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# **An allozyme survey of white hake (*Urophycis tenuis*) from the southern Gulf of St. Lawrence**

by:

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## ABSTRACT

Clay, D., M.M. Ferguson, T. Hurlbut and W. Stott. 1992. An allozyme survey of white hake (*Urophycis tenuis*) from the southern Gulf of St. Lawrence. Can. Tech. Rep. Fish. Aquat. Sci. 1908:12p.

White hake (*Urophycis tenuis*) is a demersal fish species that is exploited by directed, seasonal fisheries with the majority of the catch taken in the southern Gulf of St. Lawrence. Over the past 30 years, the mean landings in the southern Gulf have been about 6,500 tonnes, making it the third most important demersal species. The combined evidence from a discriminant function analysis of morphological characters and previous tagging and distributional studies suggests that at least two geographically separate components (stocks) are exploited in the southern Gulf: an offshore, deep-water component along the slopes of the Laurentian Channel and an inshore, shallow-water component in areas around the Northumberland Strait.

A preliminary enzyme screening using both starch and cellulose acetate gel electrophoresis was conducted to assess the amount of genetic variability between the two groups of white hake. Little allozyme variation was found in samples collected in 1989 or 1990 (27 loci were resolved in 16 enzyme systems, only 6 of these were polymorphic). White hake have inadequate numbers of polymorphic loci for a robust analysis of genetic population structure. Analysis of restriction fragment length polymorphism in mitochondrial DNA has been used to examine other marine species where low levels of allozyme polymorphism have been detected. Such an approach may reveal genetic differences between the two groups of white hake.

## RÉSUMÉ

Clay, D., M.M. Ferguson, T. Hurlbut and W. Stott. 1992. An allozyme survey of white hake (*Urophycis tenuis*) from the southern Gulf of St. Lawrence. Can. Tech. Rep. Fish. Aquat. Sci. 1908:12p.

La merluche (*Urophycis tenuis*) est une espèce de poisson démersal qui est exploitée par une pêche sélective saisonnière. Presque toutes les prises proviennent du sud du golfe du Saint-Laurent. Depuis 30 ans, la moyenne des débarquements réalisés dans le sud du golfe se situe à environ 6500 tonnes, ce qui en fait la troisième plus importante espèce démersale exploitée. Les résultats d'une analyse discriminante des caractères morphologiques et les résultats d'études antérieures d'étiquetage et de répartition semblent indiquer que l'on exploite au moins deux composantes (stocks) de merluche géographiquement distinctes dans le sud du golfe : une composante hauturière qui habite les eaux profondes le long des pentes du Chenal Laurentien et une composante côtière située dans certaines parties peu profondes du détroit de Northumberland.

Nous avons effectué une analyse préliminaire des enzymes, en employant l'électrophorèse sur gel de l'amidon et l'électrophorèse sur gel de l'acétocellulose. Notre objectif était de comparer la variabilité génétique entre les deux populations de merluche. Nous avons trouvé très peu de variation au niveau des allozymes dans les échantillons prélevés en 1989 et en 1990 (vingt-sept loci ont été trouvés dans seize systèmes enzymatiques, dont seulement six étaient polymorphes). La merluche n'a pas suffisamment de loci polymorphes pour permettre une analyse approfondie de la structure de sa population génétique. On a utilisé l'analyse du polymorphisme des sites de restriction dans de l'ADN mitochondrial afin d'examiner d'autres espèces de poisson de mer, telle la morue, où l'on a décelé de faibles niveaux de polymorphisme des allozymes. Une telle analyse pourrait révéler des différences génétiques entre les deux groupes de merluche.

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## DISCLAIMER

The use of brand names in this report should not be taken as an endorsement by either the authors, the Department of Fisheries and Oceans nor the University of Guelph.

## INTRODUCTION

Genetic variation in either protein or DNA as determined by molecular studies can be used to identify stock structure. One of the most common procedures is protein (enzyme) electrophoresis. This technique measures the migration rate (distance travelled) of polymorphic enzymes in a charged electric field. Its technical simplicity and relative ease of application make it the most widely used molecular technique (Egger and Carr, MS 1991). Other more recently developed techniques include restrictive enzyme analysis and direct sequencing via polymerase chain reaction (PCR). As this was a preliminary molecular study of white hake (*Urophycis tenuis*) in the southern Gulf of St. Lawrence, only protein electrophoresis was investigated in this screening.

Two geographically separate concentrations of white hake have been identified in the southern Gulf of St. Lawrence from analysis of annual and seasonal resource survey data (Koeller and LeGresley, 1981; Clay and Hurlbut, MS 1989; Clay, 1991). The two groups are represented by an offshore component found along the slope of the Laurentian Channel and an inshore component ranging from St. Georges Bay, Nova Scotia to Miscou Island, New Brunswick (Figure 1). To test the hypothesis that these two groups represent distinct components or 'stocks', Hurlbut and Clay (MS 1990)

used discriminant function analysis of morphometric and meristic characters. Two distinct groups were identified consisting of:

(1) fish from the southern inshore areas (depth  $\leq 200$  m) of the Gulf, principally the Northumberland Strait area, the 'Strait' component, and

(2) fish from along the slope of the Laurentian Channel (depths  $\geq 200$  m), the 'Channel' component.

These studies along with further analyses by Hurlbut (1990) and a limited tagging study (Kohler, 1971) suggest that the populations of these two areas probably represent separate stocks. If so, the traditional management unit for white hake in the southern Gulf (NAFO Division 4T) would no longer be appropriate. Additional evidence is required to determine whether the two hake populations differ sufficiently in the frequency of allozymes to be designated discrete stocks. The purpose of this co-operative research study was to conduct a preliminary enzyme screen of distinct alleles at defined genetic loci.

This allozyme survey was conducted using both starch and cellulose acetate gel electrophoresis to determine if an adequate amount of genetic variation exists to assess the degree of reproductive separation of white hake in the southern Gulf of St. Lawrence.

## MATERIALS AND METHODS

Samples were collected from the eye, liver and white muscle of white hake caught during the annual September resource survey of the southern Gulf of St. Lawrence in 1989 (RV *Lady Hammond* cruise H204) and 1990 (RV *Lady Hammond* cruise H219). Twenty-three individuals (13 females; 10 males) were taken in 1989, and 42 individuals (23 females; 19 males) in 1990 (Figure 1). The samples from both years were stored in liquid nitrogen immediately after dissection from fresh fish. These samples were then transported to the University of Guelph where the analysis was conducted.

Two methods of protein electrophoresis were used to test for genetic differences between the two groups of white hake. For most enzymes, cellulose acetate electrophoresis was performed using the methods of Hebert and Beaton (1986). Cellulose acetate gels were run for 20 minutes at room temperature, and the starch gels were run for three to four hours at 4°C. For the proteins glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase, starch gel electrophoresis was run on a 12% gel following the method described by May et al (1979). Two buffer systems were used: Tris Glycine, pH 8.5 (TG) and Amino Citrate, pH 7.2 (AC) (Hebert and Beaton 1989).

The proteins (enzymes) examined in this screening, the tissue, the buffer, the International Union of Biochemistry

enzyme classification numbers (EC), and the number of loci are listed in Table 1.

Allele designations, and frequencies are coded according to the recommendations of Shaklee et al. (1989). Alleles were designated according to relative electrophoretic mobility. According to this system, the most common allele at a locus was designated 100 and all others were given higher or lower values depending on the distance travelled through the gel matrix. Allele frequencies were calculated by collection year and according to the depth from which the fish were taken (both years combined). A goodness of fit Chi-square test was used to determine if any deviations from Hardy-Weinburg-Castle proportions existed (both years combined). Contingency Chi-square tests were used to test for differences in allele counts between white hake captured in shallow and deep water areas (sites and years combined).

## RESULTS AND DISCUSSION

Twenty-seven loci were resolved in 16 enzyme systems (Table 1). Of these, six were polymorphic: phosphoglucosmutase (PGM), glucose-6-phosphate dehydrogenase (G6PDH), glucose-6-phosphate isomerase (GPI), isocitrate dehydrogenase-1 and 2 (IDH1 and IDH2) and peptidase B (PEPB) (Table 2; see also Figure 2). Five of these (G6PDH, GPI1, IDH1, IDH2, and PEPB) displayed variation in the 1989 samples (Table 3), and three

(G6PDH, PGM and PEP B) displayed variation in the 1990 samples (Table 4).

White hake in the Gulf of St. Lawrence showed little allozyme variation in either year. In fact, only PEPB has adequate polymorphism for any statistical analysis. The other polymorphic loci had variant alleles at a frequency of less than 5% of samples. Variation at PEPB indicated no departures from random mating conditions (years combined). However, the counts of predominant allele and variant alleles (combined) differed significantly between hake collected from shallow and deep sites (Table 5). This suggests some genetic differences between white hake collected at different depths but can only be considered preliminary because it is based on a single locus.

White hake have inadequate numbers of polymorphic loci for a robust analysis of genetic population structure using protein electrophoresis. Analyses using low numbers of loci and small sample sizes could result in misleading conclusions due to a lack of discriminating power. Examining a small number of polymorphic loci means that only a small part of the genome is sampled, and that effects of evolutionary forces acting on a locus (ie. PEPB) may be missed or over-emphasized.

This indirect method of molecular analysis can detect only a portion of the potential genetic variation at each locus; this limits its detection capabilities in instances where the groups are closely related (Egger and

Carr, MS 1991) as appears to be the case for southern Gulf white hake. Thus it is unlikely that larger sample sizes would have increased the resolving power of allozyme electrophoresis for white hake. Other methods, such as restriction fragment length polymorphism analysis of mitochondrial DNA is an alternative approach to defining stock structure. Working at the DNA level may increase the power to resolve any differences that exist between shallow and deep water white hake. Such an approach has been used to examine other marine species, such as Atlantic cod (*Gadus morhua*) (Payne and Ni 1982, Mork *et al* 1985 and Smith *et al.* 1989) where low levels of allozyme polymorphism have been detected and it will probably be necessary for white hake in the Gulf of St. Lawrence. This more complex and sometimes more powerful technique also has had a great deal of uncertainty associated with it. This was recently illustrated by the discourse between Bentzen and Gauldie in the Canadian Journal of Fisheries and Aquatic Science (Vol 49, 1992:196-199) over Gauldie's (1991) paper.

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Table 1. Proteins investigated in white hake (*Urophycis tenuis*) from the southern Gulf of St. Lawrence.

Protein	EC no.	Tissue	Buffer	No. loci	Poly-morphic
Acid phosphatase	3.1.3.2	L	AC	1	no
Adenosine deaminase	3.5.4.4	L	TG	2	no
Alcohol dehydrogenase	1.1.1.1	L	AC	1	no
Creatine kinase	2.7.3.2	E/M	TG	6	no
Glucose-6-phosphate dehydrogenase	1.1.1.49	M	AC*	1	G6PDH
Glucosephosphate isomerase	5.3.1.9	M	TG	2	GPI
Beta-Glucuronidase	3.2.1.31	L	TG	2	no
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	L	AC	1	no
Isocitrate dehydrogenase	1.1.1.42	M/L	AC*	2	IDH1 IDH2
Lactate dehydrogenase	1.1.1.27	E/M	AC	2	no
Malate dehydrogenase	1.1.1.37	L	AC	1	no
Mannose-6-phosphate	5.3.1.8	L	AC	1	no
Peptidases Glycyl-L-leucine (A) DL-Leucylglycylglycine (B)	3.4.-.-	M	TG	2	PEPB
Phosphoglucomutase	5.4.2.2	M	TG	1	PGM
Sorbitol dehydrogenase	1.1.1.14	L	TG	1	no
Superoxide dismutase	1.15.1.1	L	TG	1	no

EC no. - Enzyme classification number

E = eye, L = liver, M = muscle

AC - Amino Citrate \* Run on starch gel

TG - Tris Glycine

Table 2. Genotypes at 6 polymorphic loci in white hake (*Urophycis tenuis*) collected from the southern Gulf of St. Lawrence in 1989 and 1990.

Numerals indicate the relative distance moved by the alleles, the most common distance being set to 100, those in bold type are those individuals exhibiting polymorphic enzymes.

Fish sampled in 1989 (RV cruise H204)

Fish No.	Strata*	Set	Zone	Sex	G6PDH	PEPB	GPI	IDH1	IDH2	PGM
1	433	1	s	f	100/100	100/100	100/100	100/100	100/100	100/100
2	433	1	s	m	100/100	100/100	<b>100/ 44</b>	100/100	100/100	100/100
3	433	5	s	m	100/100	100/100	100/100	100/100	100/100	100/100
4	433	5	s	f	100/100	100/100	100/100	100/100	100/100	100/100
5	437	10	s	m	100/100	100/100	100/100	100/100	<b>125/100</b>	100/100
6	437	10	s	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
7	437	11	s	m	100/100	100/100	100/100	100/100	100/100	100/100
8	437	11	s	f	100/100	100/100	100/100	100/100	100/100	100/100
9	439	17	d	m	<b>114/100</b>	100/100	100/100	100/100	100/100	100/100
10	439	17	d	f	100/100	100/100	100/100	100/100	<b>125/100</b>	100/100
11	439	17	d	f	100/100	100/100	100/100	100/100	100/100	100/100
12	415	66	d	f	100/100	100/100	100/100	100/100	100/100	100/100
13	415	66	d	m	100/100	100/100	100/100	100/100	100/100	100/100
14	415	66	d	m	100/100	100/100	<b>100/ 44</b>	100/100	100/100	100/100
15	415	66	d	f	100/100	100/100	100/100	100/100	100/100	100/100
16	416	70	s	f	100/100	100/100	100/100	100/100	100/100	100/100
17	416	70	s	m	100/100	100/100	100/100	100/100	100/100	100/100
18	416	70	s	f	100/100	100/100	100/100	100/100	100/100	100/100
19	416	70	s	f	100/100	<b>108/108</b>	100/100	100/100	100/100	100/100
20	422	89	s	m	100/100	100/100	100/100	<b>100/ 40</b>	100/100	100/100
21	422	89	s	f	100/100	100/100	100/100	100/100	100/100	100/100
22	422	90	s	f	100/100	100/100	100/100	100/100	100/100	100/100
23	422	90	s	m	100/100	100/100	100/100	100/100	100/100	100/100

s - shallow <200 m  
d - deep ≥200 m

m - male  
f - female

\* - for strata definitions see Hurlbut and Clay (MS 1991) and Figure 2.

Table 2. continued

## Fish sampled in 1990 (RV cruise H219)

Fish No.	Strata	Set	Zone	Sex	G6PDH	PEPB	GPI	IDH1	IDH2	PGM
1	433	2	s	f	100/100	-	100/100	100/100	100/100	100/100
2	433	2	s	f	100/100	-	100/100	100/100	100/100	100/100
3	433	2	s	f	100/100	-	100/100	100/100	100/100	100/100
4	433	2	s	m	100/100	-	100/100	100/100	100/100	100/100
5	403	5	s	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
6	403	5	s	f	100/100	<b>100/108</b>	100/100	100/100	100/100	100/100
7	403	5	s	m	100/100	100/100	100/100	100/100	100/100	100/100
8	403	5	s	m	100/100	<b>100/108</b>	100/100	100/100	100/100	<b>100/ 77</b>
9*	-	d5	-	f	100/100	100/100	100/100	100/100	100/100	<b>100/ 77</b>
10*	-	d5	-	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
11	439	28	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
12	439	28	d	m	100/100	<b>108/108</b>	100/100	100/100	100/100	100/100
13	439	28	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
14	439	28	d	f	100/100	100/100	100/100	100/100	100/100	100/100
15	439	28	d	f	100/100	<b>113/100</b>	100/100	100/100	100/100	100/100
16	439	28	d	m	100/100	<b>108/108</b>	100/100	100/100	100/100	100/100
17	439	28	d	m	100/100	<b>108/108</b>	100/100	100/100	100/100	<b>100/ 77</b>
18	439	28	d	m	100/100	<b>113/113</b>	100/100	100/100	100/100	100/100
19	439	28	d	f	100/100	100/100	100/100	100/100	100/100	100/100
20	439	28	d	m	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
21	439	28	d	m	100/100	<b>113/108</b>	100/100	100/100	100/100	100/100
22	439	28	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
23	439	28	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
24	439	28	d	f	100/100	100/100	100/100	100/100	100/100	100/100
25	439	28	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
26	439	28	d	m	100/100	100/100	100/100	100/100	100/100	100/100
27	439	28	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
28	439	28	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
29	439	28	d	m	<b>114/100</b>	<b>113/108</b>	100/100	100/100	100/100	100/100
30	439	28	d	m	100/100	<b>113/108</b>	100/100	100/100	100/100	100/100
31	415	103	d	m	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
32	415	103	d	m	100/100	100/100	100/100	100/100	100/100	100/100
33	415	103	d	f	<b>114/100</b>	<b>108/108</b>	100/100	100/100	100/100	100/100
34	415	103	d	f	100/100	<b>108/108</b>	100/100	100/100	100/100	100/100
35	415	103	d	m	100/100	<b>108/100</b>	100/100	100/100	100/100	<b>100/ 77</b>
36	415	103	d	f	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
37	415	103	d	m	100/100	100/100	100/100	100/100	100/100	100/100
38	415	103	d	f	100/100	<b>113/108</b>	100/100	100/100	100/100	100/100
39*	-	121	-	m	100/100	<b>113/113</b>	100/100	100/100	100/100	100/100
40*	-	121	-	f	100/100	100/100	100/100	100/100	100/100	100/100
41*	-	141	-	m	100/100	<b>108/100</b>	100/100	100/100	100/100	100/100
42*	-	141	-	m	100/100	100/100	100/100	100/100	100/100	100/100

\* Data for strata and depth of these fish were not recorded.

Table 3. Allele frequencies (of relative mobility in a charged field) at polymorphic loci in white hake (*Urophycis tenuis*) collected from the southern Gulf of St. Lawrence - 1989 samples.

Locus	Alleles	Allele frequency
Isocitrate dehydrogenase-1	100 40	0.978 0.022
Isocitrate dehydrogenase-2	100 125	0.957 0.043
Glucosephosphate isomerase	100 44	0.957 0.043
Glucose-6-phosphate dehydrogenase	100 114	0.978 0.022
Peptidase B	100 108	0.935 0.065

Table 4. Allele frequencies (of relative mobility in a charged field) at polymorphic loci in white hake (*Urophycis tenuis*) collected from the southern Gulf of St. Lawrence - 1990 samples.

Locus	Alleles	Allele frequency
Glucose-6-phosphate dehydrogenase	100 114	0.976 0.024
Peptidase B	100 113 108	0.441 0.132 0.427
Phosphoglucomutase	100 77	0.964 0.024

Table 5. Allele frequencies (of relative mobility in a charged field) at polymorphic loci in white hake (*Urophycis tenuis*) collected from shallow (N = 20 at  $\leq 200$  m) and deep (N = 35 at  $\geq 200$  m) depths in the southern Gulf of St. Lawrence in 1989 and 1990.

Locus	Alleles	Depth	
		shallow	deep
Glucose-6-phosphate dehydrogenase	100	1.000	0.957
	114	0.000	0.043
Peptidase B *	100	0.850	0.529
	108	0.150	0.371
	113	0.000	0.100
Isocitrate dehydrogenase 1	100	0.975	1.000
	44	0.025	0.000
Isocitrate dehydrogenase 2	100	0.975	0.986
	125	0.025	0.014
Phosphoglucomutase	100	0.975	0.971
	77	0.025	0.029

\* Counts of 100 and variant alleles (108 and 113) differ significantly between samples (Chi-square = 15.00, df = 1,  $P < 0.001$ ). All other loci show inadequate amounts of polymorphism for an equivalent analysis.

Figure 1. Sample locations and numbers of fish collected at each site for the allozyme survey of white hake (*Urophycis tenuis*) in the southern Gulf of St. Lawrence.

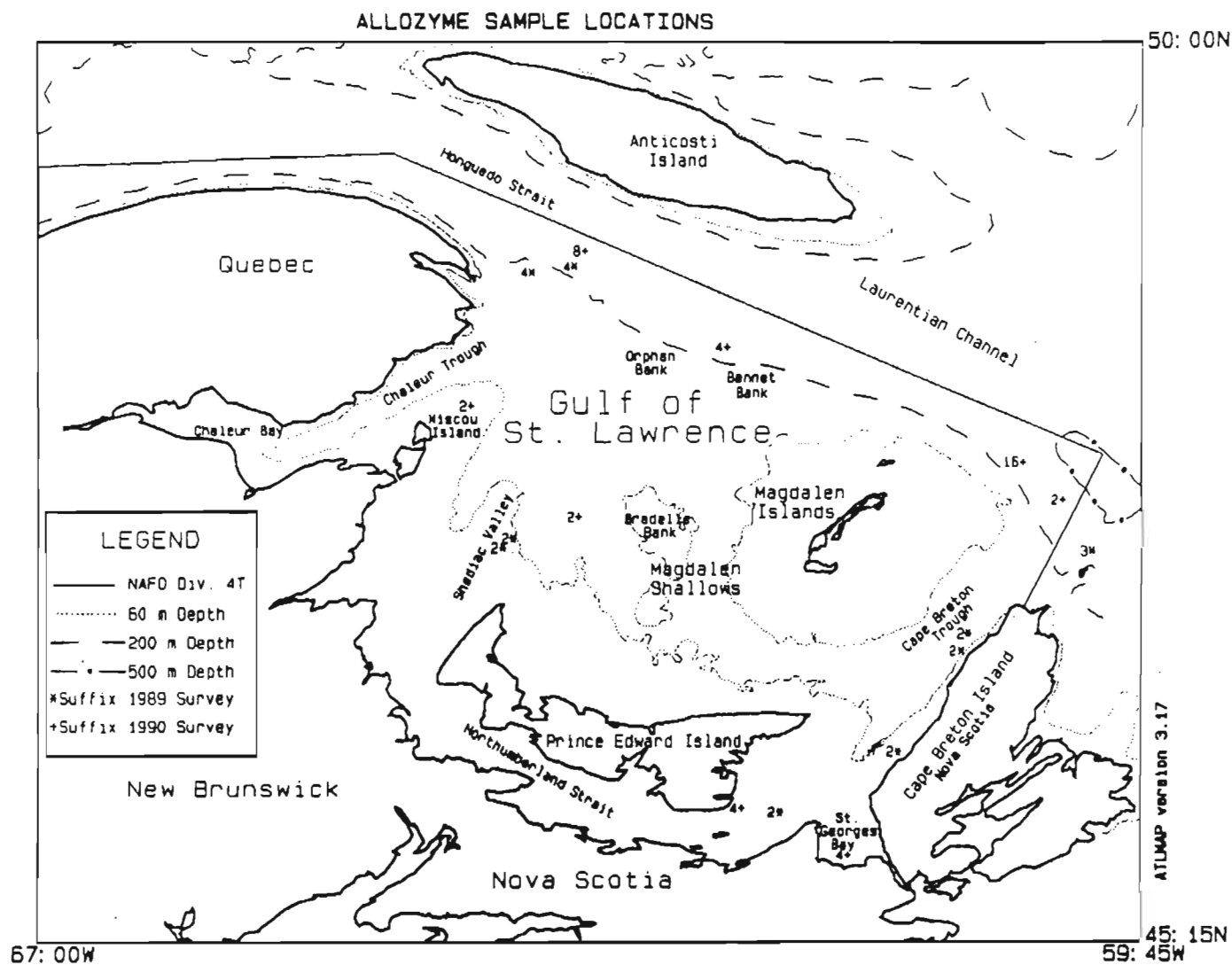


Figure 2. Map of southern Gulf of St. Lawrence showing the stratum boundaries with labels marking each stratum.

