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## Stock Composition of Cod Aggregations Near the Mouth of the Gulf of St. Lawrence in January 1996 Based on an Analysis of Otolith Elemental Fingerprints

S.E. Campana<sup>1</sup>, G. Chouinard<sup>2</sup>, M. Hanson<sup>2</sup>, A. Fréchet<sup>3</sup>, and J. Brattey<sup>4</sup>

1-Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2

2-Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6

3-Maurice Lamontagne Institute, P.O. Box 1000, Mont Joli, Québec G5H 3Z4

4-Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, NF A1C 5X1

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## ABSTRACT

Dense aggregations of cod were observed in and around the mouth of the Gulf of St. Lawrence during a synoptic survey in January 1996, in keeping with previous winter RV surveys of the Gulf since 1994. The results of previously-reported tagging studies suggested that the 3Pn4RS, 3Ps, 4T, 4Vn and 4VsW stocks probably contributed to the aggregations, but the relative contribution, distribution and biomass of each of the stocks has never been determined. As part of the *High Priority Project on the Identification of Mixed Cod Stocks in the Gulf of St. Lawrence and Approaches*, the various aggregations were surveyed and sampled in January 1996, and 781 adult cod analyzed for stock identity using otolith elemental fingerprints, microsatellite DNA and vertebral counts. Spring spawning aggregations (used as baseline or reference collections) indicated that there were large differences in otolith elemental fingerprints among stocks for all but the 4Vn stock. Maximum likelihood-based stock composition analysis of independent collections of 420 pre-migratory cod in the northern and southern Gulf indicated that the accuracy of the elemental fingerprints as stock identifiers for 3Pn4RS and 4T cod exceeded 95%. Stock composition analysis of the Jan 1996 samples indicated that all stocks were present near the mouth of the Gulf, but that considerable stock separation was maintained. 4T cod dominated the stock composition of catches on the southern edge of the Laurentian Channel from the southern Gulf down to and including the northern edge of Banquereau Bank (4Vs<sub>b</sub>). Cod from 3Pn4Rs comprised most of the catch in the Laurentian Channel, as well as along its northern edge to St. Pierre Bank. 3Ps cod were concentrated most heavily to the west of St. Pierre Bank, where they mixed extensively with 3Pn4RS cod. 4VsW cod were virtually absent from all areas, including the northern edge of Banquereau Bank (4Vs). When calculated on a stock-specific basis, the 3Pn4Rs and 4T stocks together comprised about 94% of the survey biomass of 102,786 t, with the remainder being made up of 3Ps and 4VsW cod. Most of the 4T, and in particular the 3Pn4RS stock biomass, appeared to be represented in the survey area. The distribution of the 3Pn4RS biomass was not in keeping with current management units, indicating that management of a winter fishery would require different boundaries

## RÉSUMÉ

Des concentrations denses de morue ont été décelées à la sortie du golfe du Saint-Laurent et dans les environs pendant un relevé synoptique réalisé en janvier 1996, dans le cadre des relevés d'hiver par NR réalisés dans le golfe depuis 1994. Les résultats d'études par marquage déjà signalés portent à croire que les stocks de 3Pn4RS, 3Ps, 4T, 4Vn et 4VsW ont sans doute contribué aux concentrations, mais l'apport relatif, la distribution et la biomasse de chacun des stocks n'ont jamais été déterminés. Dans le cadre du projet prioritaire sur l'identification des stocks de morue mixtes du golfe du Saint-Laurent et de ses approches, les diverses concentrations ont fait l'objet d'un levé et d'un échantillonnage en janvier 1996 et le stock d'appartenance de 781 morues adultes a été déterminé à partir de la composition en éléments des otolithes, des microsatellites d'ADN et du nombre de vertèbres. Selon les concentrations de frai de printemps (utilisées comme point de départ ou collections de référence), il existait d'importants écarts de la composition élémentaire des otolithes entre tous les stocks sauf celui de 4Vn. L'analyse de la composition des stocks par maximum de vraisemblance de collections indépendantes de 420 morues de stade pré-migratoire du nord et du sud du golfe a montré que l'exactitude de la signature élémentaire à titre d'identificateur des stocks de morue de 3Pn4RS et 4T était supérieure à 95 %. L'analyse de composition des stocks des échantillons de janvier 1996 a montré que tous les stocks étaient présents au voisinage de la sortie du golfe mais qu'il y avait maintien d'une importante séparation entre les stocks. Les morues de 4T dominaient les captures sur le bord sud du chenal Laurentien, du sud du golfe jusqu'à l'extrémité nord du banc Banquereau (4VsB). Les prises effectuées dans le chenal Laurentien, de même que le long de l'extrémité nord du banc Saint-Pierre, étaient surtout constituées de morues de 3Pn4RS. Les morues de 3Ps étaient surtout concentrées à l'ouest du banc Saint-Pierre où elles étaient fortement mélangées à des morues de 3Pn4RS. Les morues de 4VsW étaient pratiquement absentes de toutes les zones, y compris la bordure nord du banc Banquereau (4Vs). Le calcul par stock a montré que les stocks de 3Pn4RS et de 4T représentaient ensemble 94 % de la biomasse des relevés, de 102 786 t, le reste étant représenté par des morues de 3Ps et de 4VsW. La plus grande partie de la biomasse de 4T, et plus particulièrement celle de 3Pn4RS, était représentée dans la zone du relevé. La distribution de la biomasse de 3Pn4RS ne correspondait pas aux unités de gestion actuelles, de sorte que la gestion d'une pêche d'hiver exigerait de modifier les limites.

## INTRODUCTION

It has been known for some time that the approaches and mouth of the Gulf of St. Lawrence serve as the overwintering grounds for several large cod stocks in the area (Templeman 1962; D'Amours et al. 1994). While the northern Gulf (3Pn4RS), southern Gulf (4T), and Cape Breton (4Vn) stocks may use the Laurentian Channel and slope to escape ice cover in the Gulf, use of the warmer deeper waters of the Channel by St. Pierre cod (3Ps) and eastern Scotian Shelf cod (4VsW) probably reflects a seasonal movement into deeper water characteristic of many other cod stocks (Jean 1964). Annual January surveys of the Cabot Strait area which have been carried out since 1994 have confirmed the historic observations, and have documented dense aggregations of cod in various locations along both the northern and southern flanks of the Laurentian Channel (Chouinard 1997). However, the stock composition of these aggregations is unknown. Winter cod catches near the mouth of the Gulf have traditionally been large ( $> 30,000$  t in 1990), but past and current stock assessments have not been able to accurately categorize these catches by stock. Therefore, stock assessments for one or more of the 3Pn4RS, 4T, 4Vn, 3Ps and 4VsW cod stocks may be in error, and indeed, indirect methods have suggested winter catch stock assignment errors of up to 60% in the 4Vs region (Hanson 1995). Stock assignment errors would be particularly acute if the winter cod aggregations tended to be of random stock composition, or alternatively, stock-specific and extending outside of their management unit.

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. 1995). 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 probe techniques can be used to analyze the elemental composition of discrete regions of the otolith (Campana et al. 1997a), 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 once characterized for each of the groups of fish of interest, can be used with considerable confidence to track and identify those same groups of fish over the following months.

*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. Preliminary results of the otolith elemental fingerprint component demonstrated that there were highly significant differences in the fingerprints of the 3Pn4Rs, 4T, 3Ps and 4Vs cod stocks, although further sampling was required to address the issue of a discrete fingerprint for resident 4Vn cod (Campana et al. 1997b). Furthermore, the stock-specific fingerprints were demonstrated to be stable across seasons, and thus suitable for identifying the

stock origin of cod present in the mixed-stock winter aggregations near the mouth of the Gulf. The objectives of the current analysis were four-fold: 1) test the accuracy of the spring-spawner elemental fingerprints as stock identifiers through a maximum likelihood-based analysis of stock composition of fall-collected cod in the Gulf; 2) estimate the set-by-set stock composition of the cod collected during the Jan 1996 RV survey of the Cabot Strait area; 3) map the stock-specific distribution and stock proportions in the survey area; and 4) estimate the stock-specific biomass in the survey area as a whole, as well as in each of the stock management areas.

## 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. 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). Full details are provided in Campana et al. (1997b).

A second set of samples (n=420) was collected in the fall of 1995 prior to any migration out of the Gulf. 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 and 4T cod stocks. 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 provide an independent test of accuracy of the spring elemental fingerprints as stock-specific markers.

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 on the *Wilfred Templeman*, 3-25 January 1996. A total of 138 successful sets were made, of which 60 sets were sampled for otoliths (n=754) and other tissues. Survey biomass was calculated by multiplying the mean weight per standardized tow by the number of trawlable units (2,283,871) in the survey area ( $15,576 \text{ nm}^2$ ). Stock-specific biomass was calculated in the same manner after first calculating stock proportion on a set-by-set basis. Unsampled sets were assumed to contain the same stock proportions as the nearest neighbouring set along a similar depth contour. The resulting biomass distributions were contoured using Delauney triangles in ACON.

The elemental fingerprint of each otolith was characterized by a suite of 5 elements (Li, Mg, Zn, Sr, Ba) using isotope dilution inductively-coupled plasma mass spectrometry (ID-ICPMS), which offers excellent accuracy for these types of assays (Campana et al. 1995; 1997b). MANOVA was used to test for significant differences among samples, while discriminant analysis was used to prepare two-factor elemental fingerprints. Stock composition analysis of both the fall and winter samples was carried out using a maximum likelihood-based analysis programmed in S-Plus. The accuracy of the S-Plus program was confirmed through comparisons with Millar's (1990) HISEAS maximum likelihood-based stock composition analysis. The

reference data for all elemental fingerprints were the spring data, for which stock affinity was known.

## RESULTS AND DISCUSSION

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. The elemental fingerprints of the spring spawning aggregations not only differed significantly and substantially among the 3Pn4RS, 4T, 3Ps and 4Vs cod stocks, but remained stable across seasons (Campana et al. 1997b; Fig. 1). Thus the use of the spring-spawner fingerprints to track and identify cod collected in the fall or winter of the same year is justified. As an independent test of this conclusion, a maximum likelihood-based stock composition analysis of pre-migratory (fall) cod distributed throughout the northern and southern Gulf was carried out using the spring spawner samples as the reference. The results of the analysis indicated that 98.9% of the cod collected in the 4T management area were 4T cod, while 99.2% of those collected in 3Pn4Rs were 3Pn4RS cod. While the actual stock identity of the pre-migratory cod was not known definitively, it is probably reasonable to conclude that cod captured in their own stock area were primarily residents.

Analysis of the fall collections on a site-by-site basis indicated that stock identity was well indicated by location north and south of the Laurentian Channel, as would be expected. However, 3Pn4RS cod dominated the stock composition of cod throughout the Laurentian Channel (Fig. 2). Indeed, virtually no 4T cod were identified anywhere in the Channel, and even cod collected in 4T near the Channel slope were predominantly from the 3Pn4RS stock. However, cod densities were typically low at such sites (Swain and Wade 1993), and the major areas of 4T cod distribution were almost exclusively of 4T origin. Note as well that "origin" as used here refers to the stock area in which most of the life was spent, and not necessarily the genetic origin.

The distribution of cod as surveyed in the Jan 1996 RV was similar to that of previous years (Chouinard 1997), with major areas of concentration along the slopes of both sides of the Laurentian Channel (Fig. 3). Most large catches were made at depths of 250-450 m. To determine if the geographic distribution of the otolith samples was representative of that of the total survey catch, the number of cod collected in the sampled sets was contoured using the same criteria used for the overall survey. The geographic distribution and relative abundance of the sampled sets was similar to that of the complete survey (Fig. 3), indicating that the stock composition analysis results are broadly applicable to the overall survey.

Set-by-set stock composition analyses indicated that 4T cod dominated the stock composition on the southern side of the Channel, while 3Pn4Rs dominated the stock composition of most sets on the northern side (Fig. 4). All large sets were either of 4T or 3Pn4RS origin. 3Ps cod were most abundant to the west of St. Pierre Bank, in areas also occupied by 3Pn4Rs cod. The only significant numbers of 4VsW cod were observed off St. Paul's Island in 4Vn. However, as will be seen later, those numbers are almost certainly artifacts of small classification errors (<1%) associated with very high densities of 4T cod. To a large extent then, the two largest cod stocks

in the area appear to have remained largely disjunct in their distribution, with no evidence of large-scale mixing across the Channel.

When examined on a proportional rather than a numerical basis, some of the patterns in stock composition became more clear. Survey catches on the southern side of the Channel tended to be largely 4T cod, whether in 4T, 4Vn or 4Vs, and independent of fish density (Fig. 5). 4Vs cod were not particularly obvious in 4Vs, and made up only a very small percentage of the catch in the regions in which they appeared, perhaps because of the very low overall stock biomass in recent years. On the other hand, 3Ps cod made up the majority of the catch in some of the regions adjacent to St. Pierre Bank, and formed a significant minority of the catch through much of 3Ps. "Pure" 3Pn4RS catches were most common within the Laurentian Channel proper, but 3Pn4RS cod clearly dominated the catches throughout much of 3Pn and the surveyed region of 3Ps. This conclusion was corroborated by the bimodal length frequency of cod captured in 3Pn and 3Ps during the Jan 1996 survey, which was very similar to that observed in the 3Pn4RS mobile gear sentinel survey the preceding fall (Fréchet and Schwab 1998).

Set-by-set stock composition analyses indicated that individual sets with moderate to large catches were almost always dominated by a single stock. The only exception to this generalization occurred in the area to the west of St. Pierre Bank, in which many of the sets contained large proportions of both 3Ps and 3Pn4RS cod. While integration of small-scale aggregations by the trawl cannot be ruled out, this area more than others showed evidence of true stock mixing. While the area around Burgeo Bank has previously been identified as an area of stock mixing (D'Amours et al. 1994; Rollet et al. 1994), the current study indicates that stock mixing occurs further to the east (eg- between Burgeo and St. Pierre Bank) than had previously been suggested.

The biomass in the Jan 1996 survey was estimated as  $102,786 \pm 58,148$  t. When calculated on a stock-specific basis, the 3Pn4Rs and 4T stocks together comprised about 94% of the biomass, with the remainder being made up of 3Ps and 4VsW cod (Table 1). The minimum trawlable biomass for 3Pn4RS cod (calculated as the mean of the 1995 and 1996 mobile gear sentinel surveys) was somewhat lower than the stock-specific biomass estimated from the Jan 1996 survey (Table 1), suggesting that most or all of the adult stock biomass was represented in the winter survey area. The Jan 1996 survey estimate for the 4T stock was probably slightly underestimated, given the high densities of 4T cod at the extremes of the surveyed area. Nevertheless, the minimum trawlable biomass of adult fish (calculated as the mean of the 1995 and 1995 Sept RV surveys) was not unduly high compared to the Jan survey estimate. For both the 3Pn4RS and the 4T stocks, the winter biomass estimate was between 49-54% of the most recent 3+ biomass estimate from ADAPT. Thus, allowing for the catchability of the trawl, the winter survey appears to have sampled most of the available adult fish for both stocks. Such cannot be said for the 3Ps and 4VsW stocks, which were poorly represented in the survey area. Clearly, these two stocks must overwinter in other areas.

The distribution of stock-specific biomass was not in keeping with current management units, indicating that management of a winter fishery would require different boundaries (Table 1). In particular, less than 1/3 of the 3Pn4RS biomass was observed in the 3Pn4Rs management unit,

with much of the remainder being found well into 3Ps mixed with the resident fish. Similarly, only 6% of the cod collected in the surveyed region of 4Vs were actually of 4Vs origin, with much of the remainder (74%) being 4T. In contrast, the 3Ps stock seemed to be well contained within the portion of the 3Ps management unit that was surveyed, and 92% of the 4T stock was found in the appropriate management unit (4TVn Nov-Apr). The fact that the distribution of the 4T and 3Pn4RS stocks extends well to the east during the winter has long been known (Templeman 1962; Hanson 1995; Rollet et al. 1994). What is surprising though is the very low representation of 4Vs cod in the surveyed 4Vs area, and the considerable extent of mixing between 3Pn4RS and 3Ps cod to the west of St. Pierre Bank. As noted by Shelton et al. (1996), the extension of 3Pn4RS cod into the 3Ps area poses serious problems for the interpretation of the historic annual January 3Ps RV surveys (research surveys in 3Ps have been carried out in April in recent years to help avoid this problem). The high proportion of 3Pn4RS biomass found in the 3Ps survey area reinforces their concerns, and suggests that a January RV survey in 3Ps without stock identifiers would be quite problematic. To the extent that the 1996 results can be generalized to other years, it also suggests that a modified assessment and management unit for 3Ps cod, modelled after that implemented for 4TVn (Jan-Apr) cod, might be useful. However, the 1997 stock composition results (at a minimum) must be examined before any major changes can be implemented.

Additional research on the cod stock identity issue, as well as the question of stock mixing during the winter, is ongoing under the auspices of DFO's Strategic Research Program, *The Identification of Mixed Cod Stocks in the Gulf of St. Lawrence and Approaches*. 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. In particular, the results of the 1997 survey of the spring spawning and winter aggregations will be used examine the extent of inter-annual variability in both the stock-specific elemental fingerprints and the winter stock composition patterns. As well, potential contributions to the winter mixtures from previously uncharacterized stocks (eg- resident 4Vn, coastal Newfoundland) will be examined.

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Table 1. Stock-specific biomass (t) from the Jan 1996 RV survey in each of the management areas based on MLE classification of the otolith elemental fingerprints. 1996 biomass estimates from the 1997 stock assessments are tabled for comparison. The ADAPT biomass estimates are 3+, while the minimum trawlable biomasses are 0+, averaged between summer/fall 1995 and summer/fall 1996 (3+ for 4T). The minimum trawlable biomass for 3Ps cod represents the result of the April 1996 RV survey.

NAFO Area in Jan RV	Stock				
	3Ps	3Pn4RS	4T	4Vs	TOTAL
<b>3Ps</b>	3567	24014	469	542	28839
<b>3Pn4RS</b>	858	12464	2145	798	16365
<b>4T</b>	0	1955	13088	708	15743
<b>4Vn</b>	176	3487	35429	594	39733
<b>4Vs</b>	146	241	1560	169	2116
<b>TOTAL in Jan RV</b>	4747	42160	52691	2810	102796
<b>Minimum summer/fall trawlable biomass</b>	36418	34500	86127	10755	
<b>ADAPT Biomass</b>	60000	78250	107524	35662	

Fig. 1. 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.

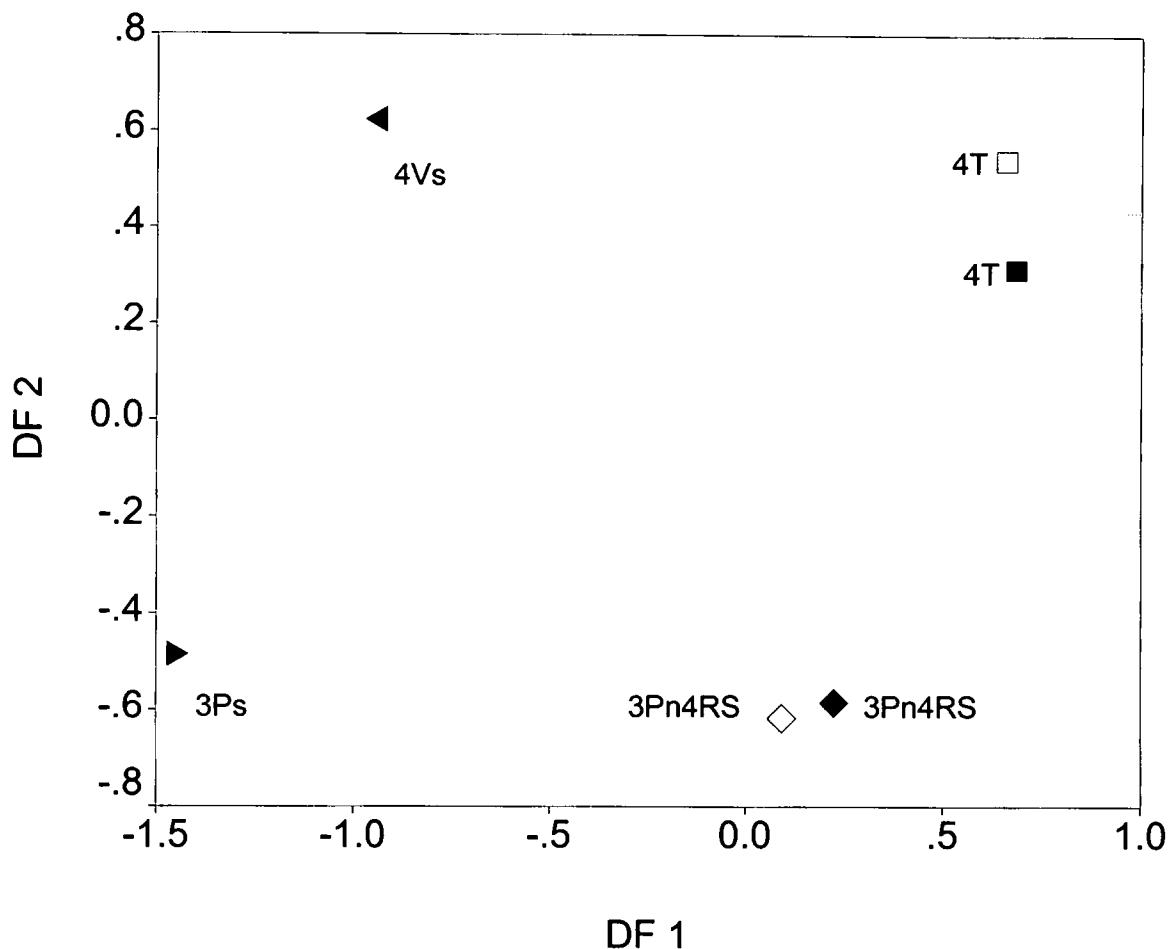


Fig. 2. Stock composition of pre-migratory adult cod collected in Fall 1995 as determined with MLE-based stock composition analysis of otolith elemental fingerprints using spring spawners as the reference. The size of the circle represents the number of fish in the sample (total n=420), while the proportional stock composition is indicated by the colours in the pie. Red=3Pn4RS; green=4T; yellow=4Vs; blue=3Ps. While 4T and 3Pn4RS cod clearly dominated their respective stock areas, 3Pn4RS cod also dominated both slopes and the centre of the Laurentian Channel.

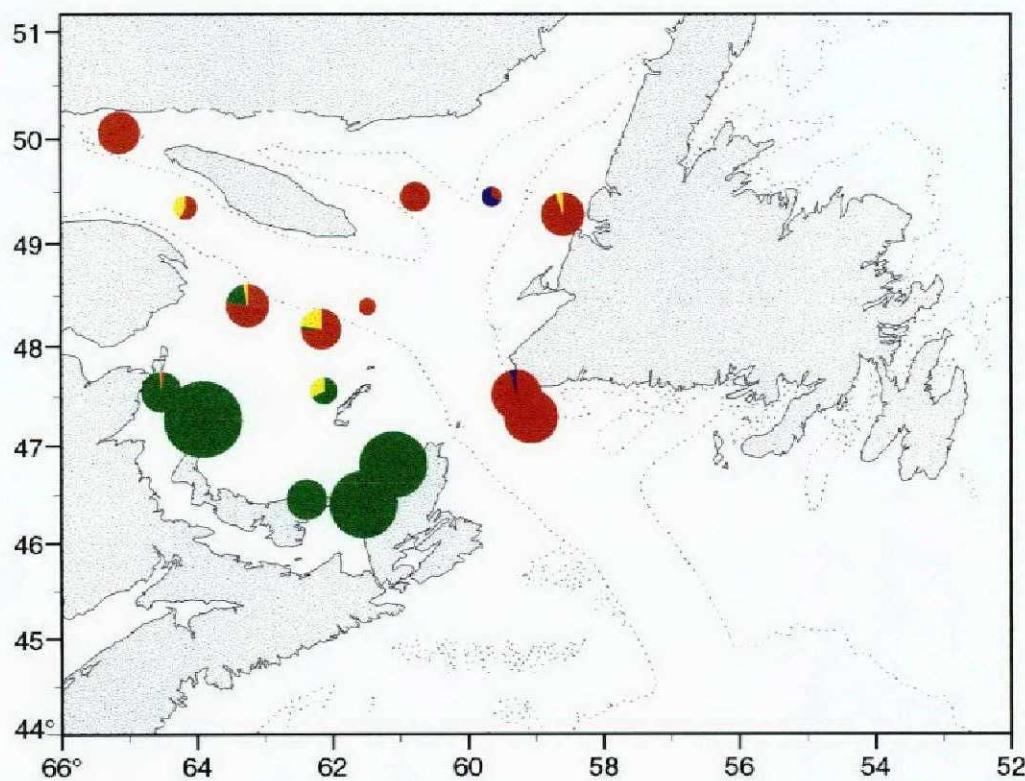


Fig. 3. Distribution and density of cod catches (number/standard tow) in the Jan 1996 RV survey of the Cabot Strait. (top panel) All sets. (bottom panel) Sets from which otoliths for stock composition analysis were sampled.

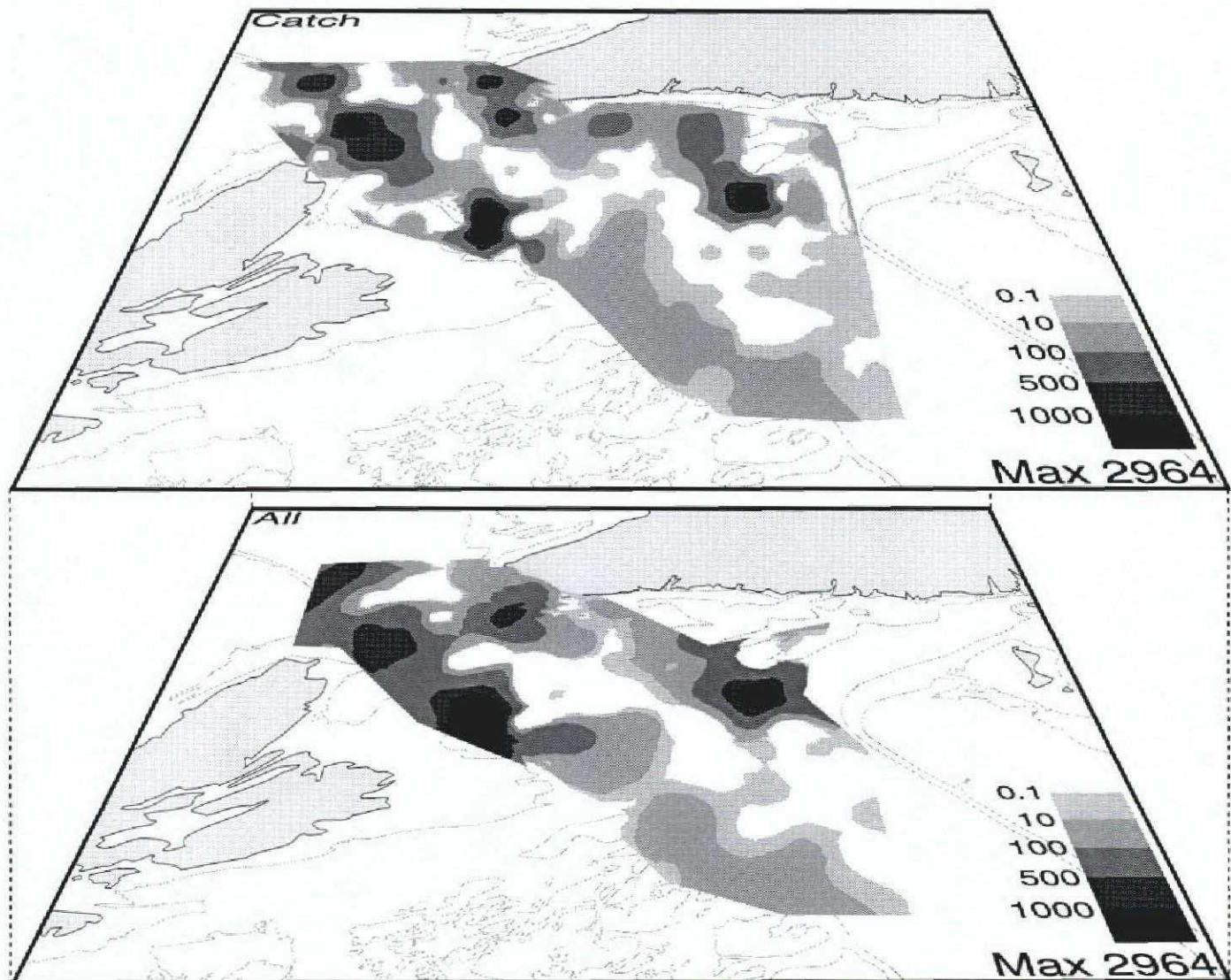


Fig. 4. Stock-specific abundance of adult cod in Jan 1996 as determined with MLE-based stock composition analysis of otolith elemental fingerprints.

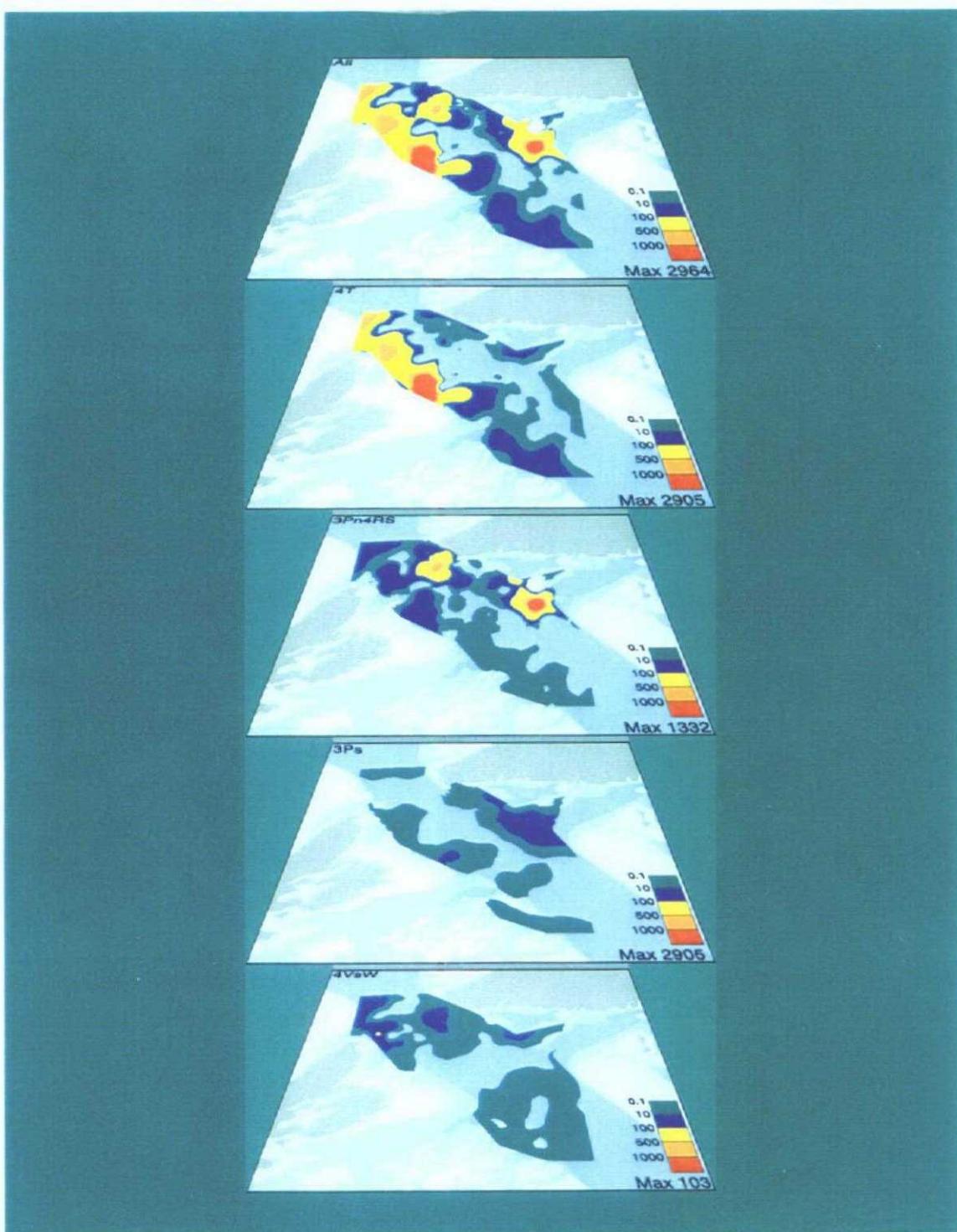


Fig. 5. Proportional stock composition by stock and set in the Jan 1996 RV survey of Cabot Strait. Darker shades represent sets which were dominated by a given stock, independent of the numbers of cod caught in the set.

