MORPHOLOGICAL AND BIOCHEMICAL HETEROGENEITY IN POPULATIONS OF MICROGADUS TOMCOD (WALBAUM, 1792)

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MORPHOLOGICAL AND BIOCHEMICAL HETEROGENEITY IN POPULATIONS OF *MICROGADUS TOMCOD* (WALBAUM, 1792)

by

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Presented to

Department of Biology

Memorial University of Newfoundland

In Partial Fulfillment

for the Degree of

Master of Science

August 1973

ABSTRACT

The tomcod, *Microgadus tomcod*, as compared to the other species of the same family, Gadidae, is a little known species. It inhabits both marine and freshwater environments and seldom undertakes long migrations.

The muscle myogen, lactic dehydrogenase (LDH) and liver esterase patterns obtained by starch gel electrophoresis and the morphometric variation in specimens of the tomcod *(Microgadus tomcod)* from five localities are compared. The pattern variations observed in the electrophoretic analysis and the variations observed in the morphological analysis are discussed in relation to geographic location and temperature.

The morphological and meristic data revealed a strong northward latitudinal cline. The main muscle myogen, LDH and esterase patterns are similar for all the populations but with polymorphism occurring in each. The populations can be separated using the ratios of the main patterns obtained.

Numerical clustering analysis revealed three populations using the morphometric data. Using the biochemical data it revealed the New York population as differing the most.

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ACKNOWLEDGEMENTS

The author wishes to thank Dr. G. I. McT. Cowan for his suggestion of the problem and his encouragement and supervision throughout the duration of the study.

I wish to thank Dr. D. G. Copeman for his continuing help throughout all stages of the project. Dr. J. M. Green gave valuable advice and helpful discussion regarding the study.

Many people assisted in the collections, but I especially wish to thank the following people: Mr. Vianney Legendre, Director, Research Laboratory, Quebec Wildlife Service, assisted in obtaining all of the Quebec specimens; Mr. Gordon Beckett, New York Fish and Wildlife Service, assisted in obtaining the New York collection; and Dr. A. W. H. Needler, Executive Director, Huntsman Marine Laboratory, obtained the New Brunswick specimens.

During the course of this study financial support was received from a Memorial University fellowship. National Research Council of Canada Grant No. 5072 awarded to Dr. G. I. McT. Cowan assisted in travel expenses.

Finally, I wish to thank my wife, Enchie, and my son, Nick, who both have been patient and understanding throughout the period of this study.

INTRODUCTION

The purpose of detecting geographic variation in biological systematics is to describe and summarize the limits of expression of the organism over an area. The most frequently studied characters are the morphological ones, but recently there have been studies of biochemical, physiological, immunological and genetic characters. These investigations can result in information useful for the allocation of unknown specimens to a given population or geographic locality.

Microgadus tomcod (Walbaum, 1792) shows several qualities which make it a suitable species to study intraspecific heterogeneity. *M. tomcod* inhabits shallow coastal and brackish water from Virginia to southern Labrador (Leim and Scott, 1966). It is landlocked in Lac St. Jean, Quebec (Legendre and Lagueux, 1948) and in Deer Lake, Newfoundland (Scott and Crossman, 1964). The species therefore occupies a wide range of habitats but since it seldom strays from shore (Leim and Scott, 1966) it may be that there is minimal movement between localities.

In December and January, tomcods usually migrate upstream where spawning takes place. The eggs have been described as being free and heavy (Baird, 1887; Booth, 1969). Booth describes tomcod larvae as relatively non buoyant. The previous authors and Svetovidov, 1948; Herman, 1963; Merriman, 1947; Percy and Richards, 1962; Perlmutter, 1939, and Richards, 1959, have only reported pelagic stages of

Microgadus larvae from estuarine situations. These characteristics of *M. tomcod* presumably give the populations of this species a degree of geographic isolation not generally found in species with pelagic eggs and larvae which can be widely dispersed.

The object of this study is to determine whether geographical isolates of the tomcod can be identified on the bases of morphometric and some "biochemical" characters. Also, the type and quantity of intraspecific heterogeneity will be considered. The use of the term Geographical Isolate conforms to Mayr's (1969) definition. All populations or groups of populations, which have only limited or no gene exchange with other populations of the species, are designated as geographical isolates.

MATERIALS AND METHODS

MATERIALS

The material studied comprised 474 specimens from 5 localities (Table I). The specimens were kept frozen at -30°C until used.

TABLE I--Localities from which *M. tomcod* Specimens were Examined.

Pop. No.	Locality	Approx. lat.N	Approx. long.W	Total Number Specimens Examined: Morphological	Total Number Specimens Examined: Biochemical
(1)	New Brunswick Passamaquoddy Bay (Digdequash River)	45°06'	67°10'	60	175
(2)	Newfoundland St. George's Bay (Harry's River)	48 30	58 30	60	154
	Quebec				
(3)	St. Lawrence estuary (Ste. Anne-de-la-Perade)	46 33	72 18	60	107
(5)	Lac St. Jean	48 34	72 18	13	6
(4)	New York Hudson River (Peekskill)	41 40	75 50	60	32

The tomcod populations will be identified either by locality name or population number, as in Table I, throughout the text.

METHODS

Meristic Characters

The following meristic characters were counted: dorsal fin rays, anal fin rays, pectoral fin rays, pelvic fin rays, vertebrae (precaudal and caudal), lateral line scales, scales above lateral line, scales below lateral line, gill-rakers, and pyloric caeca. Counts were taken as defined by Hubbs and Lagler (1958) with the following exception:

Lateral line scales: counts on the first row below the lateral line on the right side.

The vertebrae were counted from X-ray photographs.

Measurements

The following morphometric characters were measured: standard length, caudal peduncle length, caudal peduncle depth, predorsal length, preanal length, head length, snout length, eye length, upper jaw length, and first, second, and third pelvic fin ray length. Measurements were taken as defined by Hubbs and Lagler (1958) with the following exceptions:

Caudal peduncle depth: depth at the origin of caudal fin.

Preanal length: distance from the tip of the snout to the structural base of the first anal fin ray.

Upper jaw length: length from the anteriormost point of the snout to the posteriormost point of the maxillary.

First, second, and third pelvic fin ray length: distance from the structural base of the pelvic fin to the farthest tip of the ray.

Measurements were taken to the nearest tenth of a millimeter using Helios dial calipers.

Electrophoretic Procedure

Preparation of muscle samples. A 0.2 to 1.0 gm. of skeletal muscle was taken between the first and second dorsal fins from the left side of the specimen. This sample was diluted 1:1 with distilled water, homogenized with a Viritis 45 Micro Homogenizer until a smooth consistency was obtained (about 15 to 20 seconds), centrifuged at 30,000 x g for one hour at 0° to 1°C and the supernatant was decanted and stored at 0° to 1°C. Prior to use, the samples were recentrifuged at 1,700 x g for at least 4 hrs.

Preparation of liver samples. A 1.0 to 3.0 gm. of liver was taken, diluted 1:1 with distilled water, homogenized with a Bronwill Biosonik III using between 21 watts/cm^2 to 63 watts/cm^2 for 5 to 15 secs. and centrifuged one hour at 30,000 x g at 0° to 1°C. Prior to use, the supernatant was recentrifuged at 1,700 x g for at least 12 hrs.

Starch gel electrophoresis. Micro starch gel electrophoresis was performed according to Tsuyuki *et al.* (1966a). The bridge and gel buffer system was borate pH 8.5 of 0.3 and 0.023 M. respectively. The gels were made 12% using Connaught Laboratories, Toronto, hydrolized starch. Alternate buffer systems were employed but resolution was found to be best with the borate system.

Electrophoretic runs were made at 0° to 2°C using 210 volts and run time being 30 minutes for esterase, 60 minutes for muscle myogen and 90 minutes for lactic dehydrogenase (LDH).

A 0.01% solution of Amido Black 10B in gel wash (5:5:1,

distilled water, methanol and acetic acid) was used in the detection of muscle myogen patterns. LDH isozyme patterns were detected utilizing a formazine precipitation reaction as described by Colowick and Kaplan (1963). Esterase patterns were detected using the method of Markert and Hunter (1959) with alpha napthyl acetate.

Analysis of Data

The data was studied using analysis of variance (Steel and Torrie, 1960; p. 99), Student-Newman-Keuls (SNK) comparison among ordered means (Sokal and Rohlf, 1969; p. 239) and Pearson's correlation coefficients. T-tests were carried out to test for sexual dimorphism.

Graphic comparison of meristic data was plotted using a modification of the Hubbs and Hubbs (1953) method by Eberhardt (1968). The difference is that the standard error is not multiplied by two but by the square root of the number of populations studied.

The unweighted group method was used for the numerical clustering analysis (Sokal and Sneath, 1963; pp. 290-311).

Chi-square test was used to analyze the significance of bands present among the populations for the biochemical results.

Coefficient of Difference (CD) values were calculated as described by Mayr (1969). Values of 1.28 and higher are indicative of subspecific level of differentiation. At this value, 90% of the animals in each of the two populations being compared differ from one another.

RESULTS

MORPHOLOGICAL INVESTIGATION

Meristic Characters: Geographic Variation

Significant differences occurred in all the meristic characters investigated except for the first anal, pectoral and pelvic fin ray number. Results are shown in Figures 1 and 2, and Table II. Coefficient of difference (CD) values are listed in Table VII.

Meristic Characters: Sexual Dimorphism

Sexual dimorphism was examined for each locality and combination of all five localities (Table VIII).

New Brunswick: slight sexual dimorphism was expressed. Significant differences occurred in the third dorsal fin, gill-rakers, precaudal vertebrae and scales above lateral line. Except for gill-rakers, the females had the higher mean.

Newfoundland: lateral line scales in which the males had a higher mean was the single character showing significant sexual dimorphism.

New York: second and third dorsal fin ray number showed a significant difference. In both, the females have the larger mean.

St. Lawrence estuary and Lac St. Jean: no sexual dimorphism was evident.

Combination of all 5 localities: first dorsal fin, precaudal and total vertebrae showed significant differences. Females had a larger

FIGURE 1

Graphic comparison of meristic characters of *M. tomcod* for five localities. The mean is shown by short vertical line, and the range by a horizontal line. The black part of each bar indicates standard error of the mean x $\sqrt{\text{no. of localities}}$ on either side of the mean. One half of each black bar plus the white bar at either end represent one standard deviation on either side of the mean. Localities shown north to south.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

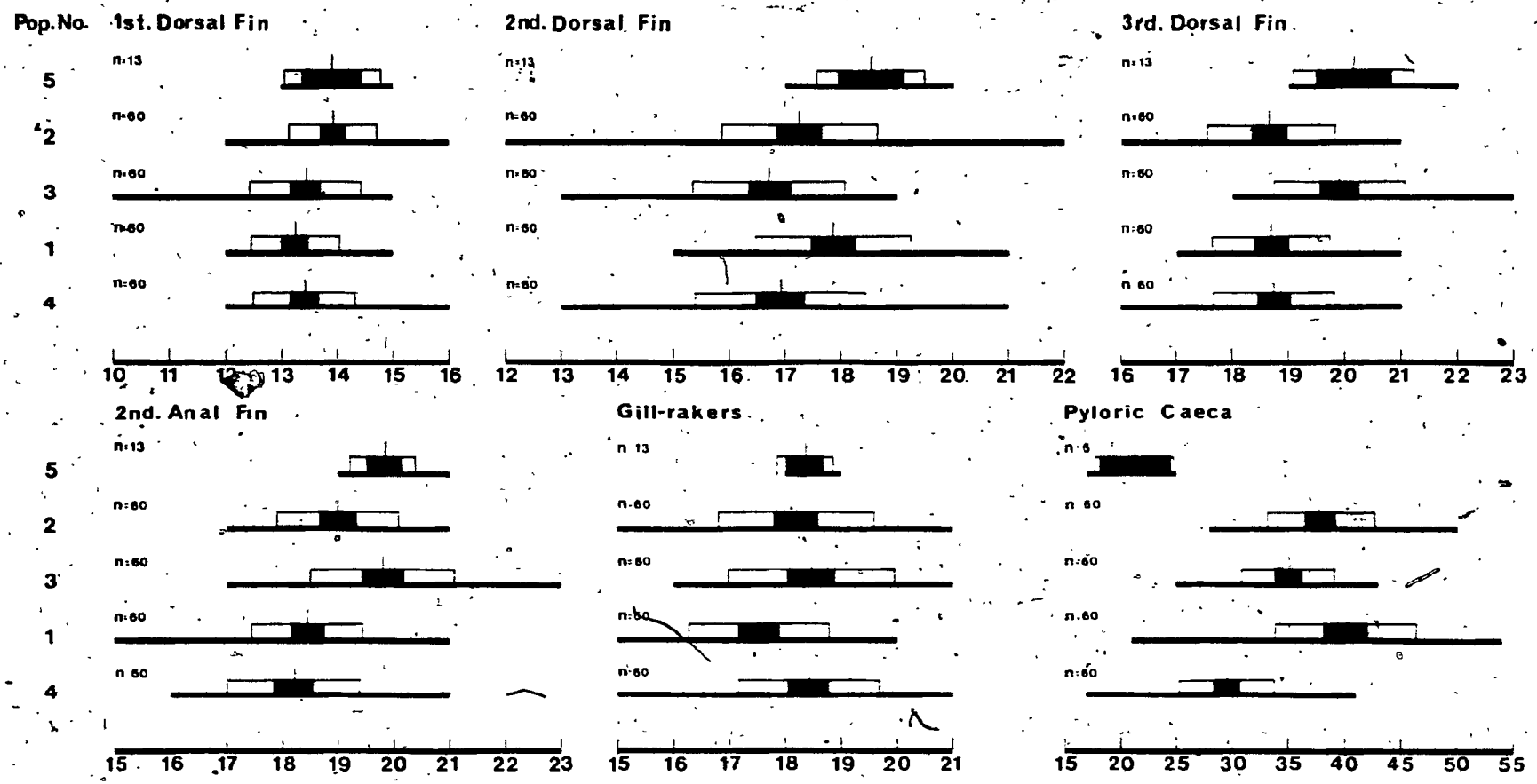

Pop.No.
1st. Dorsal Fin
2nd. Dorsal Fin
3rd. Dorsal Fin
2nd. Anal Fin
Gill-rakers
Pyloric Caeca
5
2
3
1
4
n=13
n=60
10 11 12 13 14 15 16
12 13 14 15 16 17 18 19 20 21 22
16 17 18 19 20 21 22 23
15 16 17 18 19 20 21 22 23
15 16 17 18 19 20 21
15 20 25 30 35 40 45 50 55

FIGURE 2

Graphic comparison of meristic characters of *M. tomcod* for five localities. The mean is shown by short vertical line, and the range by a horizontal line. The black part of each bar indicates standard error of the mean x $\sqrt{\text{no. of localities}}$ on either side of the mean. One half of each black bar plus the white bar at either end represent one standard deviation on either side of the mean. Localities shown north to south.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

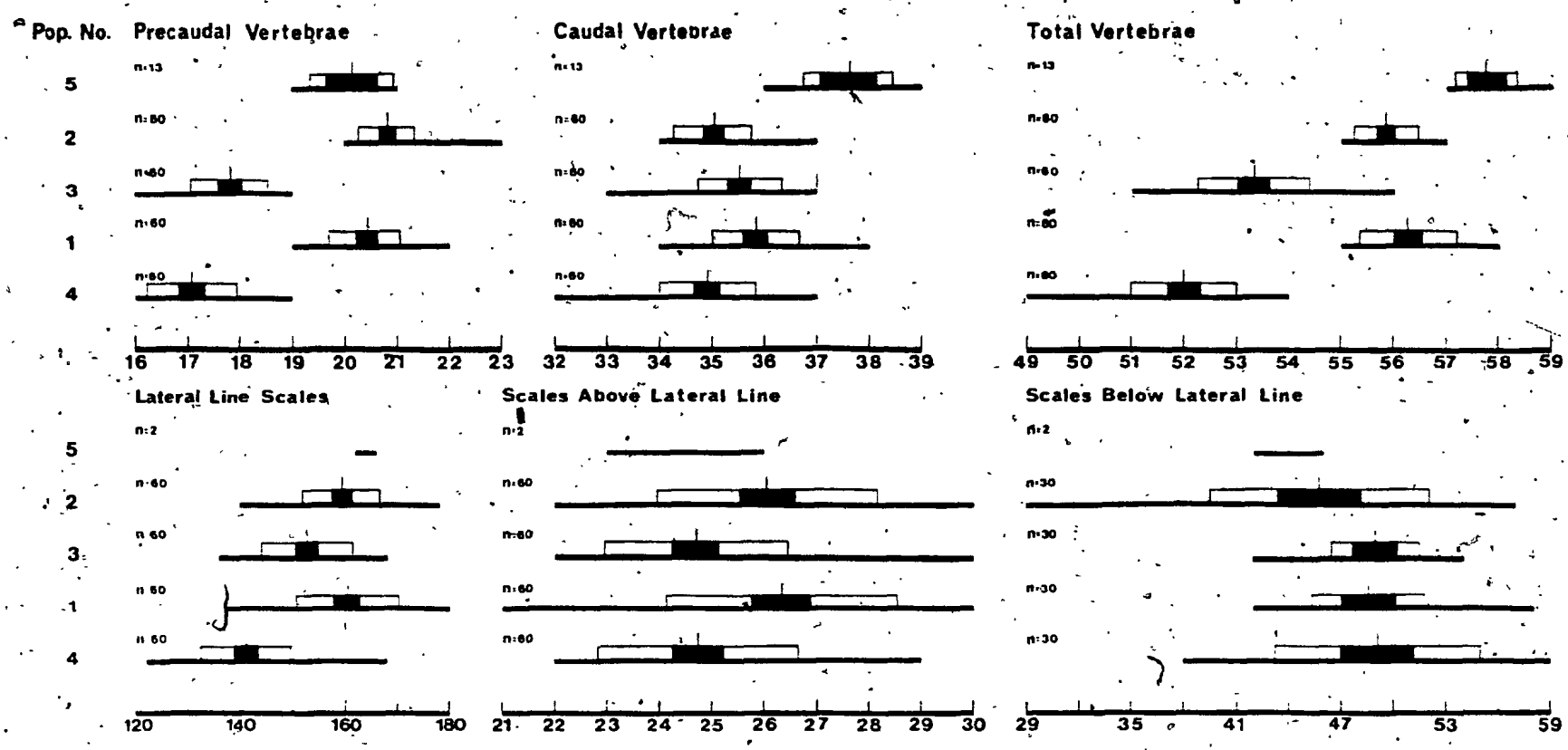

Pop. No.
Precaudal Vertebrae
Caudal Vertebrae
Total Vertebrae
5
2
3
1
4
n=13
n=60
16 17 18 19 20 21 22 23
32 33 34 35 36 37 38 39
49 50 51 52 53 54 55 56 57 58 59
Lateral Line Scales
Scales Above Lateral Line
Scales Below Lateral Line
n=2
n=30
120 140 160 180
21 22 23 24 25 26 27 28 29 30
29 35 41 47 53 59

TABLE II

Significance Matrices of Interpopulation Variation in Meristic Characters of *M. tomcod* (significance indicated by **, $p < 0.05$). Results are summarized on the right. Any means not underscored by the same line are significantly different.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

A. First Dorsal Fin Rays

2	**			
3	--	**		
4	--	**	--	
5	**	--	**	**
	1	2	3	4

2	5	3	4	1
13.93	13.92	13.43	13.42	13.27

B. Second Dorsal Fin Rays

2	--			
3	**	--		
4	**	--	--	
5	**	**	**	**
	1	2	3	4

5	1	2	4	3
18.54	17.87	17.27	16.92	16.72

C. Third Dorsal Fin Rays

2	--			
3	**	**		
4	--	--	**	
5	**	**	--	**
	1	2	3	4

5	3	4	1	2
20.15	19.9	18.73	18.7	18.67

D. Second Anal Fin Rays

2	**			
3	**	**		
4	--	**	**	
5	**	**	--	**
	1	2	3	4

5	3	2	1	4
19.85	19.8	19.0	18.45	18.22

E. Gill-Rakers

2	**			
3	**	--		
4	**	--	--	
5	**	--	--	--
	1	2	3	4

3	4	5	2	1
18.48	18.43	18.39	18.2	17.55

F. Pyloric Caeca

2	**			
3	**	**		
4	**	**	**	
5	**	**	**	**
	1	2	3	4

1	2	3	4	5
40.2	37.85	35.03	29.5	21.33

G. Precaudal Vertebrae

2	**			
3	**	**		
4	**	**	--	
5	--	**	**	**
	1	2	3	4

2	1	5	3	4
20.82	20.43	20.15	17.82	17.08

H. Caudal Vertebrae

2	**			
3	--	**		
4	**	--	**	
5	**	**	**	**
	1	2	3	4

5	1	3	2	4
37.62	35.85	35.53	35.05	34.92

I. Total Vertebrae

	1	2	3	4
2	--			
3	**	**		
4	**	**	**	
5	**	**	**	**

5	1	2	3	4
57.77	56.28	55.87	53.33	52.0

J. Lateral Line Scales

	1	2	3	4
2	--			
3	**	**		
4	**	**	**	

1	2	3	4
160.43	159.4	152.68	141.05

K. Scales Above Lateral Line

	1	2	3	4
2	--			
3	**	**		
4	**	**	--	

1	2	4	3
26.33	26.07	24.73	24.7

L. Scales Below Lateral Line

	1	2	3
2	**		
3	--	**	
4	--	**	--

4	3	1	2
49.03	49.0	48.6	45.8

mean for the first dorsal and males for the vertebrae.

Morphometric Characters (Raw Measurements): Geographic Variation

Of the twelve raw measurements studied, ten had significant differences. No differences were found in first and third pelvic fin ray length. All significant differences were due to the presence of the samples from either Lac St. Jean or New York or both. Only four of the ten had differences due to the presence of the other localities. St. Lawrence estuary accounted for four differences and New Brunswick for one. Except for one case, Lac St. Jean locality had the highest mean whereas New York had the lowest mean in all instances. Results are shown in Table III.

Morphometric Characters (Raw Measurements): Sexual Dimorphism

New Brunswick: of the twelve raw measurements investigated, differences occurred in eight. The ones that showed no differences were the lengths of first, second, and third pelvic fin ray and caudal peduncle depth. Females had a greater mean for all eight variables.

Newfoundland: five characters were significantly different. These were standard, head, snout, upper jaw and preanal lengths. Females had a greater mean for the five variables.

St. Lawrence estuary: two characters did not show any significant difference. The two were the first and third pelvic fin ray lengths. Again the females had the greater mean in all the significant variables.

New York and Lac St. Jean: exhibited no differences.

Taking all five localities as a single unit, sexual dimorphism

TABLE III

Significance Matrices of Interpopulation Variation in Morphometric Characters (Raw Measurements) of *M. tomcod* (significance indicated by **, $p < 0.05$). Results are summarized on the right. Any means (in centimeters) not underscored by the same line are significantly different.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

A. Standard Length

2	--			
3	--	--		
4	**	**	**	
5	**	--	**	**
	1	2	3	4

5	2	1	3	4
18.3	17.05	16.52	15.63	13.49

B. Head Length

2	--			
3	--	--		
4	--	**	**	
5	**	--	**	**
	1	2	3	4

5	2	3	1	4
4.45	4.28	4.06	3.99	3.66

C. Snout Length

2	--			
3	--	--		
4	**	**	**	
5	--	--	--	**
	1	2	3	4

5	2	3	1	4
1.46	1.45	1.42	1.34	1.21

D. Eye Length

2	--			
3	**	**		
4	**	**	**	
5	--	--	**	**
	1	2	3	4

5	2	1	3	4
0.70	0.69	0.68	0.61	0.56

E. Upper Jaw Length

2	**			
3	**	--		
4	--	**	**	
5	**	--	--	**
	1	2	3	4

5	3	2	1	4
1.80	1.71	1.69	1.53	1.50

F. Predorsal Length

2 --				
3 -- **				
4 ** ** **				
5 -- -- ** **				
1 2 3 4				

5	2	1	3	4
5.66	5.59	5.28	5.04	4.3

G. Preanal Length

2 --				
3 -- --				
4 ** ** **				
5 ** ** ** **				
1 2 3 4				

5	2	1	3	4
8.71	7.68	7.43	7.22	6.33

H. Caudal Peduncle Depth

2 --				
3 ** **				
4 ** ** --				
5 -- ** ** **				
1 2 3 4				

2	1	5	3	4
1.15	1.09	1.04	0.91	0.84

I. Caudal Peduncle Length

2 --				
3 -- --				
4 ** ** **				
5 ** ** ** **				
1 2 3 4				

5	2	1	3	4
2.51	2.29	2.24	2.16	1.95

J. Second Pelvic Fin Ray Length

2 --				
3 -- --				
4 -- -- --				
5 -- -- ** **				
1 2 3 4				

5	2	1	3	4
2.6	2.45	2.4	2.34	2.23

appeared in eight variables in which the females had the greater mean for all. The four variables not showing any differences were eye length and first, second, and third pelvic fin ray length. Results are summarized in Table VIII.

Morphometric Characters (Ratio Measurements): Geographic Variation

With the twelve measurements taken, fourteen ratios were obtained. Of these fourteen ratios, eleven had significant differences (Table IV). The three ratios that did not differ were first pelvic fin ray length/second and third pelvic fin ray length and second pelvic fin ray length/third pelvic fin ray length.

Morphometric Characters (Ratio Measurements): Sexual Dimorphism

New Brunswick: of the fourteen ratio measurements taken, only one had a significant difference for sexual dimorphism. This was standard length/preanal length in which the males had a greater ratio (Table VIII).

St. Lawrence estuary: five significant differences occurred. These were standard length/predorsal length, standard length/preanal length, standard length/second pelvic fin ray length, head length/eye length and caudal peduncle length/caudal peduncle depth. Males had greater ratios for the first two and females for the last three.

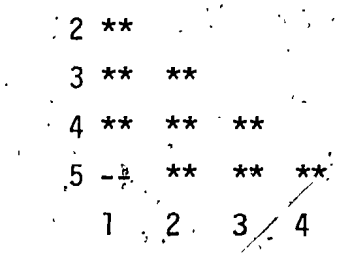
New York: four significant differences occurred. For standard length/preanal length and head length/snout length males had a greater ratio and for standard length/second pelvic fin ray length and head

TABLE IV

Significance Matrices of Interpopulation Variation in Morphometric Characters (Ratio Measurements) of *M. tomcod* (significance indicated by **, $p < 0.05$). Results are summarized on the right. Any means not underscored by the same line are significantly different.

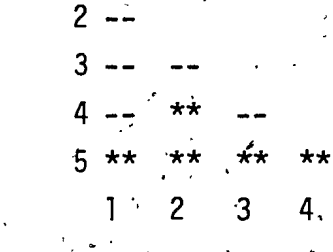
Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Québec)
4	New York
5	Lac St. Jean (Quebec)

A. Standard Length/Head Length



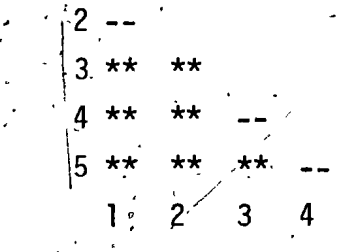
1	5	2	3	4
4.14	4.11	3.99	3.85	3.7

B. Standard Length/Predorsal Length



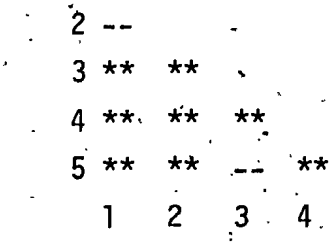
5	4	1	3	2
3.25	3.15	3.13	3.11	3.05

C. Standard Length/Preanal Length



1	2	3	4	5
2.23	2.22	2.18	2.14	2.11

D. Standard Length/Caudal Peduncle Depth



5	3	4	1	2
17.61	17.15	16.2	15.22	14.83

E. Standard Length/Caudal Peduncle Length

2	--			
3	--	--		
4	--	**	--	
5	--	--	--	--
	1	2	3	4

2	1	5	3	4
7.5	7.38	7.3	7.23	6.97

F. Standard Length/Second Pelvic Fin Ray Length

2	--			
3	--	**		
4	**	**	**	
5	--	--	**	**
	1	2	3	4

5	2	1	3	4
7.04	6.99	6.90	6.68	6.09

G. Head Length/Snout Length

2	--			
3	**	**		
4	--	--	**	
5	--	--	**	--
	1	2	3	4

5	4	1	2	3
3.06	3.04	3.01	2.97	2.87

H. Head Length/Eye Length

2	**			
3	**	**		
4	**	--	--	
5	**	--	--	--
	1	2	3	4

3	4	5	2	1
6.61	6.49	6.35	6.21	5.83

I. Head Length/Upper Jaw Length

```
2 **
3 **  **
4 **  **  **
5 **  --  **  --
  1   2   3   4
```

1	2	5	4	3
2.62	2.54	2.49	2.44	2.37

J. Head Length/Second Pelvic Fin Ray Length

```
2 --
3 --  --
4 --  **  --
5 --  --  --  --
  1   2   3   4
```

2	3	5	1	4
1.76	1.74	1.71	1.67	1.65

K. Caudal Peduncle Length/Caudal Peduncle Depth

```
2 --
3 **  **
4 **  **  --
5 **  **  --  --
  1   2   3   4
```

5	3	4	1	2
2.42	2.39	2.35	2.08	1.99

length/second pelvic fin ray length the females had the larger ratio.

Lac St. Jean: significant differences occurred for four ratios. The females had a greater ratio for standard length/head length, standard length/caudal peduncle length and head length/snout length whereas for caudal peduncle length/caudal peduncle depth males had a greater ratio.

Newfoundland: no sexual dimorphism was evident.

Lumping all five localities, five significant differences occurred. These were standard length/head length, standard length/preanal length, standard length/second pelvic fin ray length, head length/eye length and head length/second pelvic fin ray length. For the first two, males had the greater ratio and for the last three, females had the greater ratio.

A summary for sexual dimorphism is given in Table VIII.

Morphological Summary

Table V summarizes the significant differences which were detected between pairs of populations for meristics, measurements (raw and ratios) and total morphological study.

Table VI summarizes the difference for each individual population compared to all the other four localities.

Table VII summarizes the significant CDs present.

Numerical Clustering of Morphological Data

Twenty-five morphological characters were used in the cluster analysis. The resultant clustering is illustrated in Figure 3 agreeing somewhat with the statistical analysis of Tables V and VI. Two major

TABLE V.--Number and Percentage Differences Between Populations in Meristic and Morphological (Raw and Ratio Measurements) Characters: New Brunswick, Newfoundland, St. Lawrence estuary, New York, and Lac St. Jean (Population 1-5, Respectively).

Pop. No.	Meristics		Morphological				Total	
			Raw		Ratio			
	N	%	N	%	N	%	N	%
2-4	8	53.3	9	75.0	9	64.3	26	63.4
4-5	8	66.7	10	83.3	4	28.6	22	57.9
1-4	8	53.3	7	58.3	7	50.0	22	53.7
3-5	6	50.0	8	66.7	6	42.9	20	52.6
2-3	10	66.7	3	25.0	8	57.1	21	51.2
1-5	8	66.7	5	41.7	6	42.9	19	50.0
1-3	9	60.0	3	25.0	7	50.0	19	46.3
3-4	6	40.0	8	66.7	5	35.7	19	46.3
2-5	7	58.3	3	25.0	5	35.7	15	39.5
1-2	7	46.7	1	8.3	3	21.4	11	26.8

TABLE VI.--Number and Percentage Differences for Each Individual Population in Meristic and Morphological (Raw and Ratio Measurements) Characters: New Brunswick, Newfoundland, St. Lawrence estuary, New York, and Lac St. Jean (Population 1-5, Respectively).

Pop. No.	Meristics		Morphological				Total	
			Raw		Ratio			
	N	%	N	%	N	%	N	%
4	30	52.6	34	70.8	25	44.6	89	55.3
5	29	60.4	26	54.2	21	37.5	76	50.0
3	31	54.4	22	45.8	26	46.4	79	49.1
2	32	56.1	16	33.3	25	44.6	73	45.3
1	32	56.1	16	33.3	23	41.1	71	44.1

Table VII.--Significant Coefficients of Difference (CD) values for *M. tomcod*: New Brunswick, Newfoundland, St. Lawrence estuary, New York, and Lac St. Jean (Populations 1-5, Respectively).

Variable	Population Combinations									
	4-5	2-5	3-5	1-3	1-4	1-5	2-3	2-4	1-2	3-4
Pyloric Caeca	-	1.98	1.79	-	-	1.93	-	-	-	-
Precaudal Vertebrae	1.84	-	1.54	1.73	2.08	-	2.30	2.66	-	-
Caudal Vertebrae	1.52	1.61	-	-	-	-	-	-	-	-
Total Vertebrae	3.45	1.56	2.66	1.48	2.15	-	1.50	2.28	-	-
Standard Length	1.29	-	-	-	-	-	-	-	-	-
Standard/Head	1.29	-	-	1.30	1.31	-	-	-	-	-
Standard Length/Caudal Peduncle Depth	-	1.73	-	-	-	1.38	-	-	-	-

TABLE VIII

Comparison of Sexual Dimorphism Data for Meristic and Morphometric (Raw and Ratio Measurements) Characters ($p < 0.05$).

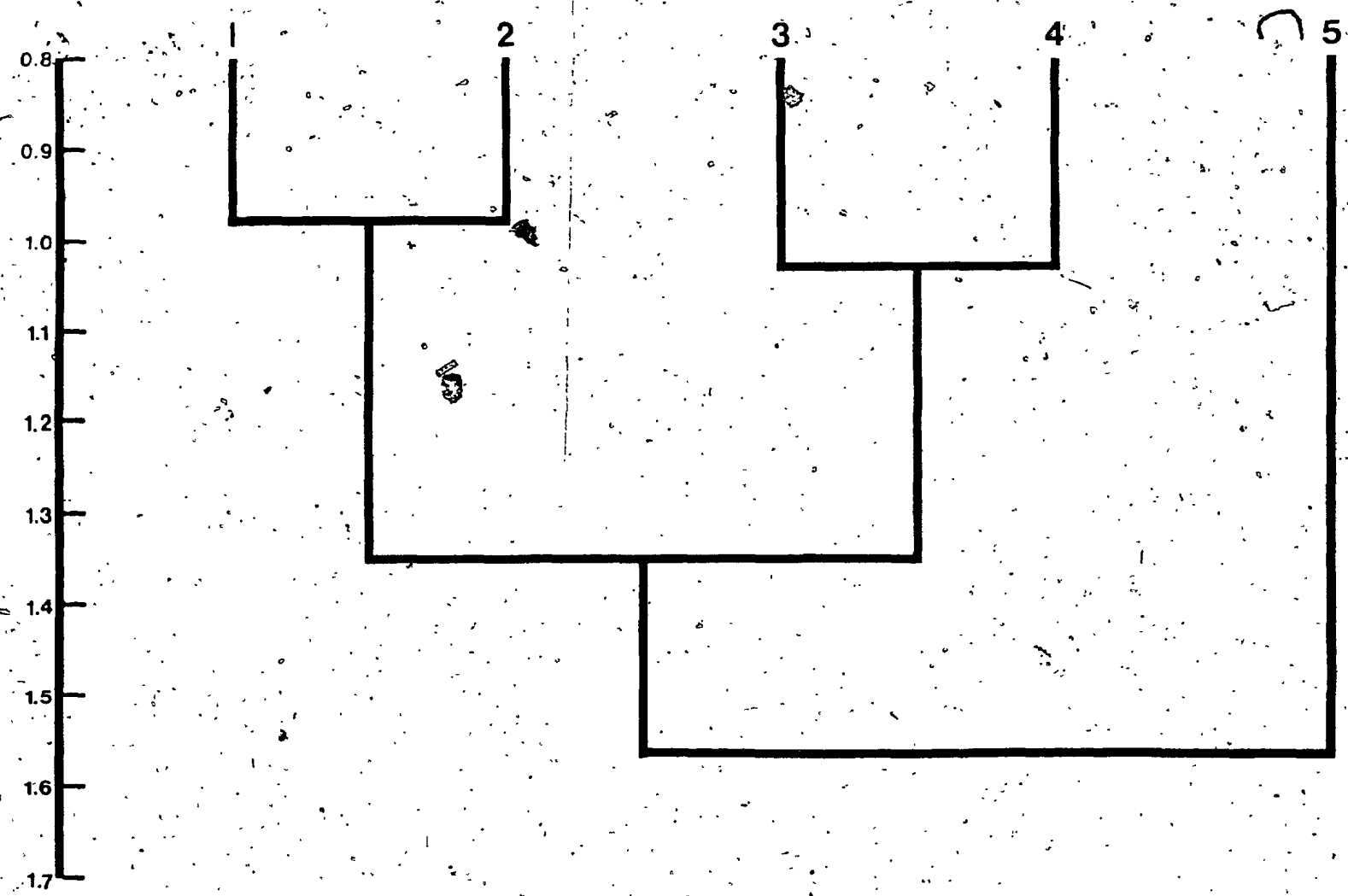
Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)
6	Combination of all localities

Population	Number	1	2	3	4	5	6
SEX	Males	50	18	35	16	4	123
	Females	10	42	25	44	9	130
	% Males	83.3%	30%	58.3%	26.6%	30.7%	48.6%
All Morphological Characters (41)	Number of Significant Differences	13	6	15	6	4	16
	% Significant Differences	31.7%	14.6%	36.6%	14.6%	9.8%	39.0%
	Number of Males with Greater Means	2	1	2	2	1	4
Meristics Characters (15)	Number of Significant Differences	4	1	0	2	0	3
	% Significant Differences	26.6%	6.6%	0%	13.3%	0%	20.9%
	Number of Males with Greater Means	1	1	0	0	0	2
Raw Measurements (12)	Number of Significant Differences	8	5	10	0	0	8
	% Significant Differences	66.6%	41.6%	83.3%	0%	0%	66.6%
	Number of Males with Greater Means	0	0	0	0	0	0
Ratio Measurements (14)	Number of Significant Differences	1	0	5	4	4	5
	% Significant Differences	7.1%	0%	35.7%	28.5%	28.5%	35.7%
	Number of Males with Greater Means	1	0	2	2	1	2

FIGURE 3

Dendrogram of Numerical Relationships based on Morphometric Data.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)


1
2
3
4
5
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7

clusters, Newfoundland with New Brunswick and New York with St. Lawrence estuary are shown. A single line depicts the Lac St. Jean population.

BIOCHEMICAL INVESTIGATION

Muscle myogen and lactic dehydrogenase were studied for 472 and 356 fish respectively, from five localities. Liver esterase patterns were studied for 336 fish from four localities.

The results for the New York population (muscle myogen and liver esterase study) cannot be taken as conclusive because the samples did not arrive in a completely frozen state. Thus there might have been some protein denaturation.

Liver esterase patterns were detected using alpha naphthyl propionate, butyrate and acetate with best results being with the latter.

Glutamic and malic dehydrogenase were tried but enzymes were not in sufficient quantity for analysis.

No sexual dimorphism existed for any of the biochemical data.

Muscle Myogen

There occurred 28 different patterns among the five populations studied and of these, 26 occurred less than 3.18% of the time. The two main patterns occurred 61.65% and 13.14% (Figure 4, B and C, respectively).

A total of 16 different bands (Figure 4A and Table IX) occurred among the five populations. Ten of the bands occurred at least 90% of the time in all the populations with the exception of New York.

FIGURE 4

Main Muscle Myogen Patterns and Band Positions for *M. tomcod*. Pattern A is a composite of all possible bands that occurred.

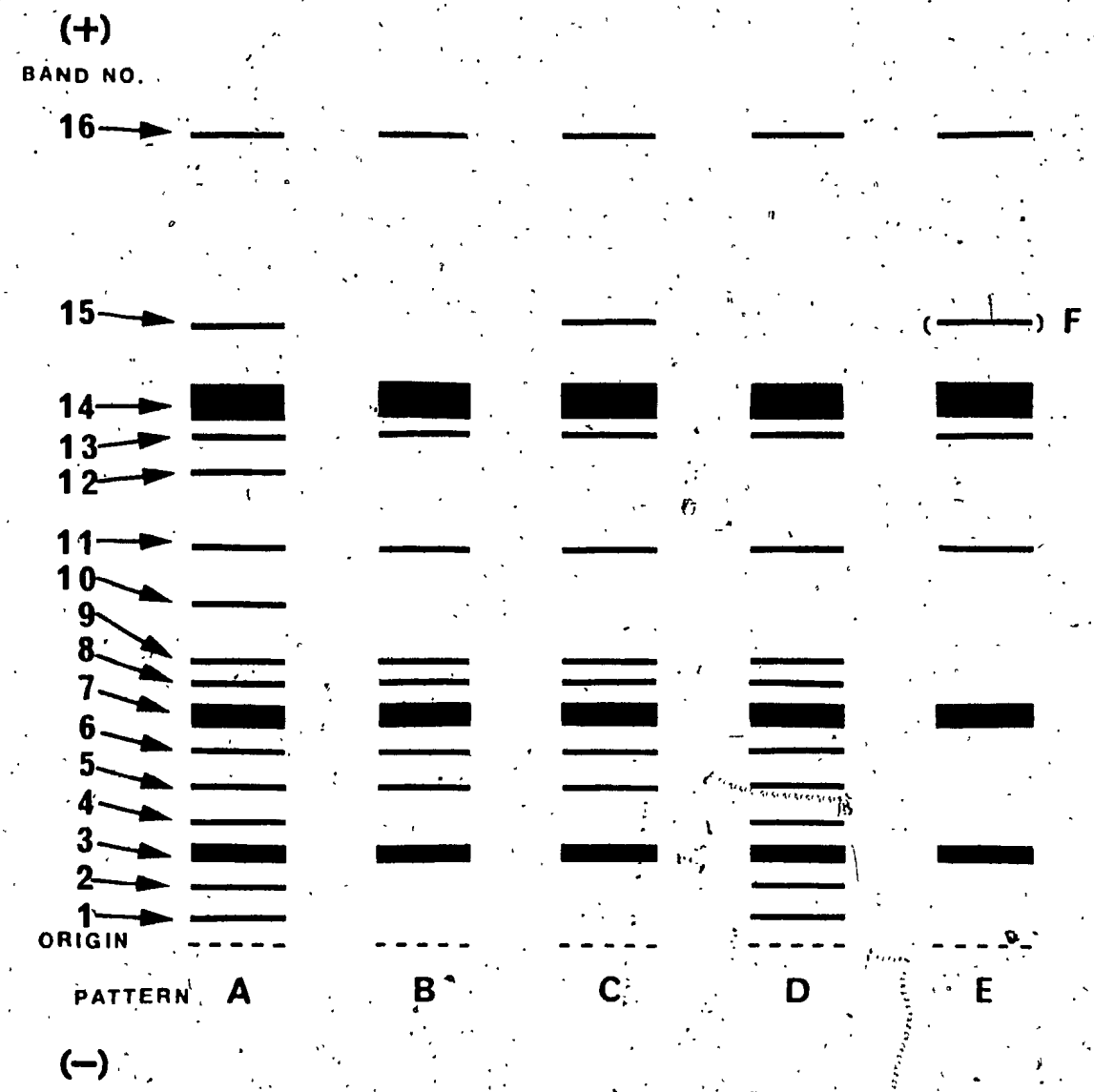

(+)
BAND NO.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ORIGIN
F
PATTERN
A
B
C
D
E
(–)

TABLE IX.--Percentage Occurrence for Main Muscle Myogen Patterns and Bands for *M. tomcod*: New Brunswick, Newfoundland, St. Lawrence estuary; New York, and Lac St. Jean (Populations 1-5, Respectively).

		POPULATION				
		1	2	3	4	5
		n=175	n=154	n=105	n=32	n=6
Total No. of Patterns		18	9	9	4	1
PATTERN	B	65.71%	65.58%	65.71%	-	100%
	C	10.28	16.23	18.09	-	-
	D	8	-	-	-	-
	E	-	-	-	46.87	-
	F	-	-	-	37.5	-
Remaining Patterns Occur Less Than		2.28	3.89	5.71	9.37	-
Total No. of Bands		14	14	15	10	10
BAND NO.	1	10.85%	0%	0.95%	0%	0%
	2	12	2.59	7.61	0	0
	3	94.20	100	97.14	90.62	100
	4	12	7.79	6.66	0	0
	5	97.14	93.5	100	6.26	100
	6	99.42	96.1	100	0	100
	7	100	100	100	100	100
	8	100	100	100	15.62	100
	9	98.28	100	100	15.62	100
	10	0	0	0.95	0	0
	11	98.28	100	100	100	100
	12	0.0	3.89	0	0	0
	13	97.7	96.10	100	100	100
	14	100	100	100	100	100
	15	10.28	16.23	19.04	53.12	0
	16	95.42	97.4	94.28	100	100

TABLE X

Significance Matrices of Interpopulation Variation in the Muscle Myogen Bands of *M. tomcod* (significance indicated by **, $p < 0.05$).

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

A. Muscle Myogen Band 1

```
2 **
3 **  --
4 --  --  --
5 --  --  --  --
  1   2   3   4
```

B. Muscle Myogen Band 2

```
2 **
3 --  --
4 --  --  --
5 --  --  --  --
  1   2   3   4
```

C. Muscle Myogen Band 3

```
2 **
3 --  --
4 --  **  --
5 --  --  --  --
  1   2   3   4
```

D. Muscle Myogen Band 5

```
2 ---
3 --  **
4 **  **  **
5 --  --  --  **
  1   2   3   4
```

E. Muscle Myogen Band 6

```
2 --
3 --  --
4 **  **  **
5 --  --  --  **
  1   2   3   4
```

F. Muscle Myogen Band 8

```
2 --
3 --  --
4 **  **  **
5 --  --  --  **
  1   2   3   4
```

G. Muscle Myogen Band 9

```
2 --
3 --  --
4 **  **  **
5 --  --  --  **
  1   2   3   4
```

H. Muscle Myogen Band 15

```
2 --
3 --  --
4 **  **  **
5 --  --  --  --
  1   2   3   4
```

Pattern and band occurrence are summarized in Table IX and Figure 4. Muscle myogen band significance was tested for each band using a chi-square test. The results of these tests are presented in Table X.

Lactic Dehydrogenase

A total of 12 patterns, of which 10 had less than 6.74% frequency, occurred for the LDH study. The two main patterns had 67.42 and 13.76% frequency (Figure 5,B and C, respectively).

Ten separate bands occurred among the 12 patterns (Figure 5A and Table XI). Three of the bands occurred at least 90% of the time (Bands 3, 4 and 7).

LDH banding and pattern frequency are summarized in Table XI and Figure 5. The results of the chi-square significance tests for LDH bands are presented in Table XII.

Liver Esterase

Six patterns were present in the liver esterase of the tomcod. The two highest frequency patterns were patterns A and B (Figure 6) with 56.25% and 33.93% frequency respectively. The remaining had 6.85% or less frequency (Table XIII).

Four bands occurred among the 6 patterns. Two of the bands occurred at least 90% (Table XIII).

Liver esterase band and pattern frequency are summarized in Table XIII and Figure 6. Table XIV presents the results of the chi-square significance tests for liver esterase bands.

FIGURE 5

Main LDH Patterns and Band Positions for *M. tomcod*. Pattern A is a composite of all possible bands that occurred.

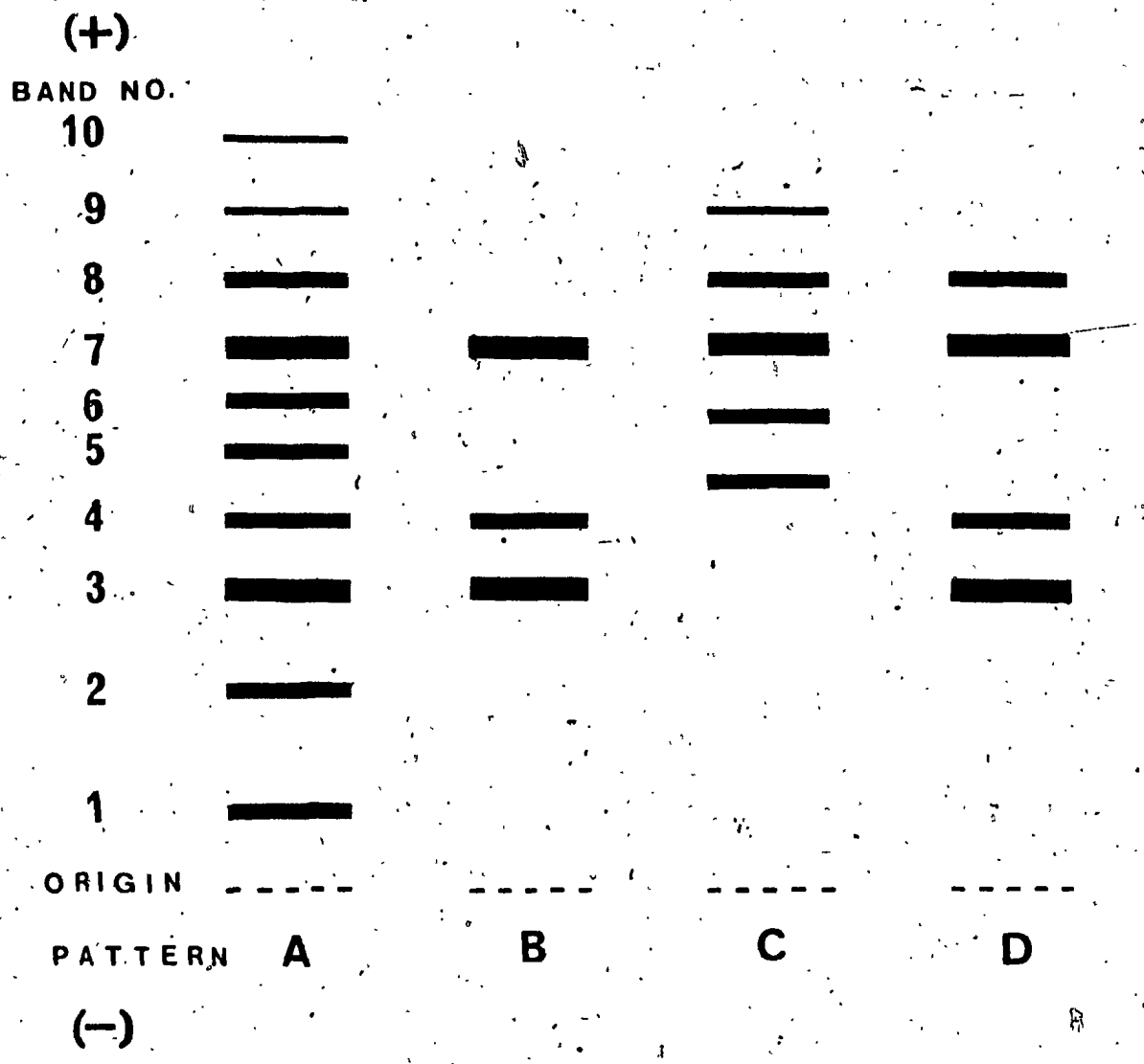

(+)
BAND NO.
10
9
8
7
6
5
4
3
2
1
ORIGIN
PATTERN
A
B
C
D
(−)

TABLE XI.--Percentage Occurrence for Main LDH Patterns and Bands for *M. tomcod*: New Brunswick, Newfoundland, St. Lawrence estuary, New York, and Lac St. Jean (Populations 1-5, Respectively).

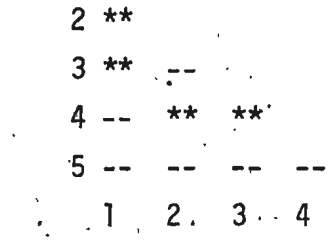
		POPULATION 1 n=151	2 n=71	3 n=103	4 n=25	5 n=6
Total No. of Patterns		8	1	5	8	2
PATTERN	B	62.91%	100%	68.93%	12.0%	-
	C	16.55	-	10.67	48.0	16.66
	D	4.63	-	8.73	12.0	83.33
Remaining Patterns Occur Less Than		5.96	-	8.73	8.0	-
Total No. of Bands		7	3	5	9	5
BAND NO.	1	11.9%	0%	0%	16%	0%
	2	0	0	0	4	0
	3	100	100	100	100	100
	4	100	100	91.26	100	100
	5	0	0	0	8	0
	6	0	0	0	4	0
	7	100	100	100	100	100
	8	33.1	0	22.33	88	100
	9	26.49	0	13.59	68	16.6
	10	0.66	0	0	0	0

TABLE XII

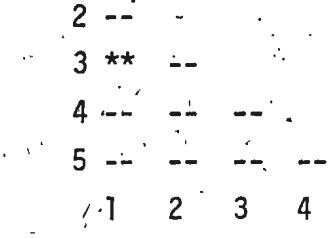
Significance Matrices of Interpopulation Variation in the LDH Bands of *M. tomeod* (significance indicated by **, $p < 0.05$)

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

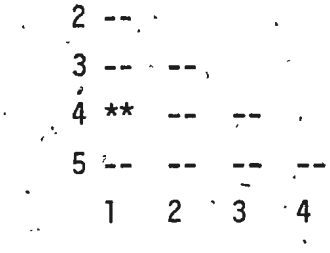
A. LDH Band 1



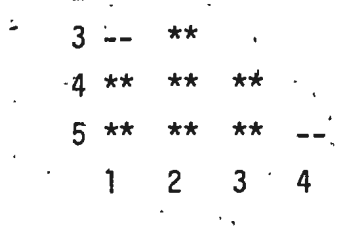
B. LDH Band 4



C. LDH Band 5



D. LDH Band 8



E. LDH Band 9

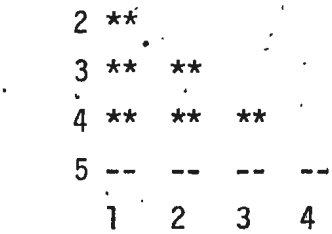


FIGURE 6

Main Liver Esterase Patterns and Band Positions for *M. tomcod*.

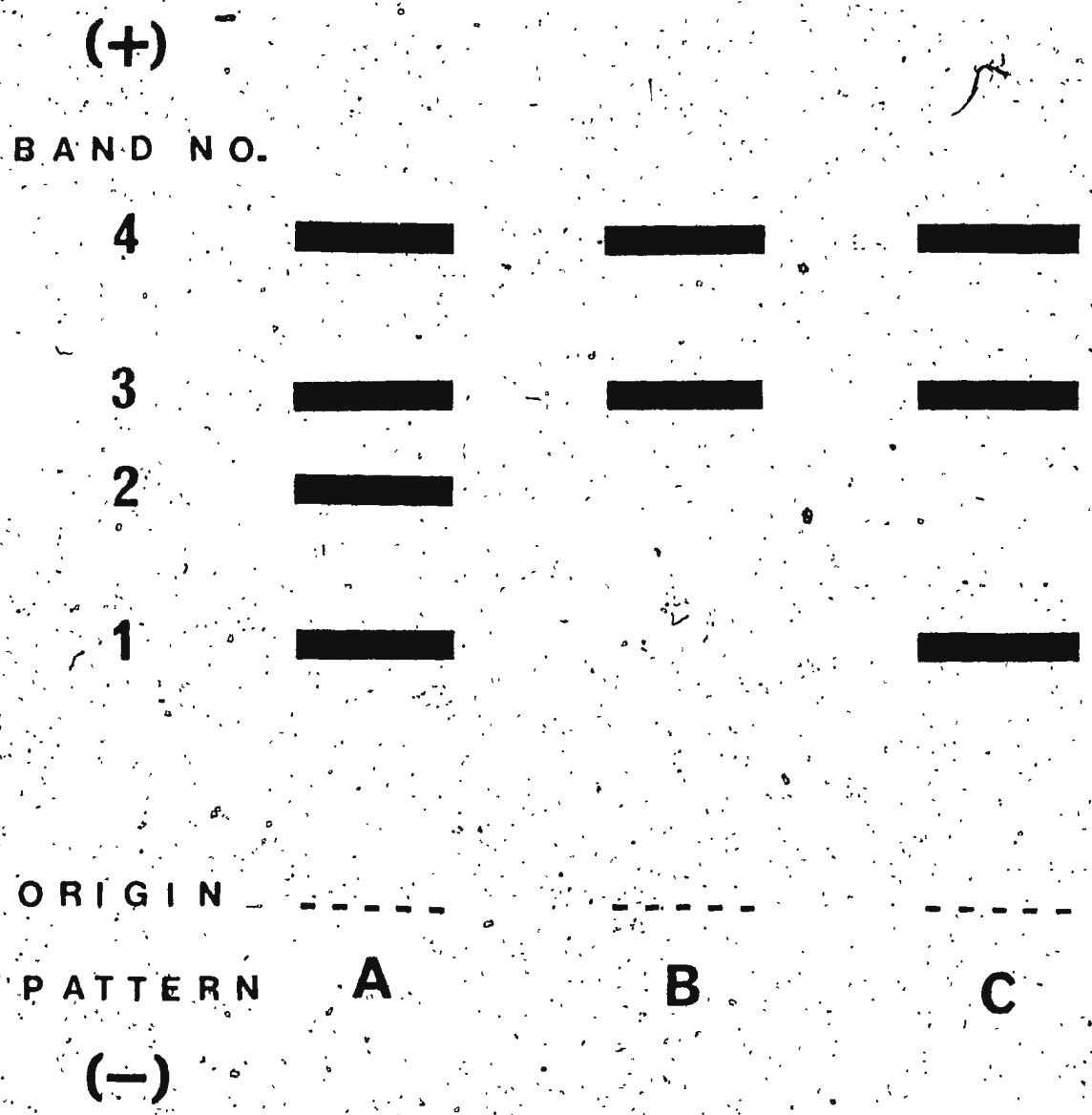

(+)
BAND NO.
4
3
2
1
ORIGIN
PATTERN
A
B
C
(−)

TABLE XIII.--Percentage Occurrence for Liver Esterase Patterns and Bands for *M. tomcod*: New Brunswick, Newfoundland, St. Lawrence estuary, and New York (Populations 1-4, Respectively).

		POPULATIONS 1 n=119	2 n=84	3 n=107	4 n=26
Total No. of Patterns		4	4	5	5
PATTERN	A	36.13%	73.8%	68.22%	42.3%
	B	61.34	16.66	23.36	7.69
	C	0.84	5.95	5.5	42.3
Remaining Patterns Occur Less Than		1.68	3.57	1.86	3.84
Total No. of Bands		4	4	4	4
BAND NO.	1	38.65%	83.33%	76.63%	92.3%
	2	37.81	77.38	70.09	46.15
	3	100	100	100	96.15
	4	98.31	96.42	97.19	92.3

TABLE XIV.--Significance Matrices of Interpopulation Variation in Liver Esterase Bands of *M. tomcod* (Significance indicated by **, $p < 0.05$). Localities shown are: New Brunswick, Newfoundland, St. Lawrence estuary (Quebec), and New York. (Population 1-4, Respectively).

A. Esterase Band 1

2 **		
3 **	--	
4 **	--	--
1	2	3

B. Esterase Band 2

2 **		
3 **	--	
4 --	**	**
1	2	3

Numerical Clustering of Biochemical Data

Muscle myogen: the resultant clustering is shown in Figure 7A. It shows Newfoundland and Lac St. Jean forming the first cluster and then joined by St. Lawrence estuary, New Brunswick and New York. There is a latitudinal cline shown.

Lactic dehydrogenase: the clustering for LDH is illustrated in Figure 7B. In this cluster, Newfoundland and St. Lawrence estuary form the first cluster and then joined by Lac St. Jean, New Brunswick, and New York. A latitudinal cline exists if not for the misplacement of St. Lawrence estuary.

Liver esterase: this cluster shows Newfoundland and St. Lawrence estuary joining and then followed by New Brunswick and New York (Figure 7C).

Total biochemical: this cluster shows again Newfoundland and Lac St. Jean forming the first cluster and then joined by St. Lawrence estuary, New Brunswick and New York (Figure 8).

Numerical Clustering of Morphological and Biochemical Data

The resultant cluster for all the morphological and biochemical is shown in Figure 9. The main cluster shows Newfoundland and St. Lawrence estuary joining, and then followed by New Brunswick, Lac St. Jean and New York.

FIGURE 7

Dendrogram of Numerical Relationships based on Muscle Myogen (A), LDH (B) and Liver Esterase (C) Data.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

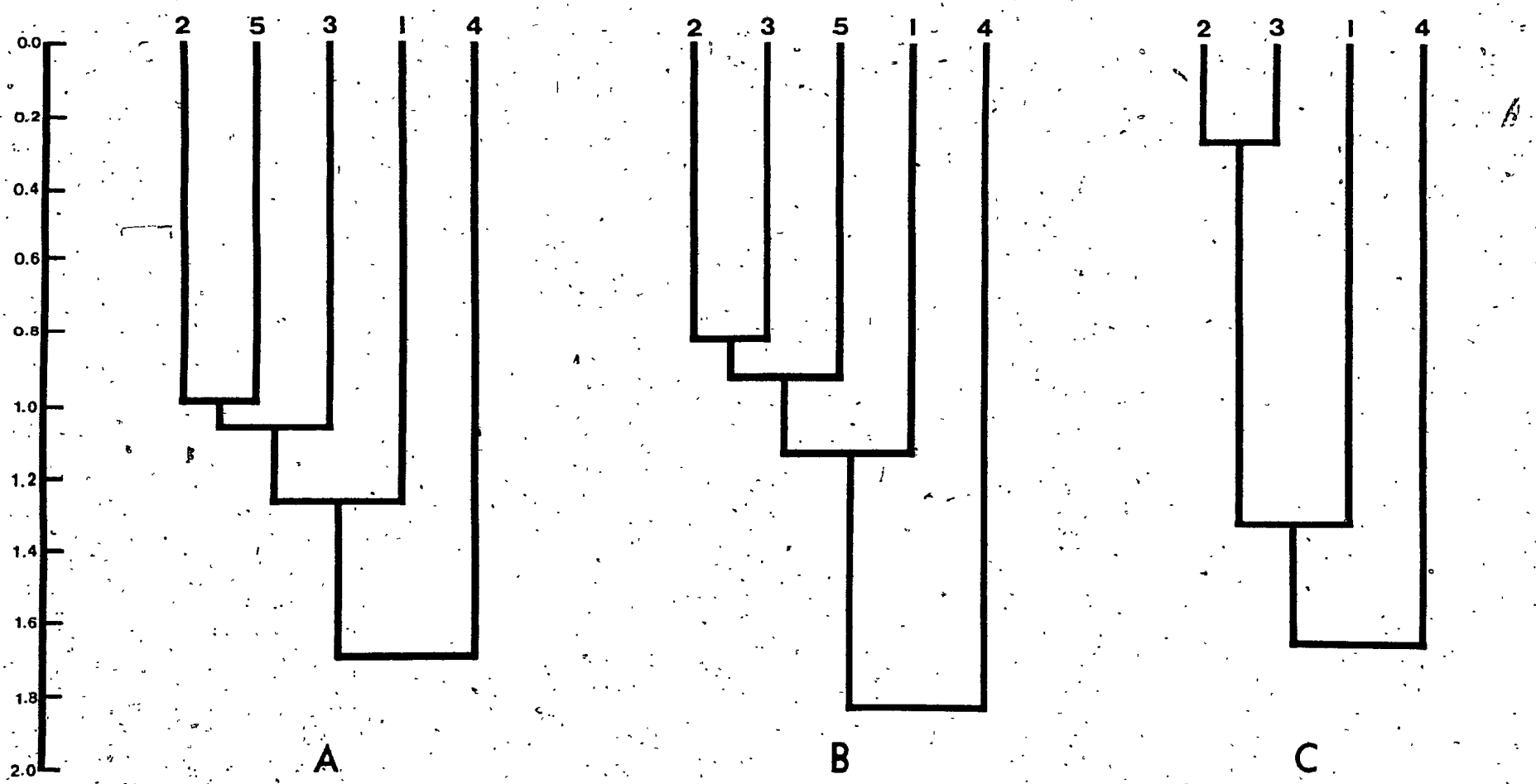

0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2 5 3 1 4
A
2 3 5 1 4
B
2 3 1 4
C

FIGURE 8

Dendrogram of Numerical Relationships based on Total Biochemical (Muscle Myogen, LDH, and Liver Esterase) Data.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)

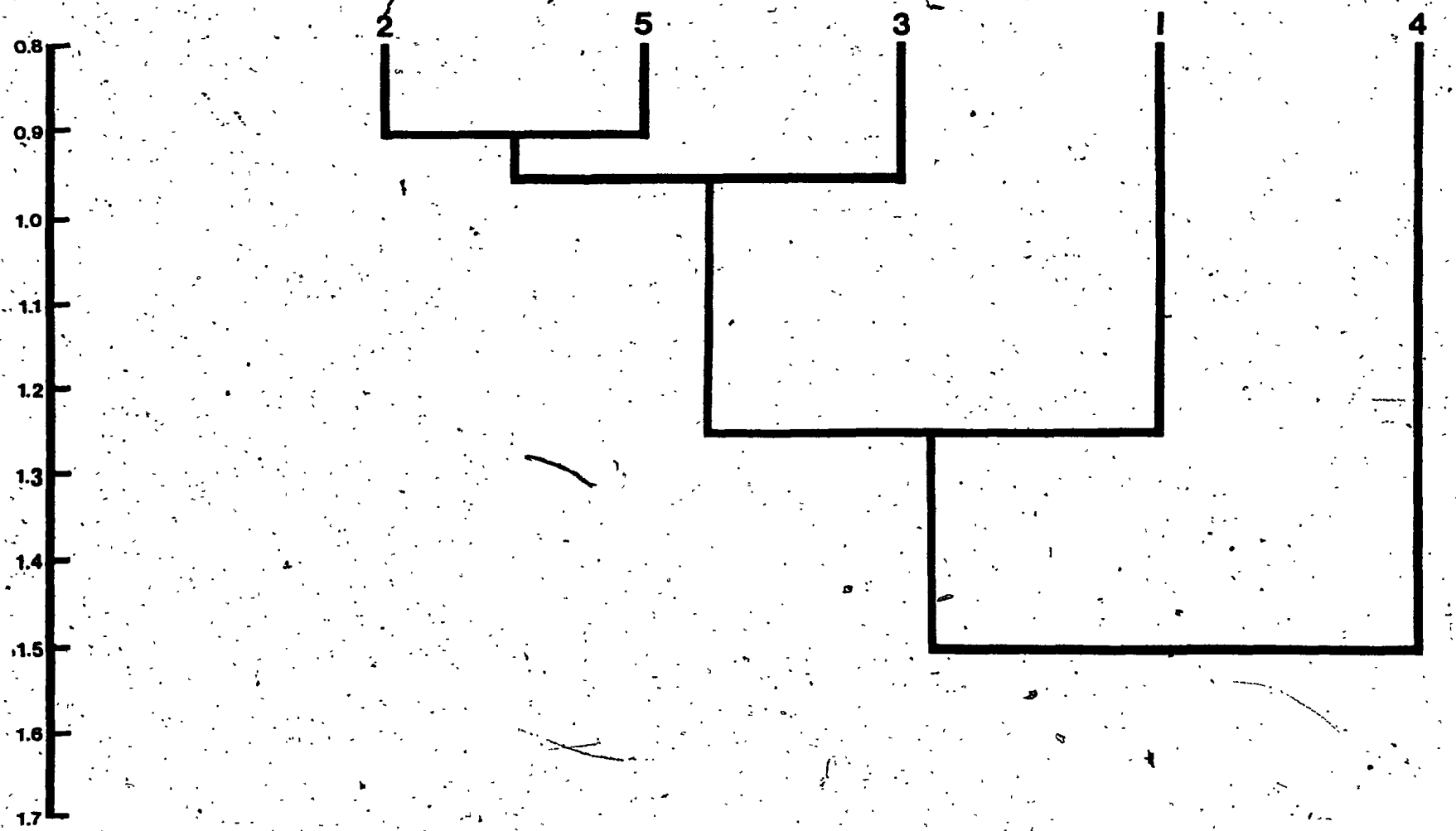
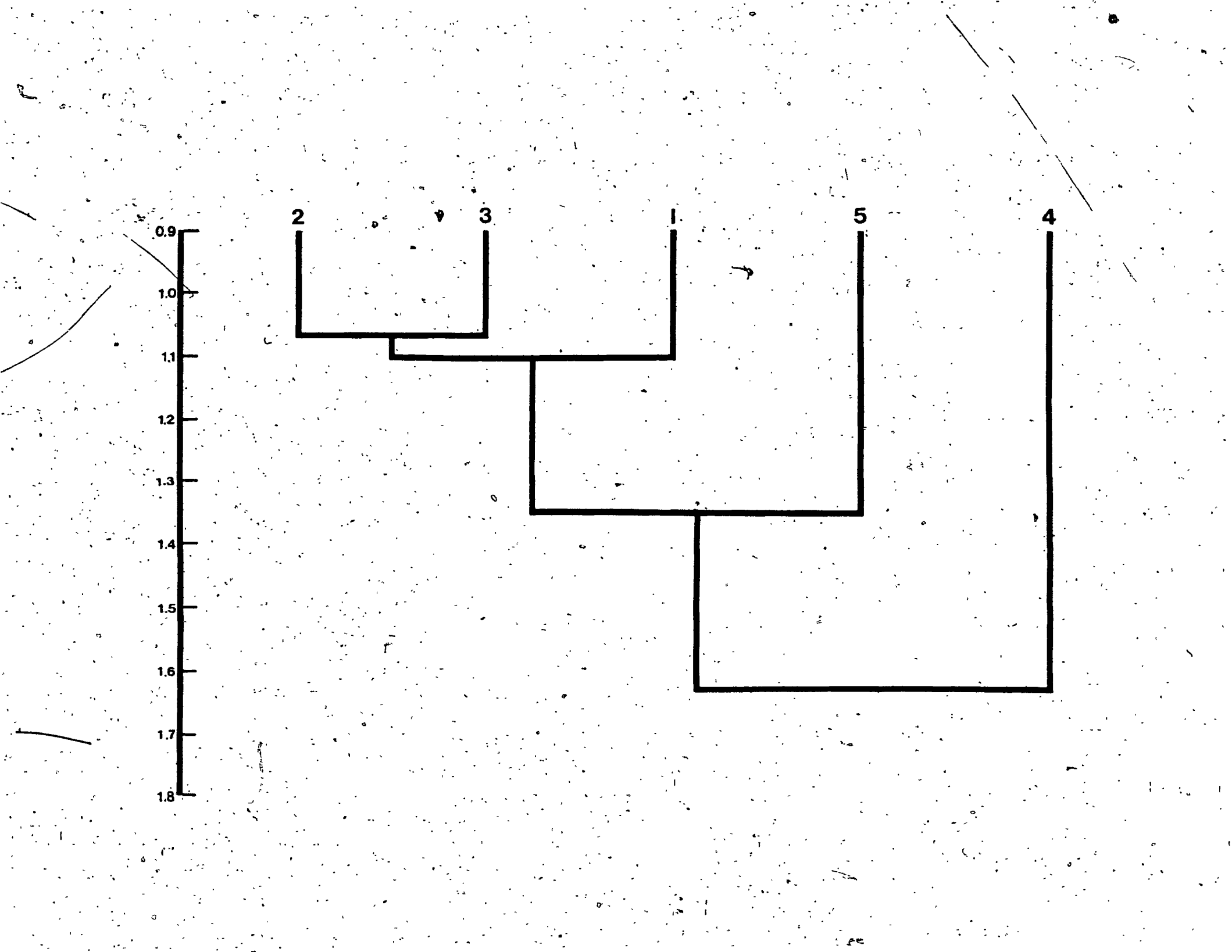

2
5
3
1
4
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7

FIGURE 9

Dendrogram of Numerical Relationships based on Biochemical and Morphometric Data.

Population Number	Locality
1	New Brunswick
2	Newfoundland
3	St. Lawrence estuary (Quebec)
4	New York
5	Lac St. Jean (Quebec)


2
3
1
5
4
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8

DISCUSSION

Geographic variation in fish species has been looked at from different points of view.

The nature of morphological variation (causes and significance) has been thoroughly reviewed by Barlow (1961). Barlow concludes that "regular changes in counts, such as in geographical clines, probably reflect adaptive changes of a genetic nature."

Biochemical analysis of fish species has been reviewed by de Ligny (1969). Electrophoretic properties of blood and tissue proteins have been used in identification of difficult species, and also for determining intraspecific polymorphism (Moller and Naevdal, 1966; Sick, 1967; Tsuyuki *et al.*, 1965, 1966b; Uthe *et al.*, 1966). Frydenberg *et al.* (1965) state that the information obtained promises to be of great value in population analysis. Payne *et al.* (1971) using serum protein analysis have revealed the existence of genetically isolated populations of salmon sufficiently distinct to merit separation into subspecies and races.

The results of this study do not clearly show any geographical isolates but do show a population continuum. Mayr (1969) describes a population continuum as

> A large part of the range of many species, particularly the central part, is occupied by a series of essentially contiguous populations. Even when there are minor breaks in distribution owing to the unsuitability of the habitat, such breaks are bridged by steady dispersal, resulting in copious gene exchange among populations. Variation in such

a population continuum is essential clinal. Terminal populations at the opposite ends of the continuum may be very different phenotypically and may deserve subspecific recognition.

Essentially both the morphological and biochemical results show a population continuum. The terminal populations show greater differences than the central populations.

Morphometrics. Of the forty-one variables studied among the five localities, between 11 (26.8%) and 26 (63.4%) significant differences were observed between populations. Most of the variation was clinal with means increasing northwards. Variation and/or similarities were difficult to attribute to any single population or groups of populations.

Meristics. Meristic variation within a species exists in many species of fish. Significant meristic differences between samples of fish from different geographic areas can usually be taken as evidence for some degree of racial segregation even though hereditary and environmental effects cannot be isolated (Lindsey, 1961). The environmental effects are varied and often interacting: temperature, light intensity, oxygen concentration, salinity, light duration and many others. All these effects have been found to cause meristic variation within a species if they have been acting during embryonic development (Lindsey, 1954, 1958, 1962; Lindsey and Ali, 1965). The number of serial elements is determined by developmental rate (Hubbs, 1926; Gabriel, 1944). Therefore longer developmental periods usually produce higher counts in meristic structures.

Significant interpopulation differences for meristics occurred

at least 40% and up to 66.7% of the time. The least variability occurred between St. Lawrence estuary and New York. Both of these populations live in somewhat warmer waters than the other three populations. Presumably their development time is more similar than if compared to the colder water populations. Newfoundland and New Brunswick, with 46.7% significant difference in meristic characters, both live in coastal estuarine waters where the temperature is lower than in the New York (Hudson River) and St. Lawrence estuary. The temperature range from December to March (1971-72) was 0° to 2°C for Newfoundland, 0° to 2° for New Brunswick and 0° to 5.5° for New York. No temperature data was available for Lac St. Jean and St. Lawrence estuary.

The population combinations differing the most (Lac St. Jean - New York, 66.7%; Newfoundland - St. Lawrence estuary, 66.7%; and New Brunswick - Lac St. Jean, 66.7%) are in different development areas: from warmer river waters to colder coastal and estuarine waters. The other population combinations differ proportionately as to their proximity in latitude.

The coefficient of difference (CD) values for the morphological measurements and meristic counts are given in Table VII. Mayr (1969) does not state how many variables should have a significant CD value before a subspecific level of differentiation is recognized. For the meristic study all the population combinations except New Brunswick - Newfoundland and St. Lawrence estuary - New York had from one to three significant CD values and this corresponds to the cluster analysis shown in Figure 3. The significant CD values occurred for total vertebrae, caudal and precaudal vertebrae and pyloric caeca. The Lac St. Jean population exhibited three significant CD values with every population except the New Brunswick population.

The Lac St. Jean population had a 66.1% higher counts (in meristics) frequency compared to the other four populations. New Brunswick was next, followed by Newfoundland, St. Lawrence estuary and New York (55.4%, 51.8%, 50% and 30.4% respectively).

Morphological. Differences in shape and size are noticeable between fish from waters of different temperatures. Almost invariably the more northern representatives of a species, or of a genus, are larger than those to the south (Hubbs, 1926; Vladykov, 1934). Northern, slower growing populations of a species, usually have smaller heads, eyes and maxillaries than do the southern species (Hubbs, 1926; Vladykov, 1934; and Martin, 1949).

Also, the environment can modify the shape and size of a fish by acceleration or retardation of the developmental rate (Vladykov, 1934; Williams, 1954; Hubbs, 1926; Martin, 1949). Variability of food, prey organisms and other factors are environmental variables affecting growth of fish.

Raw Measurements. Between 8.3% to 83.3% significant differences occurred in raw measurements. The least number of significant differences occurred between Newfoundland and New Brunswick in which only one (8.3%) occurred. Both of these populations come from similar areas, that is from coastal estuarine waters. Next there are three combinations each with 25% significant differences--Newfoundland - St. Lawrence estuary, Newfoundland - Lac St. Jean and St. Lawrence estuary - New Brunswick.

Combinations differing the most are Lac. St. Jean with New York

and with St. Lawrence estuary (83.3% and 66.7%), New York with Newfoundland and with St. Lawrence estuary and with New Brunswick (75%, 66.7% and 58.3%, respectively). The two combinations differing the most appear to be from rather different environments.

The Lac St. Jean population had, based on raw measurements, the largest fish (87.5% greater values) followed by Newfoundland, New Brunswick, St. Lawrence estuary and New York (75%, 39.6%, 37.5% and 8.3% respectively). The only significant CD obtained from the raw measurements was for standard length between New York and Lac St. Jean (the two terminal populations).

Ratio Measurements. Ratios have been the traditional way of comparing measurements of fish. Ratios have their advantages and disadvantages. One advantage is that fish can be compared irrespective of size.[1] The disadvantage is that if a raw measurement variable (significant or not) is divided by another variable (significant or not), a new significant or a non significant ratio variable might occur. There is also a lack of appreciation of variation and/or size-specific changes. Therefore, both raw measurements and ratios should be used with equal caution. Looking at the results of the ratios and raw measurements (Table V) it will be noted that there is either an increase or a decrease in significant differences going from raw to ratio measurements. For example, in the Newfoundland - St. Lawrence estuary

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[1]See Marr (1955) for a good discussion on the use of morphometric data.

combination there were 3 significant differences in raw measurements whereas 8 in ratios. For the New York - Lac St. Jean combination there were 10 significant raw measurement differences but only 4 in ratios.

For ratio measurements, Hubbs (1926), Vladykov (1934) and Martin (1949) found that the size in fishes increases from north to south. The present results conform to their findings. New York had the largest ratios (68% higher ratios) followed by St. Lawrence estuary, New Brunswick, Newfoundland and Lac St. Jean (61.5%, 47.8%, 47% and 19% respectively).

Five significant CD values occurred in the ratios data. The two variables responsible were standard/head and standard/caudal peduncle depth (Table VII).

Total Morphometrics (Meristics, Raw and Ratio Measurements). The terminal population combinations, New York with Newfoundland and with Lac St. Jean vary the most (63.4% and 57.9% respectively), whereas the two closest, Newfoundland with New Brunswick vary the least (26.8%). Newfoundland and Lac St. Jean also vary very little (39.5%) which might be due to their close relationship in latitude and temperature. The rest of the combinations are intermediate with the results varying according to the proximity (in latitude and temperature) of the populations examined.

In the dendrogram based on morphometric data (Figure 3) two main clusters were seen. New Brunswick and Newfoundland form one cluster. This cluster is composed of populations from coastal estuarine marine waters. The second cluster composed of river populations, New York and

St. Lawrence estuary, forms at about the same level as cluster one (1.03 versus 0.98). These populations are from warmer waters than the first cluster. The two clusters then join at a level of 1.35.

The Lac St. Jean population forms a single line which joins the two main clusters at a level of 1.57. It is nearly twice removed from the first two clusters and it seems as if it is slowly diverging from the other populations.

The preceding results suggest that there are three main populations. One is composed of marine dwellers (Newfoundland and New Brunswick) the second composed of river dwellers (New York and St. Lawrence estuary) and the third is the lake dweller (Lac St. Jean).

The nature of the observed differences (phenotypic and/or genotypic) remains to be resolved by other techniques. However, it is suggested that morphometric differences are of adaptive significance and therefore probably reflect genetic differences (Barlow, 1961).

Muscle myogen. Muscle myogen has been utilized as a taxonomic criterion (at generic, specific and intraspecific level) by a number of workers (Tsuyuki *et al.*, 1962, 1967, Odense *et al.*, 1966). A series of studies, particularly by Tsuyuki and his co-workers, have revealed that electropherograms of muscle myogen have a very constant appearance and species specificity. They appear to be quite independent of physiological factors such as sex, maturation, and age (Tsuyuki *et al.*, 1965). Inter-specific hybridization experiments by Tsuyuki and Roberts (1965) supported the suggestion of the hereditary nature of these muscle constituents which are derived from their species specific character. In most species

in which muscle myogens have been investigated the degree of intraspecific variation was low.

For the muscle myogen patterns for all the populations in this study the same basic pattern (Pattern B, Fig. 4) with the exception of the New York population (again it should be mentioned that the New York specimen did not arrive in a completely satisfactory state and that the results of its analysis for muscle myogen should not be taken as conclusive) occurred. There was, however, a considerable amount of intraspecific variation.

Pattern D (Fig. 4) only occurred in the New Brunswick population and therefore New Brunswick is significantly different from Newfoundland and St. Lawrence estuary for bands one and two. The dendrogram based on muscle myogen data (Fig. 7A) shows Newfoundland and Lac St. Jean forming the first cluster joined closely by St. Lawrence estuary and New Brunswick.

Examination of Table IX shows that the only bands with significant differences (excluding New York) are bands 1, 2, 3 and 5. Bands 1 and 2 are due to pattern D which occurred only in the New Brunswick population. Significant difference in band 3 is similarly due solely to the New Brunswick population which had a smaller occurrence than Newfoundland. Significant difference of band 5 is due to Newfoundland which had a smaller percentage occurrence than St. Lawrence estuary.

The two populations which form the main cluster are Newfoundland and Lac St. Jean which are extreme populations (in geographical sense) and would thus have little genetic interchange with other populations.

Newfoundland and New Brunswick differ more than either Newfoundland and St. Lawrence estuary or New Brunswick and St. Lawrence estuary. It would seem that Newfoundland and New Brunswick have less gene exchange than the other two combinations. This seems probable because tomcod have not been reported in deeper waters and thus there could be little gene exchange across the Cabot Strait, whereas Newfoundland tomcod can migrate along the coast and across the Straits of Belle Isle to the Quebec coastal waters. Frost (1939) report tomcod up to the northernmost Newfoundland and Jeffers (1932) report tomcod in Pistolet Bay.

The intraspecific variation can be accounted for by local geographic isolation with limited gene exchange occurring every so often. Tagging experiments carried out in 1945 by Vladykov (1957) might explain the limited gene exchange. Tomcods recaptured 2 years, 81 days after being tagged had moved a maximum of 135 miles.

Populations can be differentiated by using the ratios of the observed main patterns (Figure 4, Pattern B, C, and D). New Brunswick had a ratio of 8.2 : 1.3 : 1 whereas Newfoundland had a ratio of 4 : 1 : 0 and St. Lawrence estuary had a ratio of 3.6 : 1 : 0. Lac St. Jean with only one pattern present therefore had a ratio of 1 : 0 : 0.

Lactic dehydrogenase. LDH enzyme, involved in muscle metabolism and other tissue, catalyzes the reduction of pyruvate to L-lactate. The LDH molecule is a tetramer formed by the random combination of two different kinds of polypeptides, each determined by a separate locus and this explains the occurrence of the five fractions normally found (Markert, 1962). Gene mutations at either one or both of the loci may be expected

to result in intraspecific variation of the patterns involving an increase of the number of fractions in the heterozygote phenotype if the mutated polypeptide chains also combine at random (Markert, 1962).

Among thirty species of fish investigated by Markert and Faulhaber (1965) only three were found to resemble mammals and birds in possessing five LDH fractions or isozymes. In all other species only one, two or three LDH fractions were present.

The New York results are reported in the light of Odense *et al.* (1969) finding that heated cod muscle extract to 60°C had little effect on the results. The New York specimens used in this study never exceeded 10°C.

A study by Odense *et al.* (1971) reported that tomcod muscle extract showed no polymorphism for LDH. They obtained a pattern consisting of a single band (A_4)[1] whereas in the present study several patterns with up to five bands were obtained. Their single band pattern (A_4) also occurred in the present study but it was determined that it only appeared if the pH of the buffer was 8.3. When the regular pH of 8.5 buffer was used the A_4 band always separated out into either pattern B, C or D (Figure 5).

In the present study, Newfoundland, New Brunswick and St. Lawrence estuary had pattern B (Figure 5 and Table X) as the main pattern and New York had pattern C and Lac St. Jean had pattern D.

Significant differences in percentage occurrence for LDH bands occurred for band numbers 1, 4, 5, 8 and 9 (Table XI). The differences

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[1]As labelled by Odense *et al.* (1971).

were due to different populations for different bands and not to any single population.

The dendrogram derived from LDH data (Figure 7B) shows Newfoundland and St. Lawrence estuary forming the main cluster followed closely by Lac St. Jean and New Brunswick. New York follows the main group at a point 1.84 which is more than twice removed from the average of the four preceding populations.

Lac St. Jean is the only population having pattern D as its main pattern. Populations can be separated by using the ratios of the patterns observed. New Brunswick and St. Lawrence estuary had similar ratios of 13.6 : 3.6 : 1 and 7.9 : 1.2 : 1 respectively for patterns B, C and D (Figure 7). Newfoundland had a ratio of 1 : 0 : 0 whereas New York had a ratio of 1 : 4 : 1. Lac St. Jean had a ratio of 0 : 1 : 5.

The relationships indicated by this analysis are similar to those based on the muscle myogen data.

Esterase. Liver esterases have been shown by Nyman (in de Ligny, 1969) to be species and organ specific in fishes. Nyman showed by intraspecific hybridization experiments that all parental bands of esterases appear in the hybrid, indicating their genetic nature.

For all populations either two, three or four bands occurred. Newfoundland and St. Lawrence estuary had pattern A (Figure 6) as its main pattern whereas New Brunswick had pattern B. New York had an equal proportion of pattern A and C.

Significant interpopulation significance occurred for band 1, due to New Brunswick having a lower percentage frequency for that band.

Band 2 had significant differences in which New York and New Brunswick had lower percentages than Newfoundland and St. Lawrence estuary.

The esterase dendrogram (Figure 7C) shows Newfoundland and St. Lawrence estuary forming the main cluster at the level of 0.28. This cluster is joined by New Brunswick at the level of 1.33 and is thus far removed from the first cluster. New York joins New Brunswick at 1.66. These results agree with the preceding muscle myogen and LDH data.

Populations could be separated by examining the ratio of patterns which occurred. New Brunswick had a ratio of 1 : 1.7 : 0 (Pattern A, B and C, Figure 7) and Newfoundland had a ratio of 12.4 : 2.8 : 1. St. Lawrence estuary had a ratio of 12.4 : 4.2 : 1 and New York had a ratio of 5.5 : 1 : 5.5.

Total Biochemical. Cluster analysis of all the biochemical results show that Newfoundland and Lac St. Jean form the main cluster followed very closely by St. Lawrence estuary (Fig. 8). St. Lawrence estuary and Lac St. Jean presumably have had a common origin and thus their genetic makeup is similar. The Newfoundland population can be looked upon as an isolated population (the Cabot Strait separating it from the New Brunswick and New York populations) but it can probably join the St. Lawrence estuary via the Strait of Belle Isle.

New Brunswick and New York can have gene exchange but it seems as if they are slowly diverging (New York is approximately 1/5 removed from the New Brunswick population).

Biochemical and Morphometrics. For the combined total data (morphometrics and biochemical, Figure 9) Newfoundland and St. Lawrence estuary form the main cluster followed closely by New Brunswick. Lac St. Jean then joins the first cluster at 1.36 (24.4% distance away) and then New York finally joining at 1.64 (39%).

Comparing the separate results obtained (Morphometric, Total Biochemical, and Biochemical and Morphometric Data) some differences will be noted. Total biochemical versus morphometric (Fig. 8 versus Fig. 3) shows that the morphometric data are basically clinal whereas the biochemical data support the view of the genetic isolation of the population. The biochemical and morphometric data versus the total biochemical shows the weight of the morphometric data clearly being shown by moving Lac St. Jean from the primary cluster (Figure 8) to the second last position (Figure 9). Similarly for the other populations, shifts occur.

Summary. Some unanswered questions arise from the presented study. On the basis of the total biochemical data it was hypothesized that the Newfoundland population is somewhat isolated. But the cluster analysis joins in the first cluster Newfoundland and Lac St. Jean which it itself is an isolated population (landlocked). Why are Lac St. Jean and Newfoundland so closely related on the basis of biochemical data? Could it be that they originated from a common stock and since they are now isolated, there is little gene exchange and little variation. Another possible reason could be that only 6 Lac St. Jean specimens were examined biochemically.

There may also be the possibility of yearly changes that have been found in smelt populations for esterase and morphometric data (Copeman, 1973).

The results of the study show that a strong latitudinal cline exists in the morphometrics of *Microgadus tomcod*. The populations were divided into three consisting of a river population (New York and St. Lawrence estuary) a brackish salt water population (Newfoundland and New Brunswick) and a lake population (Lac St. Jean).

The biochemical results showed Lac St. Jean and Newfoundland being the most similar and New York population deviating the most.

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