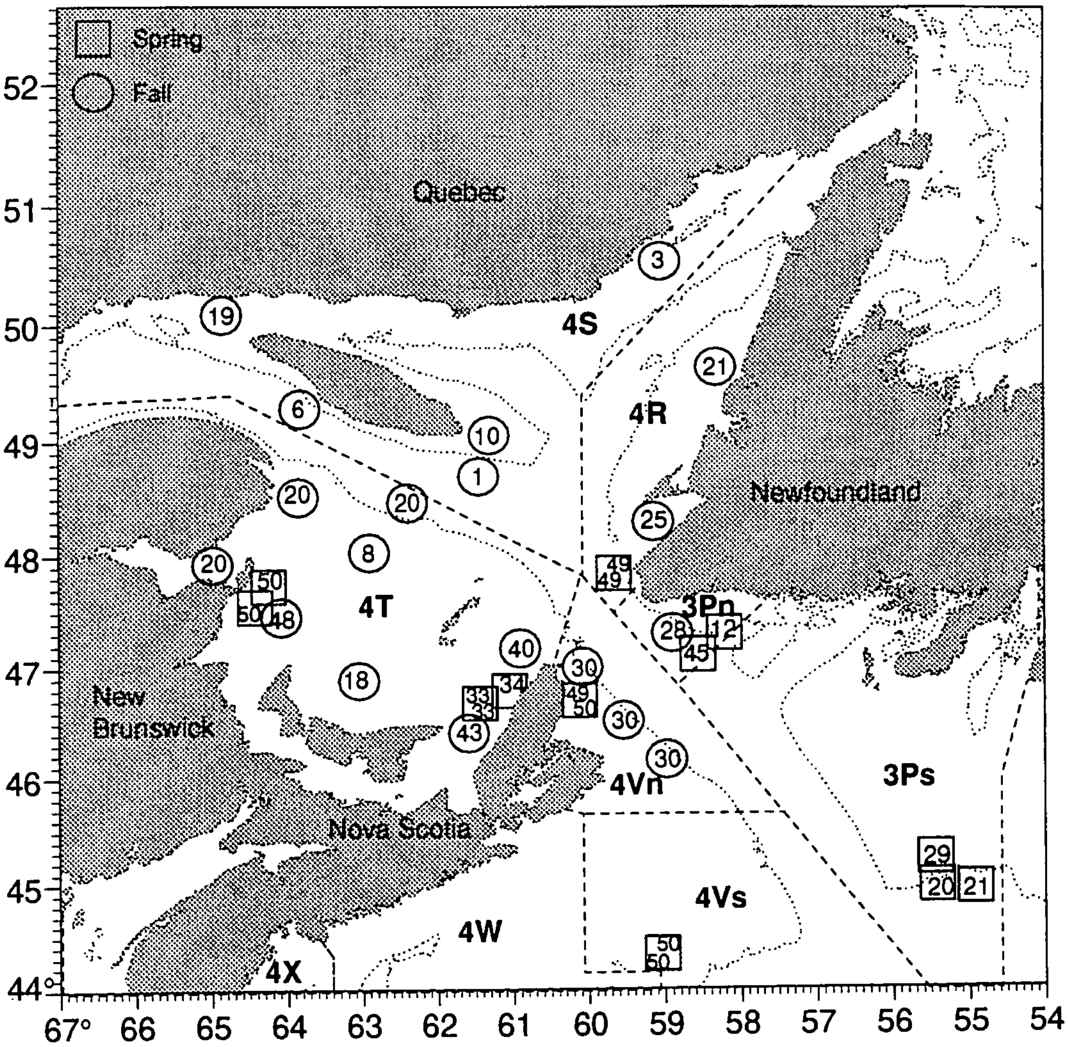

Fig. 1. Map of the study area showing the NAFO divisions and 200-m contour. Each circled number represents the number of fish collected from that site, either in the fall of 1995 (circles) prior to out-migration or the spring of 1996 (squares) at around the time of spawning.

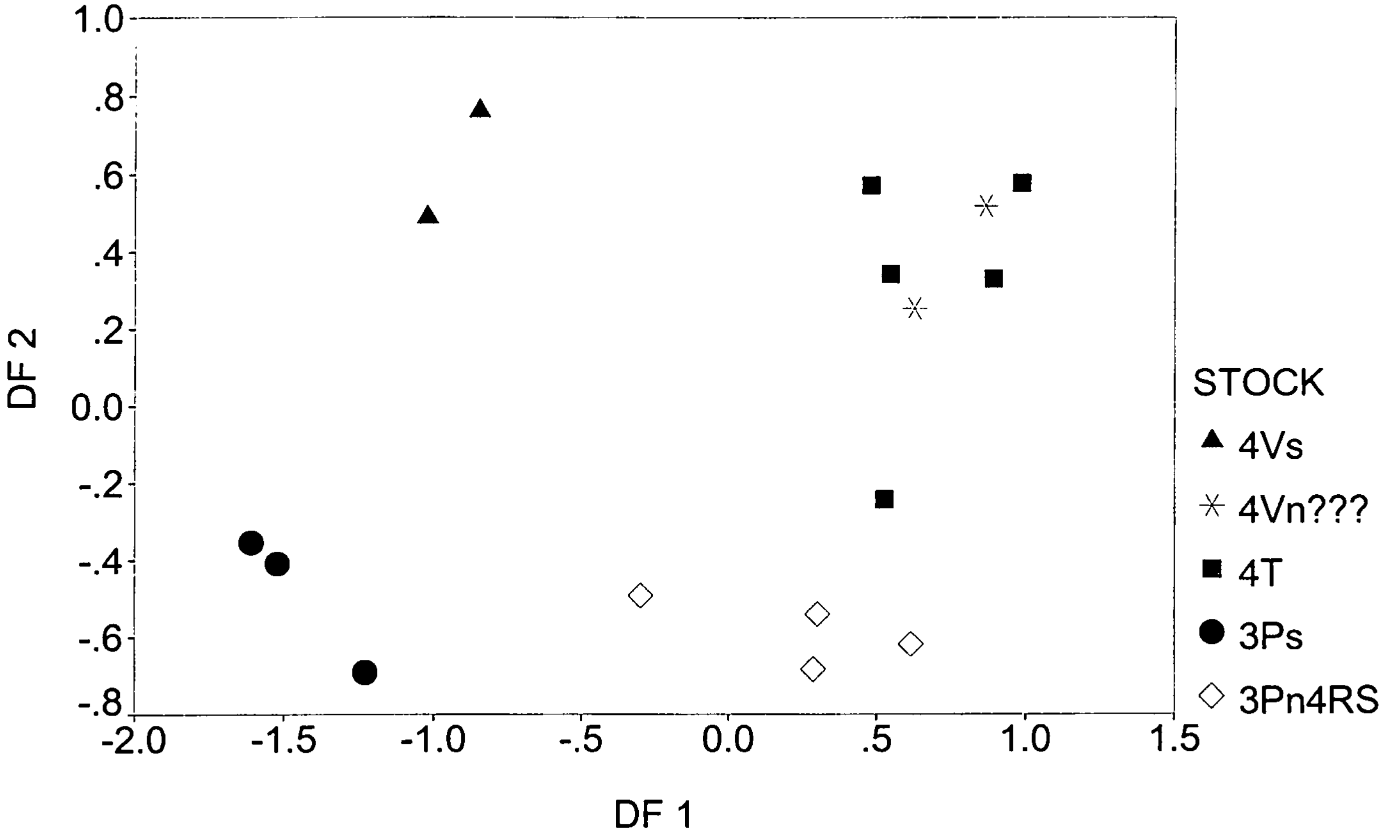

Fig. 2. Otolith elemental fingerprints (discriminant values) by set for each of the spawning stocks sampled in Spring 1996. All of the stock centroids differ significantly, except for that of the fish collected in spawning condition in 4Vn in May, which were indistinguishable from 4T cod.

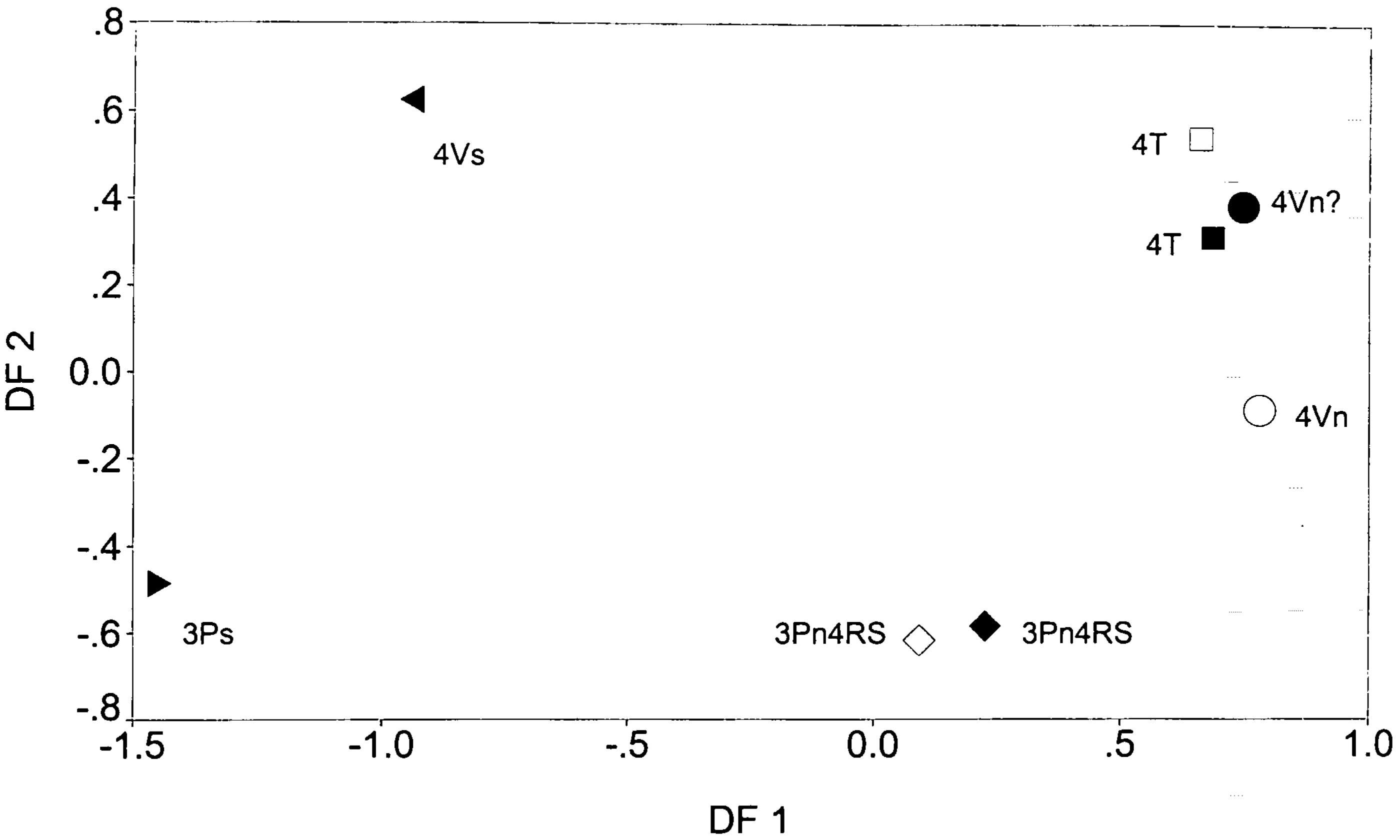

Fig. 3. Stock-specific otolith elemental fingerprints (discriminant values) of cod in Fall 1995 (pre-migration) and Spring 1996 (spawning), demonstrating the stability of the fingerprints through the course of a year. All Fall 1995 fingerprints were based on Spring 1996 discriminant functions, and thus are independent of the reference functions. The only stock where spring and fall fingerprints differed significantly is 4Vn; there, the fall centroid suggested the presence of a resident stock, while the spring centroid indicated that only 4T cod were sampled.

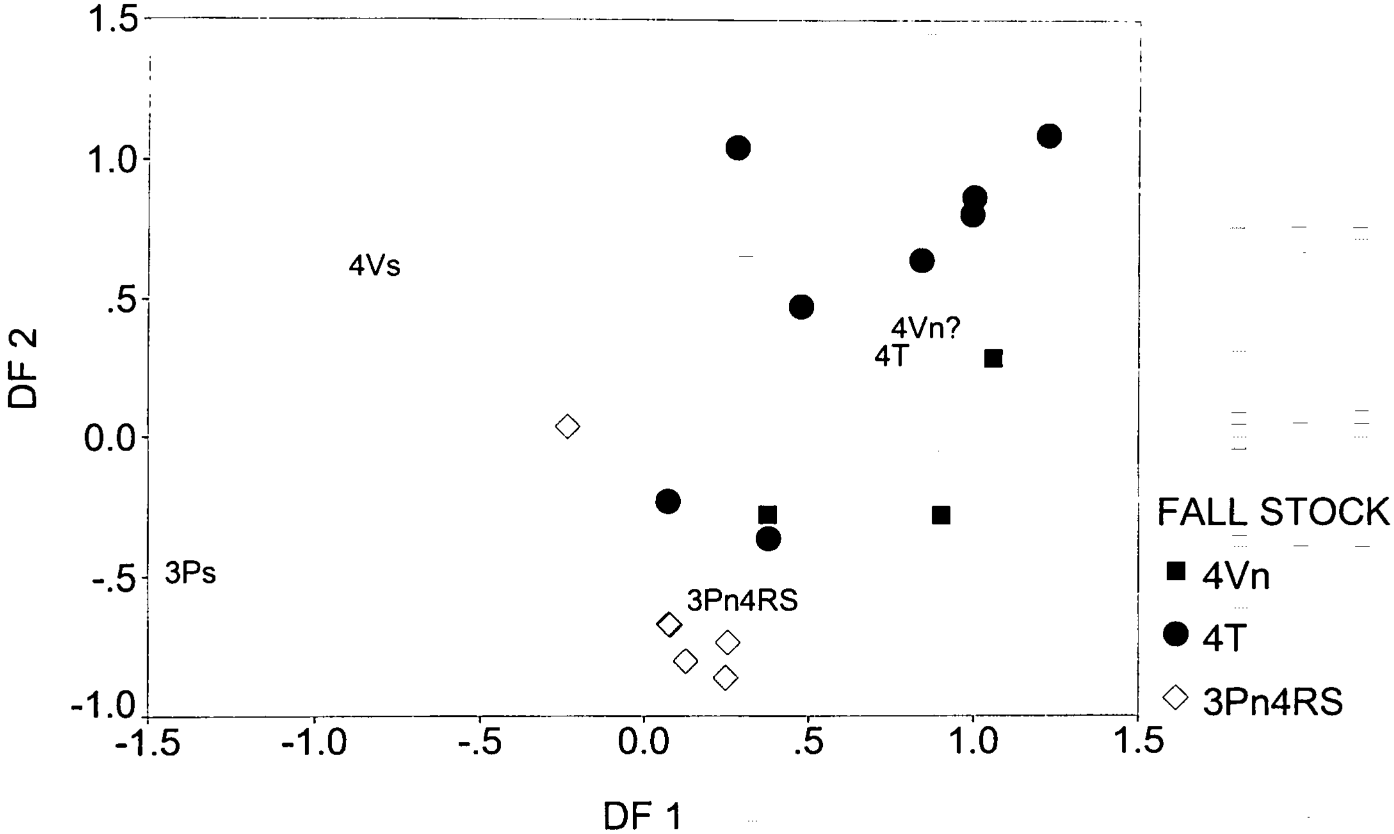

Fig. 4. Otolith elemental fingerprints (discriminant values) of cod collected prior to out-migration in Fall 1995. All Fall 1995 fingerprints were based on discriminant functions prepared with Spring 1996 data, and therefore are independent of the reference functions. Each point represents a different geographic region within each stock area. Note the correspondence between the Fall 1995 fingerprints and the centroids of the same spawning stock in Spring 1996 (shown as labels), demonstrating the stability of the elemental fingerprints as a stock-specific marker through the course of a year. However, the 4Vn fingerprints differed significantly between fall and spring, suggesting that the May 4Vn collections actually consisted of 4T fish. The two 4T symbols nearest the 3Pn4RS aggregation represent sampling sites in or adjacent to the Laurentian Channel.