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Serum esterases in mackerel, Scomber scombrus L.

by

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Introduction

A search for polymorphism of serum esterases in mackerel was undertaken independently in three fisheries laboratories, and applied to the study of mackerel stocks from the Northern North Sea to the waters South of the British Isles.

This paper describes and compares the esterase polymorphism demonstrated in each laboratory and the results of the population studies.

Material and methods

All sampling localities are shown in Figure 2. Particulars about the localities and dates of sampling are given in Tables 2 en 7, and on p. 7 for the English, Norwegian and Dutch investigations respectively. Biological data on the Norwegian samples also are given in Table 7.

Blood was collected from mackerel by cardiac puncture, or from the posterior cardial sinus (Lowestoft). A sodium citrate solution (3.8%) was used as anticoagulant in the Lowestoft Laboratory. Heparin was used to this purpose in the IJmuiden and Bergen laboratory, but omitted by the latter from the majority of samples collected. No differences were observed. Sera were stored frozen within 1 - 12 hours after collection and analyzed within 2 - 6 months, and in the Bergen laboratory also analyzed fresh, if possible.

In the Lowestoft laboratory electrophoresis was carried out in starch gel. The method used was either essentially that of Smithies (1955), using a continuous buffer system (Aaronsson and Grønwall, 1957), or that of Scopes (1968) in which a discontinuous gel and buffer system is used. With the latter method better results were obtained. In either case electrophoresis was carried out in a refrigerator at a temperature of 4°C. Unknown sera were inserted directly adjacent to control sera of known type.

Starch gel was also used as a medium for electrophoresis in the IJmuiden laboratory, and a discontinuous buffer system (Buffer 6, Smith, 1968) was applied. Gels were cooled during the electrophoretic run by a running tapwater system. All samples were run at least twice, arranged around control sera of known type.

In the Bergen laboratory the sera were analyzed by combined starch and agar gel electrophoresis using a 1 in 2 dilution of the Aaronsson and Grønwall buffer (Møller, 1966). Three sera were run on each microscope slide, the middle one being a control serum of known type.

Esterase activity was detected in the gels using alpha naphthylacetate as a substrate and Fast Blue BB (or RR in the Lowestoft laboratory) as a dye coupler.

Results and discussion

1. Serum esterase patterns

Esterase variants and patterns observed in the three laboratories are depicted in Figure 1.

In all specimens one or two of the components were present. The resulting phenotypes were designated according to a hypothesis of genetic control by a series of co-dominant alleles. The observed distribution of the phenotypes together with the expected Hardy-Weinberg distribution is given in Tables 1, 3 and 7 respectively for the samples investigated by the Lowestoft, IJmuiden and Bergen laboratory.

In section a of Figure 1, depicting the results obtained in the Lowestoft laboratory solid lines illustrate the 21 known patterns, while dotted lines represent the 7 remaining patterns.

In section b only the esterase variants that have been observed, either single or in combinations of two, in the IJmuiden laboratory are shown. The differences in migration distance between the variants a, f, m, s and y were found to be regular, the other variants migrating closely or at intermediate positions. Esterage activity at the positions 'k' and 'v' may involve more variants. The more frequently occurring phenotypes are listed in Table 3. Other phenotypes observed were: ac, as, f'k', fn, f'v', gn, 'k'm, 'k's, nn, ns and pp.

By the starch and agar gel method, used in the Bergen laboratory, several components with esterase activity could be distinguished. Only small differences in electrohoretic mobility were observed among some, however, and therefore they could not all form the basis of proper classification of the specimens. For this reason they were combined into five main components named Es B, Es F, Es M, Es S and Es Y in order of decreasing anodic mobility. Of the 15 possible phenotypes 13 were found.

2. Comparison tests

By exchange of serum samples between the laboratories it was possible to compare the esterase variants observed. Although no definite agreement on some of the rarer types and the variants showing only minor differences in migration could be obtained as yet, preliminary identification schemes resulted and could be applied in the comparison of the population data:

Lowestoft	not obs.	J	K	L	M	N	O	P
IJmuiden	a	c	e,f	g,k?	m,n	p,r,s	y	not obs.

Lowestoft	J	K,L	M	N	O	P
Bergen	B	F	M	S	Y	not obs.

IJmuiden	a,c	e,f,g	m,n,p	r,s	v,y
Bergen	B	F	M	S	Y

The frequency data obtained in the population studies and listed in Table 8 confirmed these identifications in showing a general agreement in the proportion of the more readily identifiable and more frequently occurring esterase variants.

3. Population studies

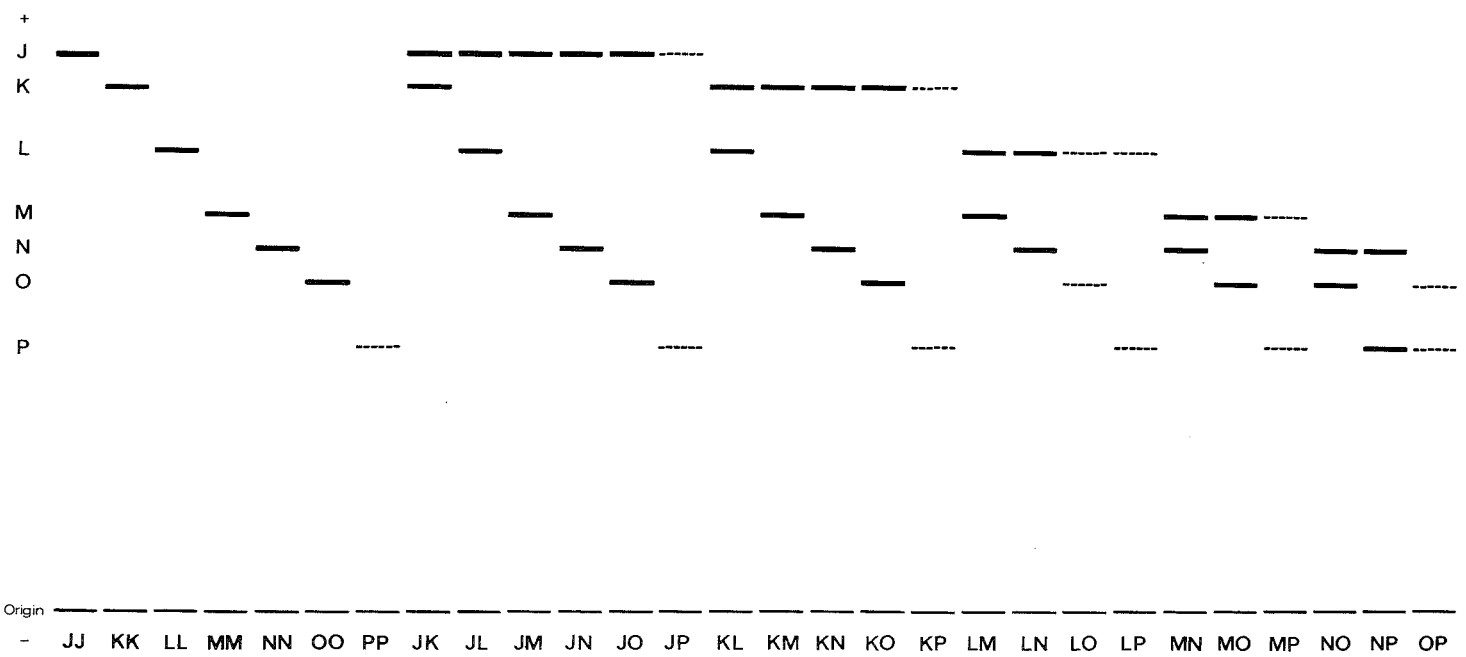
Results from the individual laboratories.

The genic composition of the samples investigated in the Lowestoft laboratory is given in Table 2 below together with the localities and dates of collection of the samples. Calculated gene frequencies may be found in Table 8, section a. The phenotype distribution observed and expected according to the Hardy-Weinberg law is shown in Table 1.

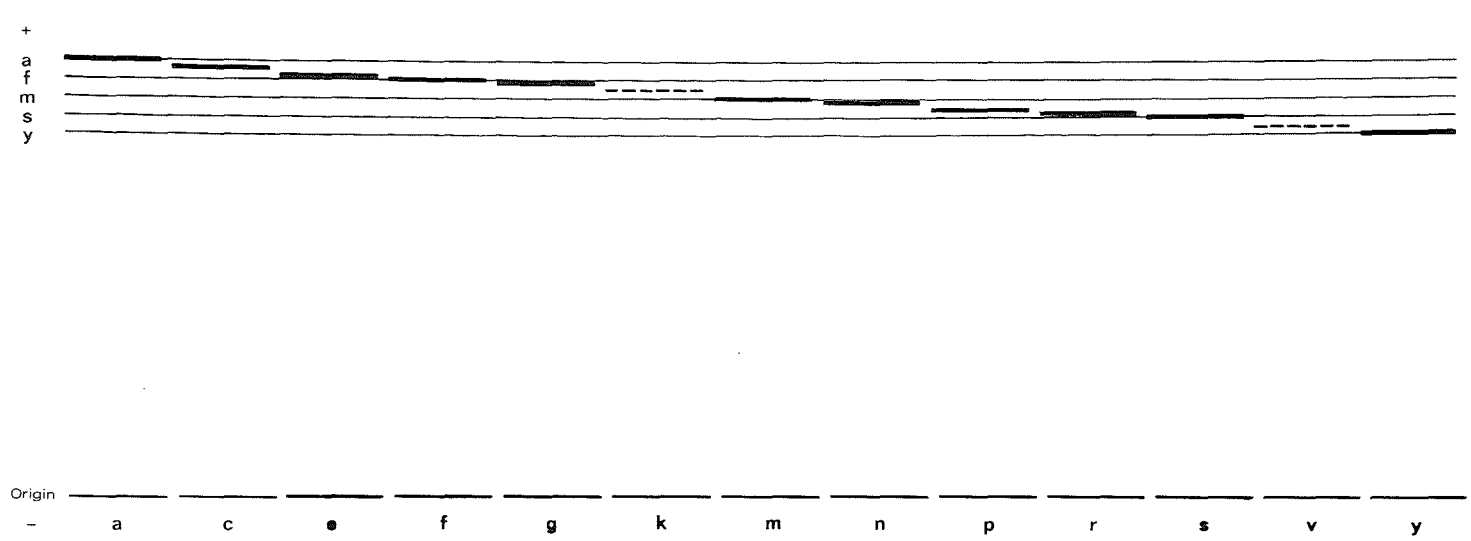
FIGURE 1

Serum esterase variants and patterns in mackerel obtained by electrophoresis in the English, Dutch and Norwegian laboratories.

a. Patterns obtained by starch gel electrophoresis in the Lowestoft laboratory
——— : observed patterns; ------ : postulated patterns



b. Migration distances of esterase variants observed in the IJmuiden laboratory in starch gel electrophoresis. – – – – – uncertain position



c. Patterns obtained by combined starch and agar gel electrophoresis at pH 9.0 in the Bergen laboratory.

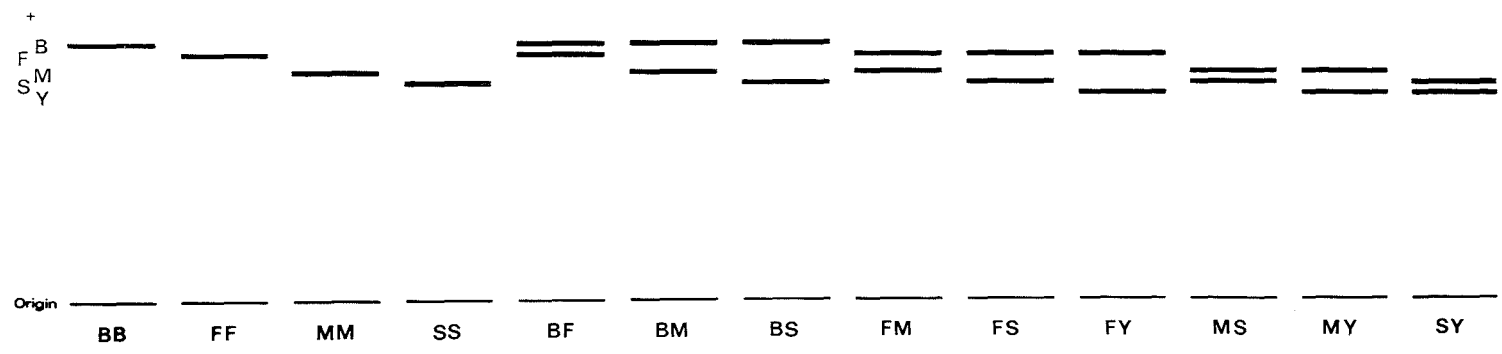


Table 1 Distribution of esterase phenotypes in mackerel - English investigations
O: observed; E: expected according to Hardy Weinberg law.

Sample no.		Esterase types																												Totals
		JJ	KK	LL	MM	NN	OO	PP	JK	JL	JM	JN	JO	JP	KL	KM	KN	KO	KP	LM	LN	LO	LP	MN	MO	MP	NO	NP	OP	
1	O	2	17	-	50	3	-	-	-	-	2	-	-	-	-	18	3	1	-	2	-	-	-	2	1	-	-	-	-	101
	E	-	8	-	39	-	-	-	2	-	4	-	-	-	1	35	3	1	-	1	-	-	-	7	1	-	-	-	-	
2	O	1	13	7	44	2	1	-	-	-	3	1	-	-	1	13	4	-	-	4	1	-	-	1	1	-	1	-	-	98
	E	-	5	1	31	-	-	-	1	1	3	-	-	-	4	25	3	1	-	11	1	-	-	7	2	-	-	-	-	
3	O	-	6	-	16	-	-	-	-	-	2	1	1	-	-	12	1	-	-	-	-	-	-	5	1	-	-	-	-	45
	E	-	3	-	15	-	-	-	1	-	2	-	-	-	-	14	2	1	-	-	-	-	-	4	1	-	-	-	-	
4	O	-	2	-	13	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	2	-	-	-	-	-	20
	E	-	-	-	12	-	-	-	-	-	-	-	-	-	-	5	-	-	-	1	-	-	-	2	-	-	-	-	-	
5	O	-	2	-	23	-	1	-	1	-	1	-	-	-	-	12	1	1	-	-	-	-	-	4	1	-	-	-	-	47
	E	-	2	-	22	-	-	-	-	-	1	-	-	-	-	13	1	1	-	-	-	-	-	3	3	-	-	-	-	
6	O	-	10	5	11	-	-	-	-	-	-	-	-	-	5	1	2	-	-	6	-	-	-	3	-	-	-	-	-	43
	E	-	5	3	6	-	-	-	-	-	-	-	-	-	7	10	2	-	-	8	1	-	-	2	-	-	-	-	-	
7	O	-	4	-	29	-	-	-	2	-	3	-	-	-	-	18	4	-	-	-	-	-	-	5	-	-	-	-	-	65
	E	-	4	-	27	-	-	-	1	-	3	-	-	-	-	21	2	-	-	-	-	-	-	6	-	-	-	-	-	
8	O	2	5	5	11	1	-	-	2	2	1	-	-	-	4	10	2	-	-	-	1	-	-	1	-	-	-	-	-	47
	E	-	4	2	6	-	-	-	3	2	3	1	-	-	5	10	2	-	-	6	1	-	-	2	-	-	-	-	-	
9	O	-	3	-	24	1	-	-	-	1	1	-	-	-	-	18	4	-	-	-	1	-	-	5	2	-	-	-	-	60
	E	-	3	-	23	1	-	-	-	-	1	-	-	-	-	17	3	-	-	1	-	-	-	7	1	-	-	-	-	
10	O	-	5	-	18	1	-	-	-	-	1	-	-	-	-	14	5	-	-	1	-	-	-	4	-	-	-	-	-	49
	E	-	4	-	16	1	-	-	-	-	1	-	-	-	-	17	3	-	-	1	-	-	-	6	-	-	-	-	-	
11	O	-	6	1	29	1	-	-	1	-	1	-	-	-	-	26	2	-	-	3	1	-	-	3	1	-	-	-	-	75
	E	-	6	-	28	-	-	-	1	-	1	-	-	-	2	25	2	-	-	4	-	-	-	5	1	-	-	-	-	
12	O	-	3	6	16	-	1	-	-	-	1	-	-	-	3	5	-	-	-	8	2	-	-	4	-	-	-	1	-	50
	E	-	1	3	13	-	-	-	-	-	1	-	-	-	4	7	1	-	-	13	2	1	-	4	1	1	-	-	-	
Total	O	5	76	24	284	9	3	-	6	3	16	2	1	-	13	149	28	2	-	25	6	-	-	39	7	-	1	1	-	700
	E	1	44	3	227	3	-	-	11	3	24	3	-	-	25	200	25	4	-	56	7	1	-	56	8	-	1	-	-	

Table 2. Genic composition, localities and dates of sampling of the samples investigated in the Lowestoft laboratory.

O: alleles counted; E: numbers allocated according to proportions of totals.

Sample		J	K	L	M	N	O	P	Totals	Location	Date
1	O	6	56	2	125	11	2	-	202	Kristiansand	29 May '68
	E	5	50	14	116	14	2	-			
2	O	6	44	20	110	12	4	-	196	Haugesund	12 June '68
	E	5	49	13	112	13	2	-			
3	O	4	25	-	52	7	2	-	90	Scapa Flow	July '68
	E	2	22	6	52	6	1	-			
4	O	-	6	1	30	3	-	-	40	North-east Bank	Oct. '68
	E	1	10	3	23	3	-	-			
5	O	2	19	-	64	5	4	-	94	Newhaven	23 May '68
	E	2	24	6	54	6	1	-			
6	O	-	28	21	32	5	-	-	86	Cherbourg	July '68
	E	2	22	6	49	5	1	-			
7	O	5	32	-	84	9	-	-	130	Chesil Bank	May '68
	E	4	32	9	75	9	2	-			
8	O	9	28	17	34	6	-	-	94	Mevagissey	30 April '68
	E	3	24	6	54	6	1	-			
9	O	2	28	2	74	12	2	-	120	Codgwith, Kennack	1 May '68
	E	3	30	8	69	8	1	-			
10	O	1	29	1	56	11	-	-	98	Newlyn	2 May '68
	E	3	24	7	56	6	1	-			
11	O	2	41	6	92	8	1	-	150	St. Ives	1 May '68
	E	4	38	10	86	10	2	-			
12	O	1	14	25	50	7	2	1	100	Barnstaple Bay	July '68
	E	3	25	7	57	7	1	-			
Totals		38	350	95	803	96	17	1	1400		

It can be observed from Table 1 that the three batches of samples from the South-West of England in the Lands End area showed genetic equilibria, and may represent a single stock (Samples 9, 10 and 11).

The totalized samples showed a massive excess of homozygotes. The greatest excesses of homozygotes in individual batches were found in stored material from Norway (Samples 1 and 2). If this is not a storage effect, the Norway material may represent mixtures of two or more stocks.

In Table 8, section a, the low frequency of the Es^{M} and the high frequency of the Es^{L} allele in samples 6, taken near Cherbourg, and 8, collected at Mevagissey may be noted. Both samples also showed excessive numbers of homozygotes, which may suggest that they contain an admixture of a southern stock not yet sampled as a simple unit stock batch.

Table 3 Distribution of esterase phenotypes in mackerel - Dutch investigations
O: observed; E: expected according to Hardy-Weinberg law.

Sample		am	a^e_f	cc	c^e_f	cg	cm	c^r_s	ee ff	$e_f{}^g$	$e_f{}^m$	$e_f{}^p$	er fs	$e_f{}^y$	gg	gm	g^r_s	mm	mp	m^r_s	mv	my	rr ss	other	N
1	O	2	-	-	2	-	2	-	14	1	29.5	-	5	-	1	10.5	3	45	1	19	-	-	6	-	141
	E	1.1	-	-	0.9	0.2	2.1	0.5	7.7	3.9	35.9	-	9.1	-	0.5	9.1	2.3	42.0	0.6	21.2	-	-	2.7		
2	O	-	-	-	-	1	3	-	9	1	20	-	5	2	1	9	1	36	1	13	-	1	-	3	106
	E	0.6	-	-	0.9	0.3	2.2	0.4	5.0	3.0	26.0	-	4.1	0.6	0.5	7.9	1.3	34.0	0.6	11.4	1.7	1.7	0.9		
3	O	-	-	-	3	0.5	10	1	20	3	55.5	1	16	-	5	16.5	2	77	-	19	-	1	2	1.5	234
	E	-	-	0.2	3.8	1.0	8.2	1.4	14.3	7.9	65.5	0.2	10.3	-	1.1	17.4	2.7	70.0	0.5	22.7	0.5	0.5	1.9		
4	O	1	1	-	-	-	3	1	11	-	19	1	5	-	4	4	1	40	-	10	-	-	-	2	103
	E	1.2	0.5	-	1.2	0.3	2.8	0.4	5.8	3.1	27.6	0.2	4.0	-	0.4	7.3	1.1	32.9	-	9.5	1.2	-	0.7		
5	O	1	-	1	2	2	7	-	33	1	80.5	-	19	-	2	15.5	-	102	2	23	2	-	5	1	299
	E	0.7	-	0.2	3.7	0.5	7.4	1.2	23.9	6.4	94.6	-	14.9	-	0.4	12.7	1.7	93.4	1.0	29.4	1.0	-	2.3		
6	O	-	1	1	4	-	4	-	10.5	1	46.5	-	7	1	1.5	12.5	-	50	-	7	1	-	-	2	150
	E	0.5	0.2	0.2	2.7	0.5	5.6	0.8	9.3	4.5	47.0	-	4.1	0.2	0.5	9.4	0.8	48.7	-	8.5	0.5	0.5	0.4		
7	O	1	-	-	-	-	3	-	5	1	20	-	4	-	-	4	-	42	-	15	-	-	1	1	97
	E	0.6	-	-	0.7	0.1	2.7	0.4	3.1	1.0	22.8	-	3.8	-	0.1	3.5	0.4	41.5	-	13.7	-	-	1.1		
8	O	-	-	-	1	-	-	-	3	-	13	-	-	-	-	5	-	19	-	7	-	1	1	-	50
	E	-	-	-	0.2	-	0.6	0.1	2.0	1.0	12.8	-	1.8	-	0.1	3.1	0.5	20.5	-	5.8	-	0.6	0.4		
9	O	-	-	0.5	-	-	6	2	12.5	2	44	1	7.5	-	2	5	1	54	1	9.5	-	-	-	1	149
	E	-	-	0.1	2.4	0.4	5.2	0.7	10.6	3.2	46.3	0.7	5.8	-	0.2	6.9	0.9	50.5	1.7	12.7	-	-	0.8		
10	O	-	0.5	1	1	-	4	-	3.5	-	10	-	2	-	2.5	1	-	15	-	4	-	-	-	0.5	45
	E	0.5	-	0.3	1.7	-	4.1	0.5	2.3	1.4	11.2	-	1.3	-	0.2	3.3	0.4	13.3	-	3.3	-	-	0.2		

The locality and date of collection of the samples investigated in the IJmuiden laboratory are given below:

Sample	Locality	Date	Sample	Locality	Date
1 - 4	S. North Sea		5 - 6	N.E. North Sea	
1	Southern Bight	21 June '67	5	Coral Bank	11 May '68
2	Texel Lightvessel	22 June '67	6	Viking Bank	13 May '68
3	Cleaver Bank	28 June '67	7	Irish Sea	15 May '69
4	Flamborough	22 Aug. '67	8 - 10	Celtic Sea	19 May '69

The observed distribution of the phenotypes and the expected Hardy-Weinberg distribution are given in Table 3. The numbers of the phenotypes involving the closely migrating variants e and f, m and n, and r and s have been combined because they may not have been differentiated with sufficient accuracy in some of the samples.

An excess of the homozygotes ee/ff and mm, together with a shortage of the e/fm heterozygote, was observed in the samples 1 - 4 and 5. The shortage of the e/fg heterozygotes in the samples 1 - 6 was considered to be due to the difficulty of differentiating this phenotype from the homozygotes of the f and g variants that migrate at close distance. The excess of the gg homozygote observed in these samples may also be due to this typing error.

In the samples 6, 7 and 8 - 10 the deviations between the observed and expected values of the ee/ff, e/fm and mm phenotypes, if existing, were minor. It may be noted that the positions at which the samples 6 and 8 - 10 were taken are located in areas where mackerel spawning is concentrated, and close to or within the spawning period.

The results of the analysis of possible associations between the esterase system and length and/or sex of the animals are given in Tables 4 - 6.

Table 4. Genic composition of different length classes in samples investigated in the IJmuiden laboratory.
O: numbers observed; E: numbers expected according to proportions of totals.

Sample length.		a	c	e f	g	'k'	m n	p	r s	v	y	Total
1 - 4												
less than 26 cm	O	1	4	53.5	22.5	2	136	1	23	-	-	244
	E	1.0	5.9	58.5	15.9	0.8	135.1	0.8	24.5	0.6	0.8	243.9
26 - 30 cm	O	2	11	92.5	22.5	1	212	1	36	1	3	382
	E	1.6	9.2	91.6	24.9	1.3	211.4	1.3	38.3	1.0	1.3	381.9
31 - 35 cm	O	-	6	43	14	-	116	-	23	-	-	202
	E	0.9	4.8	48.5	13.2	0.7	111.8	0.7	20.3	0.5	0.7	202.0
over 35 cm	O	2	7	91	17	1	182	2	35	2	-	339
	E	1.5	8.1	81.3	22.1	1.2	187.7	1.2	34.0	0.9	1.2	339.2
8 - 10												
26 - 30 cm	O	1	10.5	76.5	18	-	198	2.5	21.5	-	-	328
	E	0.7	10.3	78.1	18.5	-	193.9	2.5	23.2	-	0.7	327.9
31 - 35 cm	O	-	4	33	8	-	74	1	11	-	1	132
	E	0.3	4.2	31.4	7.5	-	78.0	1.0	9.3	-	0.3	132.0
6												
31 - 38 cm	O	-	2	35.5	10.5	1	77	-	6	1	1	134
	E	0.4	4.5	36.9	7.4	0.9	76.4	-	6.7	0.4	0.4	134.0
39 - 45 cm	O	1	8	47	6	1	94	-	9	-	-	166
	E	0.6	5.5	45.6	9.1	0.1	94.6	-	8.3	0.6	0.6	166.0

Table 5. Phenotype distribution in separate length classes and sexes. O: observed; E: expected according to Hardy-Weinberg law.

Samples length		ee ff	e f^{g}	e f^{m}	er fs	gg	gm	g$^{r}_{s}$	mm	m$^{r}_{s}$	rr ss	other	N
1 - 4													
less than 26 cm	O	12	2	19.5	6	3	11.5	3	44	13	-	8	122
	E	5.9	4.9	29.8	5.0	1.0	12.5	2.1	37.8	12.8	1.1		
26 - 30 cm	O	10	3	51.5	11	1	14.5	1	58	20	2	19	191
	E	11.2	5.5	51.3	8.7	0.7	12.5	2.1	58.8	19.9	1.7		
31 - 35 cm	O	9	-	17	8	4	4	2	38	13	-	6	101
	E	4.6	3.0	24.7	4.9	0.5	8.0	1.6	33.3	13.2	1.3		
over 35 cm	O	23	-	36	6	3	10	1	58	14	6	12	169
	E	12.1	4.5	48.6	9.3	0.4	9.1	1.8	48.7	18.7	1.8		
8 - 10													
26 - 30 cm	O	10	-	48	5	3	11	1	60	12.5	1	12.5	164
	E	9.0	4.2	46.3	5.3	0.5	10.9	1.2	59.8	13.1	0.7		
31 - 35 cm	O	7	2	14	3	1.5	3	-	23	7	-	5.5	66
	E	4.4	1.2	18.5	2.8	0.2	4.4	0.7	20.8	3.1	-		
6													
31 - 38 cm	O	5	-	19.5	3	1	8.5	-	23	2	-	5	67
	E	4.7	2.8	20.4	1.6	0.4	6.0	0.5	22.2	3.5	0.1		
39 - 45 cm	O	5.5	1	27	4	0.5	4	-	27	5	-	8	83
	E	6.7	1.7	26.6	2.5	0.1	3.4	0.3	26.6	5.1	0.2		
Samples sex, length													
1 - 4													
male	O	23	1	36	12	4	12	1	62	14	1	16	182
	E	13.2	6.2	52.7	8.0	0.7	12.1	1.9	52.7	16.1	1.2		
female	O	8	2	37.5	8	5	8.5	2	54	15	1	10	151
	E	7.5	5.0	38.7	6.2	0.8	12.8	2.0	49.8	15.9	1.3		
8 - 10													
male	O	8	1	35	5	3	6	1	32	12.5	1	13.5	118
	E	7.0	3.4	31.3	4.8	0.4	7.5	1.3	34.7	11.6	1.0		
female	O	10	1	28	4	1.5	8	-	55	7	-	7.5	122
	E	6.1	2.7	34.9	2.9	0.3	7.6	0.6	49.2	8.2	0.3		
6													
male	O	6	-	18.5	1	1	9.5	-	20	1	-	9	66
	E	4.4	3.0	18.8	0.8	0.5	6.4	0.3	20.2	1.7	0.0		
female	O	4.5	1	28	6	0.5	2	-	30	6	-	5	83
	E	6.9	1.2	28.0	3.5	0.1	2.3	0.3	28.3	7.0	0.4		
1 - 4													
male 26 - 30 cm	O	5	-	15	4	-	4	-	16	4	1	4	53
	E	4.3	1.1	16.1	2.8	0.1	2.2	0.4	15.3	5.4	0.5		
male over 30 cm	O	14	-	17	8	3	6	1	36	8	-	10	103
	E	7.1	3.4	28.8	4.7	0.4	6.9	1.1	29.4	9.6	0.8		
female 26 - 30 cm	O	1	2	13.5	2	-	4.5	-	16	2	-	4	45
	E	2.6	1.6	12.9	1.0	0.2	3.9	0.3	16.2	2.4	0.1		
female over 30 cm	O	5	-	20	4	4	3	1	32	8	1	5	83
	E	3.9	2.6	21.0	3.5	0.4	1.2	1.2	28.3	9.3	0.8		

The data from the samples from two geographic areas, the Southern North Sea (samples 1 - 4) and the Celtic Sea (samples 8 - 10), of which size and sex had been determined, were pooled to obtain a sufficient number of specimens in each group. Sample 6, from the Viking Bank, was analyzed separatedly.

The genic composition, presented in Tables 4 and 6, did not show any regular significant association with either the length groups present in the samples or the sex of the animals.

Table 6. Genic composition of different sexes in samples investigated in the IJmuiden laboratory.
O: numbers observed; E: numbers expected according to proportions of totals.

Sample sex		a	c	e f	g	'k'	m n	p	r s	v	y	Total
3 - 4												
male	O	2	12	98	22.5	0.5	196	-	30	2	1	364
	E	1.1	10.9	90.3	24.6	0.8	201.9	0.5	31.7	1.6	0.5	363.9
female	O	-	8	67.5	22.5	1	174	1	28	1	-	303
	E	0.9	9.1	75.2	20.4	0.7	168.1	0.5	26.3	1.4	0.5	303.1
8 - 10												
male	O	1	11	58	14	-	128	1.5	21.5	-	1	236
	E	0.5	8.9	55.6	12.8	-	139.1	1.7	17.0	-	0.5	236.1
female	O	-	10	55	12	-	155	2	13	-	-	244
	E	0.5	9.1	57.4	13.2	-	143.9	1.8	17.5	-	0.5	243.9
6												
male	O	-	7	34.5	11.5	1	73	-	3	1	1	132
	E	0.4	4.4	36.6	6.9	0.9	75.3		6.7	0.4	0.4	132.0
female	O	1	3	48	4	1	97	-	12	-	-	166
	E	0.6	5.5	46.0	8.6	1.1	94.7		8.4	0.6	0.6	166.1

Analysis of the distribution of the major phenotypes in the separate length groups, as presented in Table 5, shows that in the pooled samples 8 - 10 and sample 6 no major deviations of observed and expected values were found in any of the length classes. It may be recalled that these samples as a whole also showed genetic equilibrium. In the samples 1 - 4 excess of the major homozygotes, together with a shortage of the e/fm heterozygote, was found in three of the length classes, but was absent in the group from 26 - 30 cm.

Analysis of the separate sexes in the samples 1 - 4 showed a pronounced deviation of the phenotypic ratio in the males, not in the females. In the samples 8 - 10 and 6 respectively, minor deviations were found in females or no deviations in either of the sexes.

Further analysis of the samples 1 - 4 showed that the deviating phenotypic ratio did not occur in either males or females of the 26 - 30 cm length class, but it was considerable in the males of the larger length classes.

Table 7 Distribution of esterase phenotypes in mackerel - Norwegian investigations
O: observed; E: expected according to Hardy-Weinberg law.

Sample no.		Esterase phenotypes BB	BF	BM	BS	FF	FM	FS	FY	MM	MS	MY	SS	SY	Totals	Locality and date	Indications of sample
4	O	-	-	-	-	7	20	5	-	19	4	-	-	-	55	56°10' N, 05°30' E,	Mixed
	E	-	-	-	-	6.9	22.0	3.2	-	17.5	5.1	-	0.4	-		North Sea, 24 Aug. '67	
5	O	-	-	2	-	18	27	6	-	40	18	-	1	-	112	57°58' N, 08°14' E,	Adult, near
	E	-	0.6	1.1	0.2	10.6	39.1	8.0	-	36.0	14.7	-	1.5	-		North Sea, 28 May '68	spawning
6	O	-	1	2	-	32	60	6	2	84	24	2	3	-	216	57°55' N, 08°20' E,	Adult, near
	E	-	0.9	1.8	0.3	20.5	78.9	11.0	1.2	76.0	21.3	2.3	1.5	0.3		North Sea, 29 May '68	spawning
7	O	-	-	6	1	33	59	14	-	109	15	1	4	-	242	59°27' N, 04°40' E,	Adult,
	E	-	1.9	4.2	0.5	19.9	85.8	11.0	0.3	92.4	23.6	0.6	1.5	0.1		North Sea, 12 June '68	spawning
8	O	-	-	-	-	3	11	3	-	16	5	1	-	-	39	60°31' N, 00°05' E,	Immature
	E	-	-	-	-	2.6	12.5	2.1	0.2	15.4	5.0	0.6	0.4	0.1		North Sea, 20 June '68	
9	O	-	1	2	-	11	24	7	2	46	11	-	-	1	105	Stall, Austfj.,	Adult,
	E	-	0.8	1.8	0.3	7.5	34.4	5.0	0.8	39.6	11.6	1.8	0.9	0.3		Hordaland, 1 July '68	spawning
10	O	-	-	2	1	11	30	5	-	39	9	1	1	-	99	Vernøy, Bjørnefj.,	Adult,
	E	-	0.9	1.8	0.3	8.2	34.6	4.9	0.3	36.4	10.3	0.6	0.7	0.1		Hordaland, 13 July '68	spawned
11	O	-	1	3	-	7	44	4	-	33	11	-	1	1	105	55°50' N, 06°45' E,	Immature
	E	-	1.2	2.4	0.3	9.5	37.2	5.4	0.3	36.6	10.7	0.6	0.8	0.1		North Sea, 9 Aug. '68	
12	O	-	1	4	-	12	24	3	-	42	12	-	2	-	100	Asgard, Austfj.,	O-group
	E	0.1	1.3	3.1	0.5	6.7	32.2	4.9	-	38.4	11.8	-	0.9	-		Hordaland, 12 Oct. '68	
13	O	1	2	10	-	41	96	18	-	95	31	1	3	1	299	60°00' N, 03°50' E,	Mixed, mainly
	E	0.2	4.8	7.9	1.3	32.8	108.5	18.6	0.6	89.8	30.8	1.0	2.6	0.2		North Sea; 24 April '69	immature
14	O	1	2	6	1	17	33	10	1	65	9	1	1	-	147	57°56' N, 08°00' E,	Adult, near
	E	0.2	3.0	6.6	0.8	10.9	48.7	6.0	0.6	54.5	13.1	1.3	0.8	0.1		North Sea, 10 June '69	spawning

The observed distribution and the expected Hardy-Weinberg distribution of the phenotypes in the samples investigated in the Bergen laboratory, are given in Table 7, together with locality, date and composition of the samples.

Significant deviations between observed and expected distributions were found in samples 5, 6, 7 and 14, where a clear excess of hypothetical homozygotes was observed. Also in the other samples, except sample 11, an excess of homozygotes was noted, but the overall agreement between observed and expected distributions was rather good.

The samples which showed the greatest deviation between observed and expected distributions were all collected from adult mackerel near spawning or in spawning condition. Considerable deviations from the expected distribution were also observed, however, in for instance sample 12, which was collected from the 0-group.

Existence of populations with different gene frequencies was not indicated by the results of any of the analysed samples, as shown in Table 8, section c.

4. Discussion of the joint results

In comparing the results obtained in the three laboratories in which the esterase polymorphism in mackerel has been investigated the following common observations may be noted:

1. There was much similarity in the overall range of variation in the esterasetypes throughout all of the batches of mackerel tested.
2. Most of the batches tested showed general agreement in their proportions of the more readily identifiable and more frequently occurring esterase bands.
3. The majority of the batches tested showed an excessive number of single band patterns. This effect was common to tests made in different laboratories.
4. In the remaining samples a good agreement between the observed and expected distribution of the phenotypes was found.

The hypothesis of genetic control of the esterase polymorphism in mackerel by a series of co-dominant alleles, based on the latter observation, also finds support in the existence of genetically controlled variations of serum esterases in tuna species (Sprague, 1967; Fujino and Kang, 1968), which resemble the variations observed in mackerel.

Assumption that the excess of homozygotes observed in a large part of the samples may be caused by sampling of mixed populations appears to be contradicted by the absence of regional stocks, markedly differing in gene frequencies. According to Jamieson the numerical type data may suggest that the samples as taken, bled and tested indicate much physical mixing of specimens following a degree of inbreeding among the parents of the sampled fish. This apparent contradiction may be explained by postulating isolation by spacial dispersal alone or by assortative or preferential mating habits or by genetic incompatibility between sympatric stocks. Postulating sympatric stocks would explain the apparent excess of homozygotes coincident with the lack of any convincing evidence for regional stocks.

The existence of populations with different gene frequencies not being indicated, Naevdal calls attention to the possibility of selection against heterozygotes (negative heterosis) or methodical errors as possible reasons for the excess of homozygotes observed. Negative heterosis has been observed in some cases of protein polymorphism (Manwell and Baker, 1969). It should, however, be expected to affect all samples to the same degree. Methodical error may arise because the two bands of the hypothetical heterozygotes sometimes appeared very unequal in strength. In specimens of generally weak esterase activity the weaker band might be overlooked and heterozygotes might be classified as homozygotes. All specimens were however analyzed twice and weak bands were carefully looked for. Therefore it seems improbable that this type of error could account for the observed deviations.

The observation made in the IJmuiden laboratory that the deviations of the phenotypic distribution observed in the samples from the Southern North Sea were not equally present in all size groups, may be envisaged against data on the length/age relationship provided by Postuma (pers. comm.). Accordingly the 26 - 30 cm length class, that did not show any deviating phenotypic distribution, appears to represent a single, particularly strong year class. The larger length classes in which deviations of the phenotypic distribution were found, consisted of various year classes overlapping in length. In the group below 26 cm in which also only one year class appears to be present a deviation of the phenotypic distribution was also found however. These observations indicate that it may be useful to include information about the age composition of the samples in the analysis of the esterase system in mackerel. Individual year classes of fishes may, particularly in the first stages of their life history, be subject to variable environmental conditions of considerable importance in determining their survival. The demonstration by Koehn (1969) that temperature is the component of selection in maintaining esterase polymorphism in a freshwater fish may be thought of in this connection.

Conclusions

The recent tests for serum esterase variants in mackerel and our attempts to distinguish, compare and equate the variants observed in the three testing laboratories have proved a useful pilot exercise. The exchanges of material and results between laboratories gave impetus to accuracy, and a measure of confidence in particular in the population studies in areas where the individual laboratories only could collect scattered samples.

On the basis of the joint results it can be said that the present observations do not appear to have any direct and practical applications as simple regional stock 'tags'. Nevertheless, the possible interpretations of the data raise new questions which, when satisfactorily answered, could give a new appreciation of the biology of the mackerel.

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References

Aaronsson, T. and Gronwall, A. 1957 Improved separation of proteins in paper electrophoresis. Scand. J. Clin. Lab. Invest., 9: 338

Fujino, K. and Kang, T. 1968 Serum esterase groups of Pacific and Atlantic tunas. Copeia, 1968: 56 - 63.

Koehn, R. 1969 Esterase heterogeneity: Dynamics of a polymorphism. Science, 163: 943 - 944.

Manwell, C. and Baker, C.M.A. 1969 Hybrid proteins, heterosis and the origin of species - I. Unusual variation of polychaete Hyalionoecia "nothing dehydrogenases" and of quail Coturnix erythrocyte enzymes. Comp. Biochem. Physiol., 28: 1007 - 1028.

Møller, D. 1966 Polymorphism of serum transferrin in cod. FiskDir. Skr. Ser. HavUnders., 14: 51 - 60.

Scopes, R.K. 1968 Methods for starch-gel electrophoresis of sarcoplasmic proteins. Biochem. J. 107: 139 - 150.

Smith, I. 1968 Techniques of starch gel electrophoresis. In: Smith, I., Editor, "Chromatographic and electrophoretic techniques - Volume 2, Zone electrophoresis", Heinemann Medical Books Ltd, London, 2nd Edition, p. 217 - 324.

Smithies, O. 1955 Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochem. J., 61: 629 - 641.

Sprague, L.M. 1967 Multiple molecular forms of serum esterase in three tuna species from the Pacific Ocean. Hereditas, 57: 198 - 204.

Table 8 Esterase gene frequencies in mackerel samples.

a. English investigations.

Sample	Es^J	Es^K	Es^L	Es^M	Es^N	Es^O	Es^P
1	.03	.28	.01	.62	.05	.01	-
2	.03	.22	.10	.56	.06	.02	-
3	.04	.28	-	.58	.08	.02	-
4	-	.15	$.02^5$	.75	$.07^5$	-	-
5	.02	.20	-	.68	.05	.04	-
6	-	.33	.24	.37	.06	-	-
7	.04	.25	-	.65	.07	-	-
8	.10	.30	.18	.36	.06	-	-
9	.02	.23	.02	.62	.10	.02	-
10	.01	.30	.01	.57	.11	-	-
11	.01	.27	.04	.61	.05	.01	-
12	.01	.14	.25	.50	.07	.02	.01

b. Dutch investigations.

Sample	a	c	e f	g	'k'	m n	p	r s	v	y
1	.007	.014	.233	.059	--	.546	.004	.138	--	--
2	.005	.019	.217	.066	.014	.566	.005	.095	.014	.014
3	--	.032	.256	.068	.001	.547	.002	.089	.002	.002
4	.010	.024	.237	.063	.005	.565	.005	.082	.010	--
5	.002	.022	.283	.038	.002	.559	.003	.088	.003	--
6	.003	.033	.275	.055	.007	.570	--	.050	.003	.003
7	.005	.021	.180	.028	.005	.654	--	.108	--	--
8	--	.010	.200	.050	--	.640	--	.090	--	.010
9	--	.030	.267	.040	--	.582	.010	.071	--	--
10	.011	.083	.228	.067	--	.544	--	.067	--	--

c. Norwegian investigations.

Sample	q_B	q_F	q_M	q_S	q_Y
4	--	.355	.564	.082	--
5	.009	.308	.567	.116	--
6	.007	.308	.593	.083	.009
7	.014	.287	.618	.079	.002
8	--	.256	.628	.103	.013
9	.014	.267	.614	.090	.014
10	.015	.288	.606	.086	.005
11	.019	.300	.590	.086	.005
12	.025	.260	.620	.095	--
13	.024	.331	.548	.094	.003
14	.037	.272	.609	.075	.007

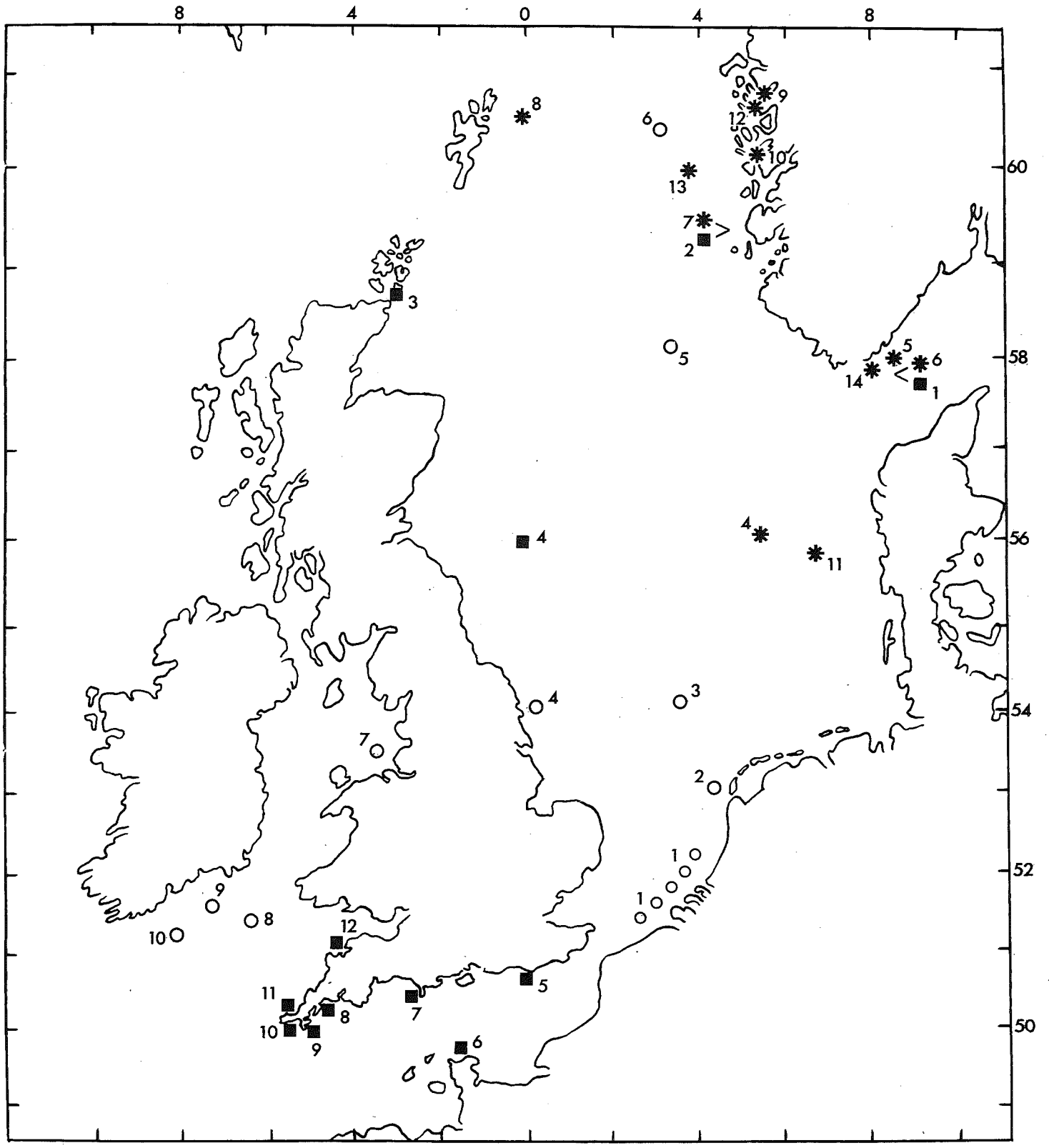

FIGURE 2

Sampling localities of blood samples in mackerel

■ English investigations

O Dutch investigations

✱ Norwegian investigations