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DFO Atlantic Fisheries
Research Document 94/27

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MPO Pêches de l'Atlantique
Document de recherche 94/27

DIFFERENTIATION OF 4T AND 4Vs COD USING OTOLITH ELEMENTAL FINGERPRINTS

by

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Abstract

Trace elements incorporated into the growing surface of the fish otolith (ear stone) are derived primarily from the surrounding water, thus reflecting the composition of the ambient environment. Since otoliths grow continuously throughout the life of the fish and otolith material is not resorbed after deposition, fish populations growing up in different water masses should produce otoliths of different elemental composition. The otolith elemental composition ("fingerprint") determined with a particularly accurate form of ICPMS proved to be an effective discriminator of adjacent cod stocks, with a jackknifed classification accuracy of 100% between the main body of the NAFO Division 4T stock (western component) and NAFO Sub-division 4Vs. Samples from the eastern portion of 4T differed significantly from those of western 4T, suggesting that cod within the Gulf of St. Lawrence do not necessarily form a unit stock. Classification of samples collected from the 4Vs winter fishery near the Stone Fence (4Vsc) indicated a high preponderance of eastern Gulf fish, and the virtual absence of western Gulf fish. If the seasonal 4T cod migration out of the Gulf is limited by distance, cod from eastern 4T and 4Vn may migrate considerably further into 4Vs than do cod from western 4T. The validity of unit stock status for the 4Vn (May-Dec) cod management unit is open to question.

Résumé

Les éléments-traces incorporés dans la surface de croissance des otolithes (os de l'oreille) de poisson proviennent essentiellement des eaux avoisinantes, et reflètent donc la composition du milieu ambiant. Comme les otolithes ne cessent de croître durant la vie du poisson et que les matières qui les composent ne se résorbent pas une fois déposées, les populations de poisson qui grandissent dans différentes masses d'eau devraient produire des otolithes de composition différente. Or, cette composition des otolithes (caractérisation), établie à l'aide d'une forme particulièrent précise d'ICPMS, s'est avérée un moyen efficace de différencier les stocks de morue adjacents les uns aux autres, produisant une classification selon la méthode jackknife exacte à 100 % en ce qui a trait à la majeure partie du stock de la division 4T de l'OPANO (secteur ouest) et à la sous-division 4Vs de l'OPANO. Les échantillons prélevés dans le secteur est de 4T différaient sensiblement de ceux du secteur ouest, donnant à penser que la morue du golfe du Saint-Laurent ne constitue pas nécessairement un stock unitaire. La classification des échantillons provenant de la pêche d'hiver dans 4Vs à proximité de Stone Fence (4Vsc) révélait une forte prépondérance de poissons de l'est du Golfe et l'absence virtuelle de poissons de l'ouest du Golfe. Si la migration saisonnière de la morue de 4T hors du Golfe est limitée par la distance, la morue de l'est de 4T et de 4Vn peut s'aventurer beaucoup plus loin dans 4Vs que celle de l'ouest de 4T. Il convient donc de s'interroger sur la validité du caractère de stock unitaire en ce qui concerne l'unité de gestion de la morue de 4Vn (mai-décembre).

Measures of growth, survival, and reproductive success in marine fishes all assume that a single population is being monitored. Unfortunately, there appear to be few (if any) natural markers, whether genetic or morphological, which can be used to consistently differentiate among mixed populations. Recent studies have pointed to the potential of the otoliths (ear stones), found in all fishes, as natural population markers (Edmonds et al. 1989; Campana and Gagné 1994). While otoliths are well known for the formation of the annual (Casselman 1987) and daily growth rings (Campana and Neilson 1985) used in their age determination, it is their elemental composition which has attracted attention as a potential means to track and identify fish populations. The potential is based on two observations: a) 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); and b) the calcium carbonate and trace elements that make up 90% of the otolith appear to be mainly derived from the water (Simkiss 1974). Accordingly, the elemental composition of the otolith reflects that of the water in which the fish lives, although not necessarily in a simplistic fashion (Kalish 1989; Fowler et al. 1995). Since the elemental composition of seawater varies from place to place (Johnson et al. 1992), fish populations occupying different water masses should contain otoliths with different exposure histories to the ocean environment, despite any periodic inter-mixing. The objective of this study was to test this hypothesis on adjacent cod (*Gadus morhua*) stocks at a time of complete separation, and then use the results to discriminate between the two stocks during a time of mixing.

Site-specific differences in otolith elemental composition have been reported previously, but interpretation has generally been confounded by ontogenetic effects, assay limitations, and unexpected differences among samples (Papadopoulou et al. 1978, 1980; Edmonds et al. 1989, 1991, 1992; Grady et al. 1989; Kalish 1989; Gunn et al. 1992; Campana and Gagné 1994; Campana et al. 1994). It now appears that the choice of elements, more than anything, has limited the value of these studies. For instance, all six of the minor elements detectable with the commonly-used electron microprobe are under physiological control (Kalish 1989; Fowler et al. 1995), and thus are relatively poor indicators of population identity. A far larger suite of trace elements can be detected with inductively coupled plasma mass spectroscopy (ICPMS), but instrument artifacts and the relative purity of the fish otolith have limited past attempts to detect what are expected to be relatively small differences among populations (Edmonds et al. 1992; Campana and Gagné 1994). Yet it is these trace elements which appear to be least regulated during uptake, and therefore most reflective of the environment (Fowler et al. 1995). For this reason, we decided to adopt the use of a much more specialized form of ICPMS for our assays, to take advantage of its reputation for extreme accuracy, precision and sensitivity.

Methods

The timing of sample collection was designed in light of the annual winter migration of cod from the Gulf of St. Lawrence (NAFO Division 4T) onto the area of the Scotian Shelf occupied by the Division 4VsW cod stock (Fig. 1). Samples of Gulf cod were collected by fisheries observers in June, which is around the time of spawning for that stock, and a time when no other cod stocks are known to be in the Gulf (Table 1). Scotian Shelf cod were sampled later in the summer, when the stock is presumed to be clear of all other stocks. Peak stock mixing is believed to occur in late winter, which is when our samples of unknown stock identity were collected. All cod samples were drawn from several independent collections at each site, and all were restricted to sexually mature fish between the lengths of 44-85 cm. Immediately after capture, sagittal otoliths were removed from each fish, cleansed of adhering tissue, and stored dry in paper envelopes until they could be examined further. After decontamination and drying in a Class 100 clean room (Campana and Gagné 1994), otoliths were weighed, dissolved in 5 ml sub-boiling distilled nitric acid, diluted to 250 ml, and prepared for analytical assay. The assay sequence was systematically mixed by sample site to avoid the possibility of instrument drift (Campana et al. 1994), although none was later observed.

Results

Of the 28 elements known to be present in the otolith (Campana and Gagné 1994), 6 elements featured the multiple isotopes free of spectral interferences which are required by our assay technique. Detection limits (3σ in ppm per otolith weight) for Sr, Mg, Li , Zn, and Ba were all well below (<1%) observed concentrations in the otolith; that for Pb censored 44% of the observations. Coefficients of variation for replicate assays were less than 3% for all elements except Pb, which was 8%. Three of the elements (Li, Mg, and Sr) showed small but statistically significant relationships with otolith weight (ANCOVA, $p<0.05$). The effect of otolith weight was subsequently removed using the common within-group slope, although this procedure did not modify any of the results (all statistical analyses were repeated using both the undetrended data set and undetrended data restricted to an otolith weight range of 0.35-0.45 g; all results were virtually identical).

All 6 of the elements differed significantly among sample sites (ANOVA, $p<0.05$) (Table 2); inter-site differences were even more pronounced when analyzed as a multivariate fingerprint, rather than as individual elements (MANOVA, $p<0.001$). None of the inter-element correlation coefficients exceeded 0.5. Differences in elemental concentration within sites (drawn from multiple independent collections over a period of several days within a 10-km radius) were not significant, indicating that the elemental fingerprints represented a sample site and not just a school of fish.

Stepwise discriminant analysis of the four reference sample sites (Sample Sites 1-4) resulted in highly significant inter-site differences based on 4 elements: Li, Sr, Ba and Zn ($p<0.001$). Virtually all of the explained variance was accounted for by the first two discriminant functions (88% and 10%, respectively). Discrimination between 4Vs and western 4T (the largest concentration of 4T fish) was virtually complete (Fig. 2). The sample from eastern 4T (Sample #2) was significantly different from both the western 4T sample and those from 4Vs ($p<0.01$), but lay in between the two in discriminant space (Fig. 3). Virtually all of the stock discrimination resulted from separation along the first discriminant axis, which appeared to correspond to an environmental gradient between the Gulf of St. Lawrence and the open ocean; the second axis served merely to separate the two 4Vs samples, which were collected in different years.

Using the discriminant functions developed from the reference samples (Samples 1-4), Samples 5-

6 from the 4Vs winter fishery were classified as to most likely sample site origin. Less than 7% of either sample was classified as being part of the western 4T sample. However, 33-65% of each sample was identified as being from eastern 4T (Table 3), the remainder being from 4Vs. When plotted in the same discriminant space as the reference samples, both of the unknown samples were characterized by distributions in keeping with an eastern 4T - 4Vs mixture.

Conclusions

The otolith elemental fingerprints associated with the 4T and 4Vs cod stocks were sufficiently distinct to serve as natural tags in monitoring their relative distribution, and are among the most powerful stock discriminators yet reported for adjacent cod stocks. Elemental fingerprints are not genetic markers, and cannot be used to determine population identity in terms of spawning site. However, based on this and other studies, they are robust discriminators of aggregations of fish which have lived the greater part of their lives in separate water masses. The fact that there was a linear trend in discriminant scores between the inner Gulf and the Scotian Shelf (Fig. 3) is completely consistent with current knowledge of the response of the otolith to changes in temperature and salinity (Fowler et al. 1995).

Whole-otolith fingerprints represent the integration of an individual fish's exposure to the environment throughout its life. As such, they are not sensitive to short-term effects. This point was highlighted by the similarity of multiple independent samples from each sample site, both within and across years. The fact that samples from the western and eastern Gulf of St. Lawrence had very different fingerprints indicates that the two groups of fish lived much of their life apart. The fingerprints differed too much to have been the product of a single season apart. While segregation such as this does not necessarily imply that the two groups constitute separate stocks, it does point to significant heterogeneity within the Gulf. Studies based on morphometrics, meristics and tag returns have also suggested that eastern Gulf cod are more closely allied with 4Vn cod than with the western 4T fish with which they are now managed (Templeman 1962).

The finding that cod collected in 4Vsc (Sample Sites 5 & 6) in February and March did not originate from the main body of the 4T cod stock (western component) is consistent with interpretations based on the Kimura-Chikuni method (Hanson and Nielsen 1992). However, the finding that about half of the 4Vsc winter samples were classified as being from eastern 4T is surprising. There are two possible explanations for these somewhat unexpected results: 1) Cod migration out of the Gulf is not targeted at a common site as it is in many pelagic fishes and birds, but rather the extent of migration is limited on an individual basis by energetic considerations. This would imply that eastern Gulf cod would migrate considerably further out along the Shelf than would those from the western Gulf. Hanson and Neilsen's inability to identify the former would then stem from the relatively small representation of eastern Gulf cod in their reference Gulf cod sample; 2) The cod in Samples 5 & 6 identified as being from the eastern Gulf were misidentified, and actually originated in 4Vn. Given the proximity and similar environmental conditions of Sydney Bight to the eastern Gulf shore, it is unlikely that cod living in the two locations could be differentiated using otolith elemental fingerprints. However, under either of the above scenarios, the net effect is the same: a significant proportion of the cod caught on the northeastern Scotian Shelf (4Vsc) in winter were not of Scotian Shelf origin. Using otolith elemental fingerprints as markers in individual fish, it should now be possible to use historical otolith collections to map the extent of the cod migration in relation to annual variations in water temperature and ice cover.

Analysis of tag return data by Lambert and Stobo (pers. comm.) indicated that many of the cod tagged in eastern 4T were recovered in the summer 4Vn fishery. This, in conjunction with the elemental fingerprint results and the presence of cod spawning grounds in both the western and eastern Gulf (M. Hanson, pers. comm.) (Fig. 4) all beg the question: is there a distinct 4Vn cod stock, or does that region merely represent a portion of the home range of cod in the eastern Gulf? There are several variations on this question, one of which involves localized spawning in Sydney Bight, supplemented by ichthyoplankton advected from the Magdalen Islands (Fig. 4). Another possibility is that cod occupy favourable habitat on both sides of the 4T-4Vn line, particularly down the western side of Cape Breton (in 4T) and in Sydney Bight, but do not necessarily return to the same place after their winter migration. As a result, the same group of fish may "slop" back and forth between eastern 4T and 4Vn on an annual basis. A third (and probable) scenario is "all of the above" - a small number of resident inshore 4Vn cod, akin to those found elsewhere along the N.S. coastline, supplemented by larger numbers of eastern 4T fish whose numbers vary with environmental conditions and the year. Recruits arrive from both local spawning and from the Magdalen Islands. If valid, this stock structure scenario would suggest that there is little value to attempting to manage 4Vn in isolation. It would also suggest that 4Vn summer abundance will co-vary with the abundance of cod in the eastern Gulf, but be less closely linked with overall 4T cod abundance. Finally, it would provide a partial explanation for the decline in abundance of 4Vn summer fish as having being impacted by the post-1987 increase in the 4Vs winter fishery.

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Table 1. Sample collection information. Sample locations are mapped in Fig. 1.

Sample no.	Location	Date	Fish length (mean ± SE cm)	Otolith weight (mean ± SE g)
1	Western Gulf	June 1992	52.2 ± 1.0	0.40 ± 0.02
2	Eastern Gulf	June 1992	53.7 ± 1.1	0.40 ± 0.01
3	4Vs	Aug. 1993	57.1 ± 1.4	0.39 ± 0.02
4	4Vs	July 1992	53.3 ± 1.2	0.36 ± 0.02
5	4Vs	Mar. 1993	55.1 ± 1.4	0.38 ± 0.02
6	4Vs	Feb. 1992	59.6 ± 1.5	0.44 ± 0.02

Table 2. Elemental concentrations (mean ± SE in ppm) in otoliths collected from each of the 6 sample sites (n = 33 for each site, except for Site 5 where n = 31).

Sample Site	Ba	Li	Mg	Pb	Sr	Zn
1	3.72 ± 0.13	0.61 ± 0.02	13.6 ± 0.5	0.0105 ± 0.0014	3394 ± 82	1.75 ± 0.18
2	3.49 ± 0.25	0.55 ± 0.03	16.9 ± 0.9	0.0106 ± 0.0021	2969 ± 80	0.58 ± 0.06
3	4.59 ± 0.24	0.56 ± 0.03	18.6 ± 1.4	0.0107 ± 0.0014	2541 ± 46	0.33 ± 0.02
4	5.40 ± 0.28	0.42 ± 0.02	17.0 ± 0.5	0.0155 ± 0.0016	2621 ± 53	0.44 ± 0.04
5	3.85 ± 0.19	0.65 ± 0.04	15.8 ± 0.4	0.0090 ± 0.0013	2865 ± 56	0.49 ± 0.04
6	3.52 ± 0.17	0.60 ± 0.07	16.1 ± 1.1	0.0094 ± 0.0010	2864 ± 82	0.31 ± 0.02

Table 3. Jack-knifed classification accuracy of cod samples identified through discriminant function analyses of otolith elemental fingerprints. Samples 5 and 6 comprised fish of unknown origin collected during the winter fishery for 4Vs cod on the Scotian Shelf.

Actual Group	No. of Cases	Predicted Group Membership (%)			
		1	2	3	4
1 - Western Gulf	33	70	30	0	0
2 - Eastern Gulf	33	18	61	12	9
3 - 4Vs	33	0	9	64	27
4 - 4Vs	33	0	9	21	70
5 - Unknown	31	6	65	23	6
6 - Unknown	33	3	33	33	30

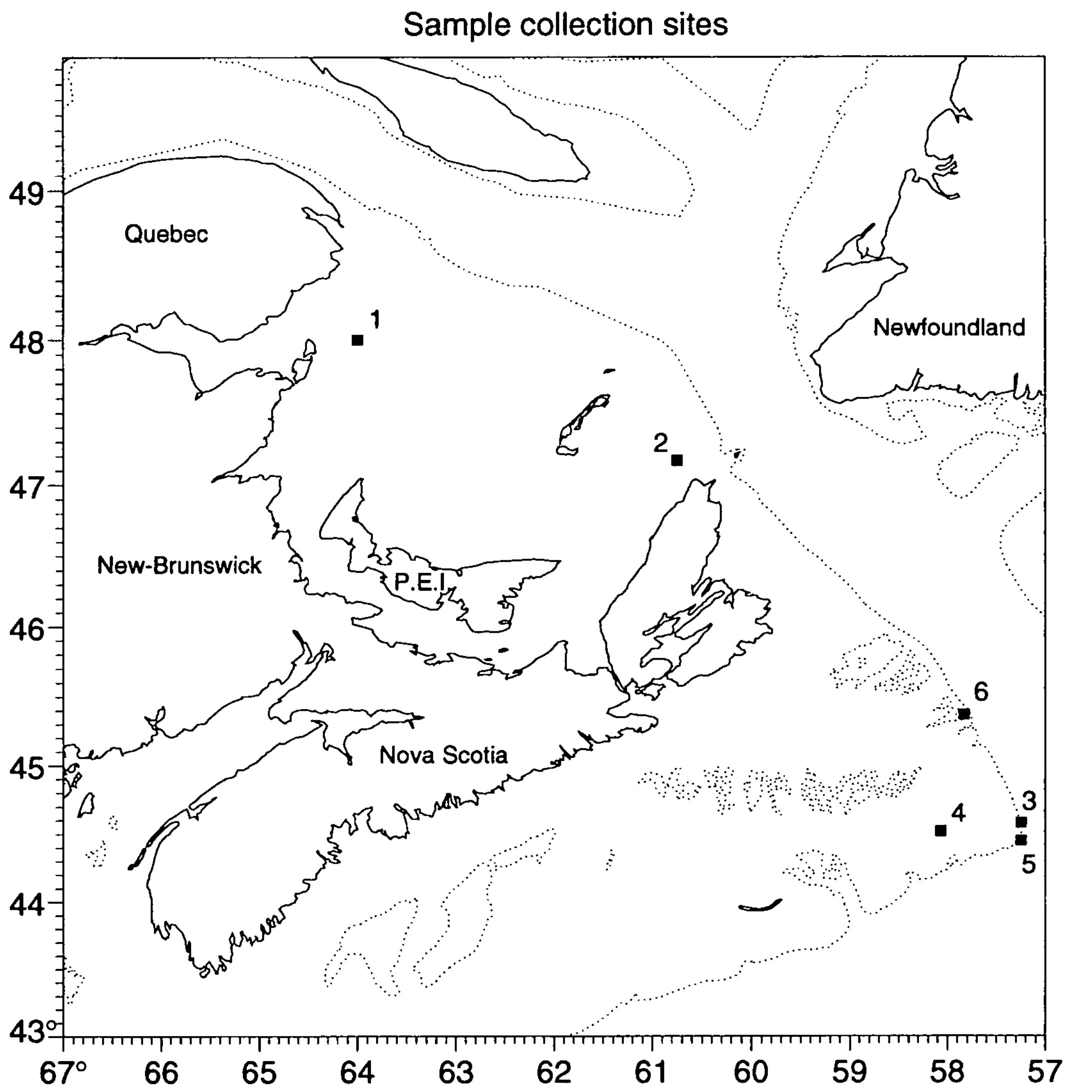

Fig. 1. **Sampling Sites.**

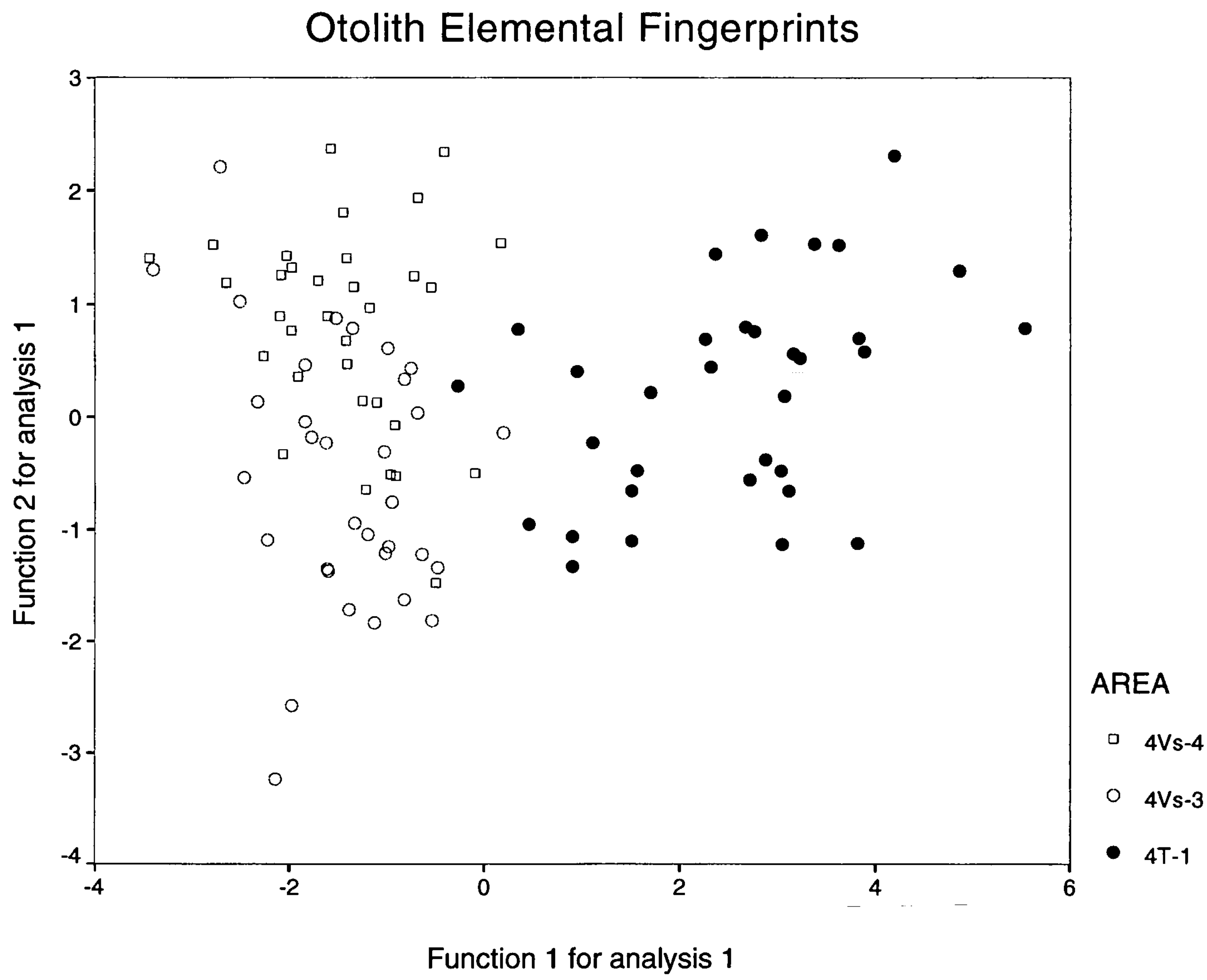

Fig. 2. **Discrimination of western 4T cod from 4Vs cod collected in 1992 (Sample 4) and 1993 (Sample 3) using otolith elemental fingerprints.**

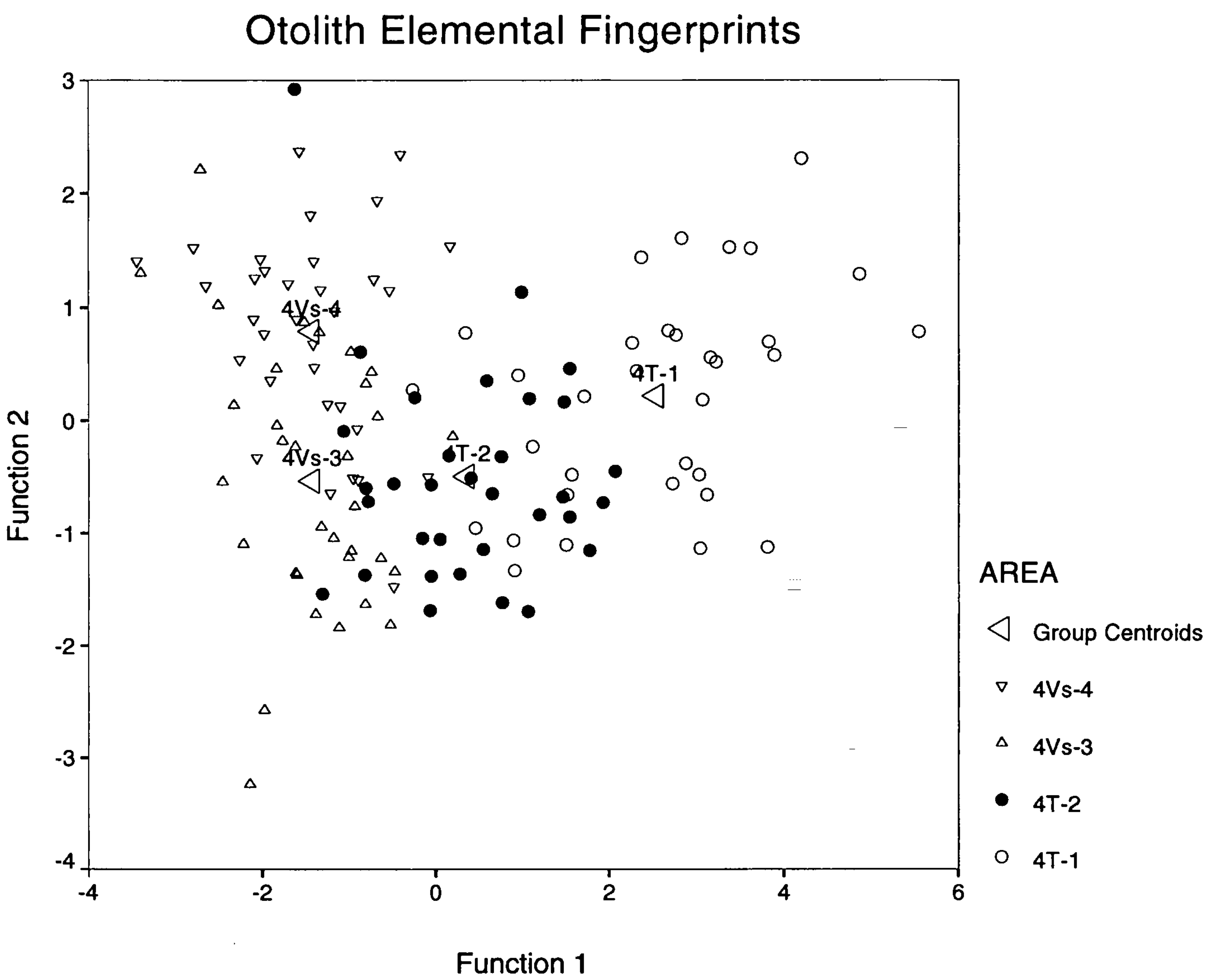

Fig. 3. **Discrimination of 4T cod (4T-1 = west; 4T-2 = east) from 4Vs cod (4Vs - 3 = 1993; 4Vs-4 = 1992) using otolith elemental fingerprints.**

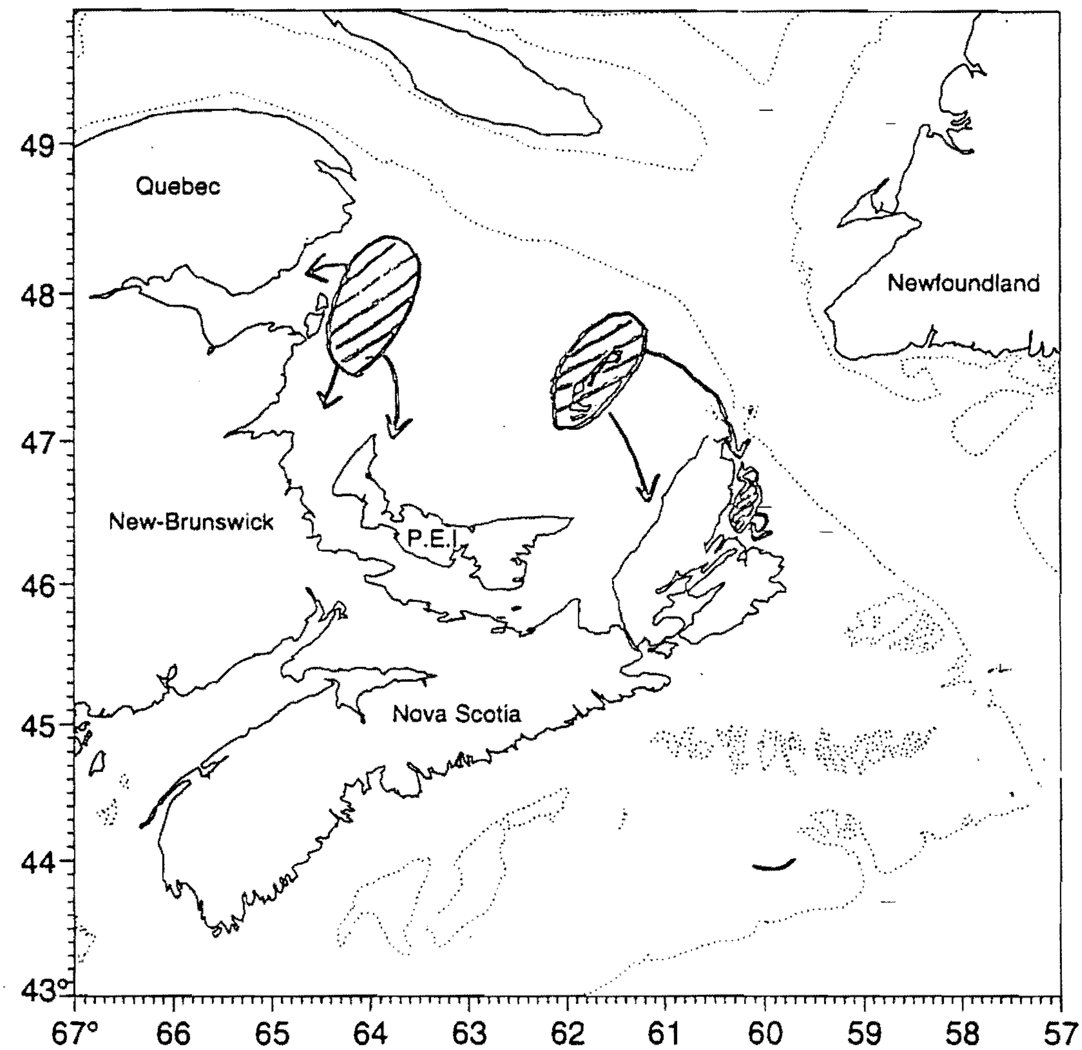

Fig. 4. A possible stock structure for 4T and 4Vn cod.