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Techniques to identify continental origin of Atlantic salmon caught at sea

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Abstract

Starch gel electrophoresis revealed hereditarily determined differences between the proteins of Atlantic salmon (*Salmo salar* L.) from North America and Europe. These differences could be used to classify individual fish caught at sea. Analyses of salmon samples of mixed origin caught off West Greenland (mainly Disko Bay) indicated 43% North American fish. Collections in the southern part of the Labrador Sea indicated that salmon of European origin occur close to the Canadian continental shelf. Division of salmon according to continental origin is supported by evidence from smolt age distribution, mean fork length and abundance of the two parasites *Anisakis simplex* and *Eubothrium crassum*. Consistent qualitative differences in the proteins of salmon from the two continents indicate they belong to different gene-pools and suggest that the two types are different subspecies according to accepted definitions. The name *Salmo salar salar* is suggested for the nominal subspecies (European) and *S. s. americanus* for its North American counterpart.

Introduction

In this paper biochemical differences in Atlantic salmon are critically examined and used to identify individual North American and European salmon off West Greenland. Studies were carried out on the smolt age composition, fork length, and parasite fauna of the same specimens to supplement the biochemical technique. Results of these investigations were then compared with data from tagged Canadian salmon (control) recovered at Greenland to establish the validity of the original separation.

Materials and methods

Analyses on blood, liver and kidney tissues were performed on 242 adult salmon of unknown continental origin caught off West Greenland by the Canadian Fisheries Research vessel *A.T. Cameron* during the fall of 1969 and 25 salmon caught in the Labrador Sea by the same vessel during the spring of 1970 (Fig. 1). In these two samples only fish belonging to the same sea-age classes (1 and 2 sea winters respectively) were considered for examination of age, length, and abundance of the adult tapeworm *E. crassum* and the larval nematode *Anisakis simplex*. This age selection eliminated possible variation resulting from different sea ages. Thus, 178 1-sea-winter salmon were examined in 1969 and 23 2-sea-winter fish in 1970. Similar studies were made on a total of 93 tagged Canadian salmon caught in Greenland in 1969. They originated in several Canadian rivers but a high proportion came from the Miramichi River system.

Tissue sampling

Blood sera, livers and kidneys were sampled from most fish.

A. Blood sampling - Blood was withdrawn from the caudal vein employing B-D's Vacutainer system, with liquid EDTA serving as anticoagulant. These tubes were submerged in ice-water until each catch had been sampled. Then the blood was transferred to the polyethylene tubes of the Beckman Spinco Analytical system and spun for 35 seconds in the Microfuge at 20,000 rev/min. The supernatant sera were then immediately transferred to new test tubes and stored in deep freeze at -20 C until analysis.

B. Livers and kidneys - Both organs were collected as soon as possible after the blood sampling, placed in plastic bags and frozen. Only a 3 cc portion of each organ was needed. Before analysis each piece was ground in a Sorvall homogenizer in an equal amount of gel-buffer (Ashton and Braden, 1961) for two minutes at top speed. The resulting slurry was transferred to polyethylene test tubes and centrifuged for 15 minutes. The supernatant was used for electrophoretic analysis, either directly or after storage in a freezer.

Electrophoretic procedure:

A standard model of water-cooled horizontal starch gel electrophoretic apparatus was employed. Detachable buffer vessels were used for rapid changing of buffer systems. Platinum was used for electrodes and Wettex sponge cloths were employed to connect the electrode buffer with the gel surface. The gel was covered with Parafilm M (American Can Co., Neenah, Wis., U.S.A.) and subjected to 400 volts DC for 105 minutes. The gel plate allowed 40 specimens to be analysed simultaneously. After the completion of the electrophoresis the gel slab was sliced horizontally, one slice being stained with Amido Black, the other with Coomassie Brilliant Blue (or for detection of transferrin zones: Nitroso R salt according to the method by Mueller (1962)).

Buffer systems:

A. Ashton and Braden (1961); (105 min electrophoresis) (400 V)
Electrode buffer: 0,029M lithium hydroxide
0,191M boric acid

Gel buffer: 0,0076M citric acid
0,051M 'Tris' (2-Amino-2-(hydroxymethyl--1,3-propanediol)

B. Aronsson and Grönwall (1957), modified according to de Ligny (1967): (3.5 hr electrophoresis) (350 V)

Standard solution: 0.5M Tris (see above)
0.075M boric acid
0.021M EDTA (disodium salt)

Electrode buffer: 3-fold dilution of standard buffer, adjustment of pH to 8.9

Gel buffer: 125 ml stnadard buffer
1.5 g EDTA
1.15 g boric acid
875 ml aq. dest.
Adjustment of pH to 8.2

C. Gahne (1966): (2 hr electrophoresis) (300 V)

Electrode buffer: pH 8.5
0.06M lithium hydroxide
0.229M boric acid

Gel buffer: pH 8.5
1 volume electrode buffer
5.4 volumes Tris-citric acid buffer (0.079M Tris + 0.007M citric acid)

The buffer by Ashton and Braden was employed in all studies on livers and kidneys and for some of the sera; the other two buffer systems were used only for sera.

Staining procedures:

A. Amido Black (stock solution):

300 ml methanol
300 ml aq. dest.
60 ml HAc
150 ml glycerine
5 g Amido Black
(2 g nigrosine)

A small amount of this solution is spread over the gel surface. After 2 minutes excess dye is rinsed away in a 5:5:1 solution of aq. dest., methanol, and acetic acid. Agitation in this destaining solution overnight before photographic recording.

B. Coomassie Brilliant Blue: according to Chrambach et al. (1967):

1:20 dilution in 12.5% TCA (Trichloroacetic acid) of a 1% aqueous stock solution of Coomassie Brilliant Blue R 250. After 30 min of staining the gel is transferred to 10% TCA and photographed after 48 hr.

C. Nitroso R reagent: according to Mueller et al. (1962):

Ferric ammonium sulphate is first added to the serum in an amount equivalent to about 5 µg of Fe +++ per ml serum. Incubate for 15 min in the following solution:

0.5 g Nitroso salt
1 g hydroxylamine hydrochloride
2.7 g sodium acetate ($3H_2O$)
1.5 ml glacial acetic acid

The gel is then washed with the same destaining solution as used for Amido Black.

D. Nonspecific esterases: Incubation in a sodium phosphate buffer of pH 7.0 (Burstone, 1962) for 15 min.

Then add: 100 mg Fast Red TR salt
50 ml aq. dest.
1 ml 1% solution of alpha naphthyl acetate in equal amounts of aq. dest. and acetone.

Destaining same as above.

Tissue sampling and electrophoretic procedures have been previously described (Nyman, 1967b).

Abundance of the larval nematode *A. simplex* free in the body cavity, encysted on the visceral peritoneum or in the muscle layers of the alimentary canal was determined with the use of ultraviolet light (Sinnhuber and Law, 1949) as described by Pippy (1970). The presence or absence of the adult tapeworm *Eubothrium crassum* was determined by slitting open a sample of 15 pyloric caecae and opening and examining the pyloric region of the intestine. Preliminary studies had shown these to be the normal localities for the tapeworm.

Results

Studies on salmon of known continental origin

Genetically controlled differences were described in the proteins of North American and European salmon (Nyman, 1966, 1967a, b). However, these studies were performed on 2- and 3-year-olds. Thus, for determination of the potential value of these characters for separation of individual salmon off Greenland, analyses of adult fish from both continents were performed. Samples of river- and sea-run adult fish from both mainland Canada and Newfoundland as well as landlocked sexually mature salmon (Newfoundland) proved identical in two serum protein systems. These were also identical to the electropherograms found in the North American immature forms described by Nyman (1966). Similarly, the serum electropherograms of juvenile and adult European salmon were identical. Comparisons of the liver esterase zymograms in sea-run fish, indicated that these patterns were influenced by environmental factors, and thus seemed of limited value for stock separation. The differences between American and European fish located in serum and kidney esterases were completely absent in adult salmon, indicating they are ontogenetically controlled. Whole frozen or otherwise haemolysed blood could not be used because haemoglobin electropherograms interfere with systems I and II (see below).

North American and European salmon could be separated primarily on the basis of the two independent systems in the blood sera as revealed by staining with Amido Black or Coomassie Brilliant Blue. Distinct liver esterase zymograms, roughly 20% of the total number, could be classified as to continent of origin.

System I - Separation with this system was based on the relative position of band '4' (Fig. 2,I = zone 'a' - Nyman, 1966) in the blood serum electropherogram when employing the buffer system by Ashton and Braden (1961) and subsequent staining with Amido Black or Coomassie Brilliant Blue. This zone is located close to the transferrin group, and stained positively (faint green) when employing Nitroso R salt, a sensitive iron indicator. However, interference with the slowest migrating transferrins seems possible. Classification of individual sera was made by measuring the distance from this zone (G1) to the slowest migrating albumin (Alb) (Fig. 3). A test of 50 randomly chosen fish of each group showed no overlap, the 'American' fish having a range of 3.58-3.68 cm (mean 3.63) the 'European' salmon ranging from 3.80-3.88 (mean 3.85).

Improved separation of the two locations was obtained when using the buffer system by Aronsson and Grönwall (1957) as modified by de Ligny (1967). This method allowed direct visual classification of individual fish (Fig. 4). Separation with system I was always performed with both buffer systems to minimize reading errors.

System II - Separation with this system was performed on blood sera using the buffer by Gahne (1966) and staining with Amido Black or Coomassie Brilliant Blue (Fig. 2,II and Fig. 5). The difference is located in the more cathodic portion of the electropherogram, and consists of a quantitatively much more intense 2-band zone in European salmon. This system, located at positions '6' and '7' corresponds to zone 'b' according to Nyman (1966) using buffer by Ashton and Braden (1961). However, employing the buffer by Gahne augmented the difference. Comparison with human sera indicated that this zone consists of globulins.

System III - The differences found in liver esterases of juvenile salmon are shown in Fig. 2,III. The somewhat slower migration of band 4 and the much lower enzyme activity of band 3 in American salmon is clearly visible after 5 minutes of staining - when only the most prominent bands have developed (Fig. 6). These differences may probably be environmentally influenced as there is extensive quantitative variation in adult sea-run fish (which at times obscures classification).

The locations of some of the faint bands in the blood serum electropherograms of salmon from both sides of the Atlantic are variable ('Var', Fig. 2). Some of these variations are probably genetically controlled (transferrins) and may be of value for interpopulation comparisons (Møller, 1969, personal communication).

Identification of salmon stocks

A. Off West Greenland (1969)

In each electrophoretic run six control specimens, three from each continent, and about 35 fish of unknown origin were analysed simultaneously. All salmon could be typed with systems I and II. Only 46 (20%) of the salmon examined could be positively typed according to liver esterase patterns alone. In only two of these instances were there any discrepancy with the three sera systems. All others gave identical results. The two exceptional cases were grouped with the European salmon according to the sera electropherograms. North American salmon made up 43 per cent of all the salmon examined. However, the proportion of salmon from each continent varied greatly with time and locale (Table 1). This wide variation is likely the result of small sample sizes.

Evidence supporting stock identifications

Comparison of smolt ages: Student t-tests indicate that the mean smolt age of biochemically identified European salmon (2.27) was lower ($P < .01$) than that of similarly identified North American salmon (2.87). Also, the mean smolt age of tagged Canadian salmon (2.94) caught in Greenland during the same period was the same ($.6 < P < .7$) as that of biochemically identified North American salmon but higher ($P << .005$) than that of European salmon. Similarly, a chi-square test on smolt age distributions indicated that biochemically identified European and North American salmon had different ($P << .005$) age distributions. However, the North American salmon also had an age distribution which was different ($P < .005$) from the Canadian tagged salmon. Since a high proportion (.98) of these tagged salmon originated in the Miramichi River system, this difference may be indicative of multiple origins of North American salmon in Greenland.

Comparison of fork lengths: Mean fork lengths of Canadian tagged (62.98 cm) and biochemically identified (65.58 cm) North American salmon in West Greenland were lower ($P < .01$) than those of European salmon (68.43 cm) (Fig. 7). These results tend to confirm biochemical separation of eastern and western Atlantic salmon stocks

on the high seas. Also, the mean fork length of tagged Canadian salmon was significantly lower ($P < .01$) than the mean for biochemically identified American salmon. This difference may be the result of lower mean fork length among the Miramichi River salmon, but is more likely related to reduced size resulting from tagging. Lower fork lengths have been previously observed in tagged Atlantic salmon (Saunders and Allen, 1967) and redfish (Kelly and Barker, 1963).

Abundance of *A. simplex* larvae: Frequency distributions of *A. simplex* larvae were skewed (Fig. 8) and had variances much greater than their means (Table 3), indicating they would require logarithmic transformation before analytical methods appropriate to normal distributions could be applied. The correct transformation to be used was determined from estimates of K (Barnes, 1952) where:

$$K = \frac{\bar{x}^2}{S^2 - \bar{x}}$$

when x is the distribution mean and S the standard deviation. Since K values for *Anisakis* in biochemically identified American and European salmon were below 2 (.72 and 1.17) the following logarithmic transformation was indicated (Barnes, 1952):

$$Y = \log_e (x+1).$$

The K value for North American tagged fish was greater than 2 (2.30), and the transformation used (Barnes, 1952) was

$$Y = \log (x+\tfrac{1}{2}K).$$

These transformations stabilized the variance (Table 3) and altered the distributions (Fig. 8). The following discussion is based on transformed values unless stated otherwise.

Student t-tests were performed on *A. simplex* abundance in biochemically identified American and European salmon as well as tagged American salmon (Table 4). There was a highly significant difference in the abundance of *A. simplex* in biochemically identified European and American salmon in West Greenland. This was supported by a similarity in abundance of *A. simplex* in tagged American and biochemically identified American salmon. These results support the conclusion based on smolt age distributions and adult fork lengths that biochemically identified North American and European salmon belong to different populations.

Incidence of *Eubothrium crassum*: The incidence of *E. crassum* in biochemically identified North American salmon was significantly higher ($.05 < P < .1$) than in the European salmon. Although the incidence of *E. crassum* in salmon in Greenland varies from year to year (Table 5), the above conclusion conforms with data collected during 1966 and 1968 when its incidence in small samples of tagged North American salmon was consistently higher than that in random samples collected at the same time. Apparently, during the past few years, incidence of *E. crassum* has been higher in North American than in European salmon in Greenland.

However, likely because of the small samples of tagged salmon involved, this cannot be shown statistically. The available data (Table 5) support biochemical separation of North American and European Atlantic salmon stocks in the sea.

B. South Labrador Sea (1970)

Six samples (Table 6) consisting of a total of 25 salmon, were collected in the Labrador Sea during April 1970 (Fig. 1, stations F-J). The classification of these salmon according to serum protein electropherograms are shown in Table 6. The percentage of American salmon in the whole sample (48) was only slightly higher than the percentage in the 1969 samples the previous fall (43). The high proportion of European fish so close to the North American continent was unexpected, and may possibly be because European salmon, migrating to and from the West Greenland area, follow the two main oceanic currents of this area, the Gulf Stream and the Labrador current (Dr A. W. May, personal communication). Such migration routes would take the salmon close to the shelf and as far south as Flemish Cap, where the easternmost part of the Labrador current meets the Gulf Stream.

Mean fork length, abundance of *A. simplex* and incidence of *Eubothrium* in the biochemically identified European and North American salmon show a trend similar to that found in the large Greenland sample the previous fall. However, presumably because of the small sample size, the differences encountered here were not significant at the 95% level. The mean smolt age was almost identical for the two groups.

Discussion

Numerous variations have been reported in the proteins of Atlantic salmon. The blood serum in particular seems subjected to complexity. Variations in protein patterns include a sexual dimorphism (Drilhon and Fine, 1963), extensive variations not correlated with sex (MacKenzie and Paim, 1969), a diallelic polymorphism in the transferrins (Møller, 1969, personal communication) and influence by disease (Mulcahy, 1967). The haemoglobin system is also highly complex and displays variations correlated with ontogeny and affected by diet (Koch et al., 1964, 1967). Polymorphisms also occur in enzymes of other tissues (Odense, 1969, personal communication). However, the complex composition of the Greenland sample which has many different breeding populations contributing, precludes gene frequency comparisons in these samples unless it can be shown that overall gene frequencies are different in North American and European fish. The only instances of geographic variation in Atlantic salmon yet reported are those reported by Nyman (1966, 1967a).

It is evident that prior to the use of selected protein characters in population studies on salmon, care must be taken to evaluate (1) the hereditary background through hybrid analysis and (2) to prove that these proteins are unaffected by sex, diet, disease, ontogeny and seasonal variations. Tests of the mode of inheritance of the proteins employed in this study as well as tests of the effect of the factors mentioned above have shown that the serum proteins used are unaffected, at least where the qualitative pattern is concerned, but that the enzymatic activity of liver esterase is evidently affected by type of diet or other environmentally controlled variation, at least in sea-run salmon.

No apparent schooling of fish from the two continents could be detected, the salmon being distributed at random in the nets. The samples from the southern Labrador Sea (Fig. 1) are particularly interesting because it presents the first evidence of European salmon so close to the North American continent.

North American rivers typically produce 3-year or older smolts and only a small proportion of 2-year-olds (Templeman, 1967). In contrast, England, Scotland and Ireland have a very high percentage of 2-year-old smolts (Templeman, 1967). In this study 41 per cent of the biochemically identified North American salmon had 3 years of river life and only 38 per cent had spent two years in the river. However, 70 per cent of the biochemically identified European salmon had spent 2 years in the river. Observations on the smolt age composition of North American and European salmon in Greenland therefore conform with previously published data on the subject.

Templeman (1967) observed that the percentage of salmon off West Greenland possessing *E. crassum* was higher in salmon of higher river age and concluded that they may be more frequent in Canadian than in European salmon. In 1969 the incidence of *E. crassum* was almost twice as high in North American salmon than in European salmon, thereby confirming Templeman's suggestion.

The total proportion of North American salmon in 1969 samples (43%, Table 1) cannot be considered as indicative of the proportion along the entire coast of West Greenland, particularly as most of the material was collected in a single area (Disko Bay). In addition to possible geographic variations in the relative proportions of North American and European salmon in Greenland, seasonal and annual fluctuations may exist. More extensive sampling along the coast of Greenland is necessary before reasonably accurate overall proportion estimates are feasible.

From the results obtained it is evident that there are two types of Atlantic salmon, one which breeds in North America, the other in northwestern Europe. The status of the salmon from Iceland and the single salmon-producing river in Greenland (Kapisigdlit) is not known.

The characters here used to distinguish the two types consist of qualitative differences in the two genepools, which are reflected in different homozygous loci for some of the proteins. Since no geographic overlap of the genepools has so far been detected, the two types of salmon appear completely allopatric and thus replace each other geographically. Although the two types mix on their oceanic feeding grounds the distributional gap is maintained by the strong homing instinct of the species, which prevents interbreeding. The above criteria justify a subdivision of the species *Salmo salar* L. into two subspecies, according to the widely accepted definition by Mayr (1969): "A subspecies is an aggregate of phenotypically similar populations of a species, inhabiting a geographic subdivision of the range of a species, and differing taxonomically from other populations of the species."

Fork length data so far obtained show that the two groups are statistically different, but because of the considerable phenotypic variability among most species of fish, the establishment of subspecific criteria based entirely on morphological characters is very complicated. However, the above definition states that two potential subspecies should differ taxonomically, which is a neutral term indicating any measurable character which reflects genotypic differentiation.

Another way of recognizing subspecies is by applying the so-called "75% rule". This rule states (Mayr, 1969): "... a population is recognized as a valid subspecies if 75% of the individuals differ from 'all' (= 97%) of the individuals of a previously described subspecies. At the point of intersection between the two curves where this is true, about 90% of population A will be different from about 90% of the individuals of population B." In the present case 100% of the individuals of North American populations can be separated from 100% of European salmon on the basis of protein differences, which are direct transcriptions of their genotypes. This basis for taxonomic classification thus offers a simpler way than most morphological criteria which are based on characters coded by polygenes which usually produce great variability and overlap. Thus,

although no morphological differences have yet been detected (except possibly fish size) these qualitative hereditary differences between North American and European salmon seem to fulfill the criteria given in the two definitions.

It is evident that each subspecies is divided into many local populations, basically one per river system, which differ at least at the gene frequency level (Møller, personal communication). The results of successful transplants of fish from for instance Canada to Sweden have shown the similarity in ecological demands and simplicity of obtaining hybrids (Nyman, 1967a, b) as well as cytological criteria (Nygren et al., 1968) and indicate the conspecific origin of the two subspecies.

Since the Atlantic salmon originally named by Linné was of European origin, this type should constitute the nominal subspecies, i.e. *Salmo salar salar*, while we suggest the North American type be called *Salmo salar americanus*.

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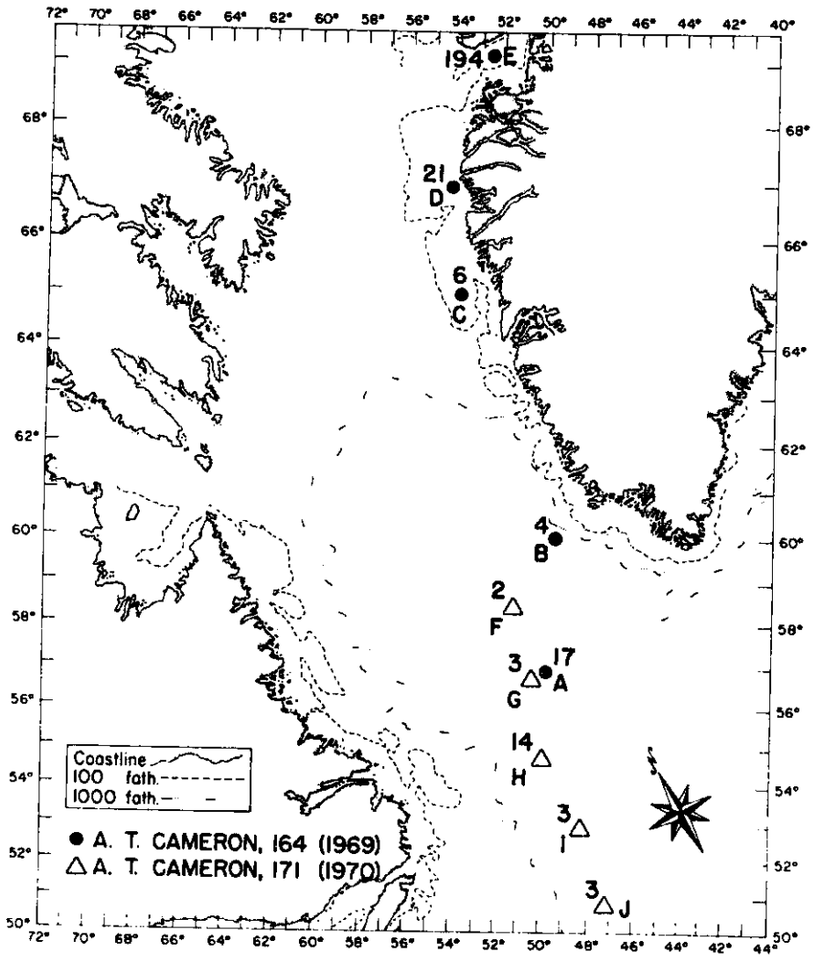


Fig. 1. Location of the ten major sampling areas. Numbers indicate number of salmon caught, the capital letters refer to sampling stations mentioned in Tables 1 and 6.

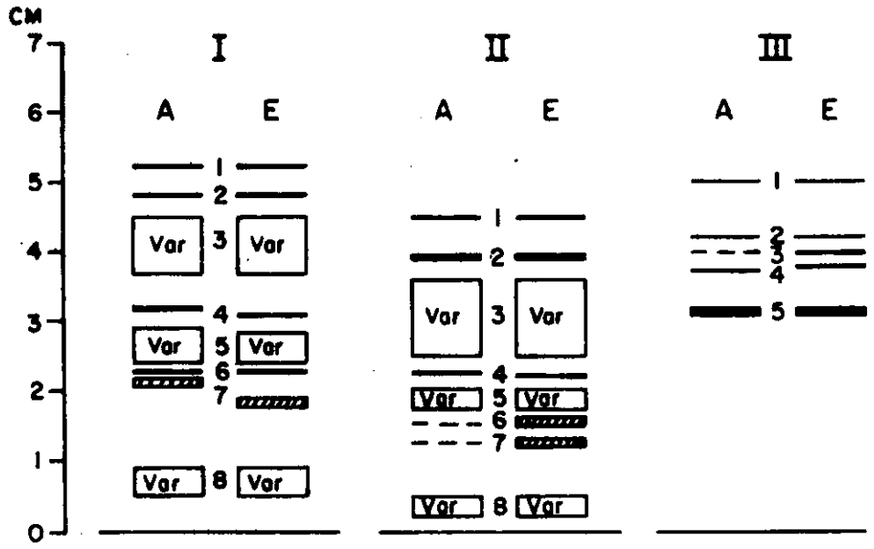
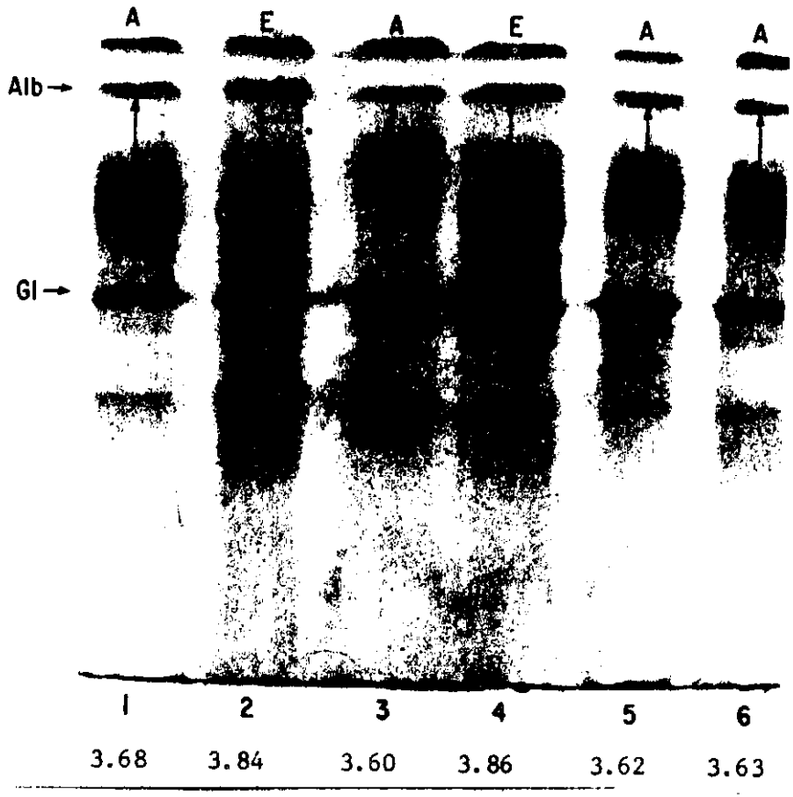


Fig. 2. Generalized electropherograms of the three protein systems discussed in this paper. A = North American, E = European. The scale to the left indicates anodic migration in centimetres. Var = zones with intraspecific variation. System I = blood serum proteins, buffer system by Ashton and Braden (1961). System II = blood serum proteins, buffer system by Gahne (1966). System III = liver esterases, buffer system by Ashton and Braden (1961). Bands with same number in Systems I and II are identical.



*Distance equals

1	2	3	4	5	6
3.68	3.84	3.60	3.86	3.62	3.63

Fig. 3. Classification of individual sera (System I) by measuring the distance between a globulin zone (Gl) and the slowest migrating albumin (Alb).* Photograph of electropherogram enlarged twice to facilitate measuring. Distance given to the nearest one hundredth of a centimetre. Specimens 1 and 5 are from New Brunswick, Canada, 3 is from a landlocked salmon and 6 from a sea-run salmon, both from Newfoundland, Canada. Specimen 2 is from Ireland and 4 from Sweden.

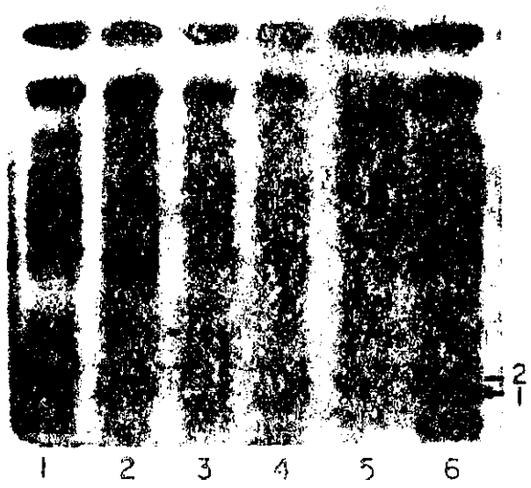


Fig. 4. Original size of the fastest portion of sera electropherograms employing a modification of the buffer by Aronsson and Grönwall (1957). Note the more anodic position of band 2 in specimens 1, 3 and 5 which are North American salmon as compared to the position of band 1 in specimens 2, 4 and 6 which are European. (1 and 3 from New Brunswick, Canada, 5 from Newfoundland, Canada; 2 and 4 from Ireland, 6 from Sweden).

Fig. 5. Original size of blood serum electropherograms employing the buffer by Gahne (1966). Note the strong bands at position "1" and "2" in specimens 2, 4 and 6 which are European as compared to specimens 1, 3, 5 and 7 where these bands are almost completely missing. (1-6 are the same fish as in Fig. 4, specimen No. 7 is from Newfoundland, Canada).

Fig. 6. Liver esterase zymograms of juvenile salmon after five minutes incubation. A = North American, E = European salmon. The differences at positions 3 and 4 are discussed in the text.

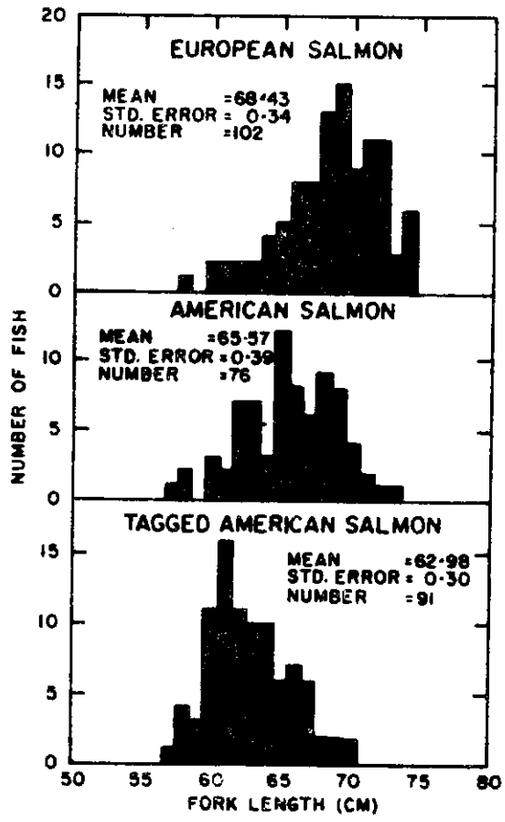


Fig. 7. Distribution of fork lengths of tagged North American salmon and biochemically identified North American and European salmon caught off West Greenland in 1969. (The number of tagged American salmon examined (91) is less than that reported in Tables 3 and 5 (93) because fork lengths were not available for two of these fish.)

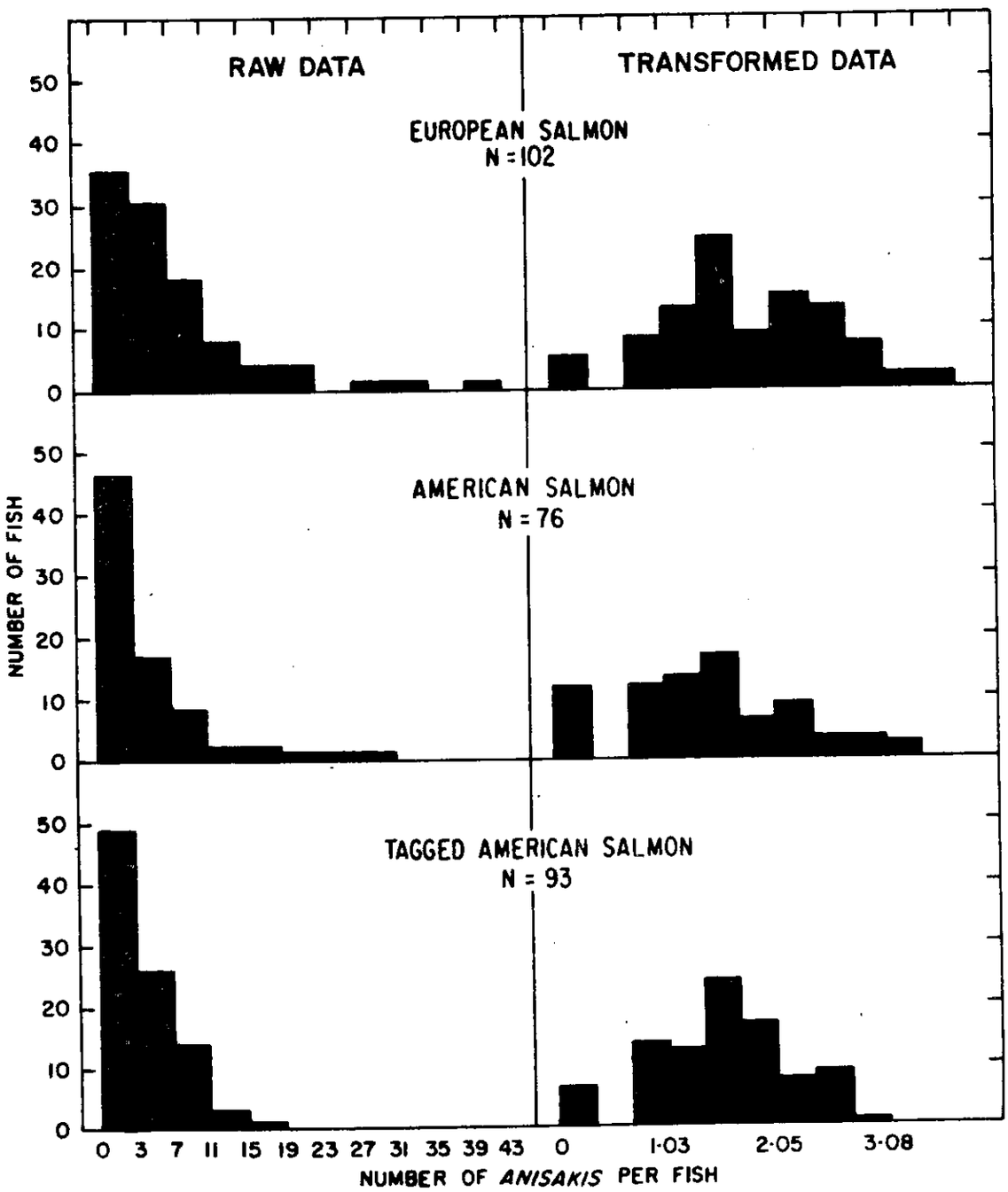


Fig. 8. Distribution of *A. simplex* in biochemically identified North American and European salmon and tagged North American salmon caught off Greenland in 1969. Transformed data are based on the equation $y = \log_e (x + 1)$ (N.A. and Eur.) and $y = \log_e (x + \frac{1}{2}K)$ (tagged).

Table 1. Continent of origin of Atlantic salmon in samples collected off West Greenland, September-October 1969.

Station (Fig. 1)	Location	Date (1969)	No. of fish analysed	Geographic composition A = North America E = Europe		Percentage of North American origin
A.	Halfway between Labrador and Greenland	6/9	17	A = 6	E = 11	35
B.	Off Kap Farvel	10/9	4	1	3	25
C.	Sukkertoppsbank	15/9	6	5	1	83
D.	Store Hellefiske	18/9	20	6	14	30
	" "	19/9	1	...	1	...
E.	Disko Bay	22/9	3	1	2	33
	" "	23/9	57	26	31	46
	" "	24/9	48	25	23	52
	" "	26/9	41	17	24	41
	" "	27/9	3	3	...	100
	" "	29/9	3	1	2	33
	" "	30/9	24	7	17	29
	" "	1/10	15	5	10	33
		Total:	242	103	139	43

Table 2. Smolt ages of 231 1-sea-year salmon caught off West Greenland in September-October 1969.

Identification	Mean smolt age	Smolt age in years, %						No. fish examined
		1	2	3	4	5	6	
European salmon	2.27	6	70	16	7	1	0	100[1]
American salmon	2.87	1	38	41	13	5	1	76[1]
Tagged Canadian salmon	2.94	0	15	76	9	0	0	55[2]

[1]Two fish were omitted from the total examined (178) because smolt ages could not be determined.

[2]Only those salmon not originating in hatcheries were considered for comparison because the smolt ages of wild salmon were considered to be more representative of the actual stock composition in Greenland.

Table 3. Intensity of infestation of *A. simplex* sp. in salmon samples from West Greenland, 1969.

	A Biochemically identified American	B Biochemically identified European	Total (A + B)	Tagged North American
No. salmon examined	76	102	178	93
No. parasites found	344	736	1080	403
Raw data				
Mean	4.53	7.22	6.07	4.33
Variance	31.91	50.38	44.27	12.16
Transformed data				
Mean	1.321	1.791	1.590	1.475
Variance	0.747	0.658	0.749	0.680

Table 4. Comparison of intensity of infestation by *A. simplex* larvae in salmon of different continental origins in West Greenland.

Sample	Sample mean	Difference	t	DF	P
iochemically identified European	1.791	.470	3.693	176	<< .01
iochemically identified American	1.321				
agged American	1.475	.154	1.174	167	.2 < P < .3
iochemically identified American	1.321				

Table 5. Incidence of *Eubothrium crassum* in Atlantic salmon caught in West Greenland, 1966-69.

Year	Per cent infested (Number examined in brackets)			
	A Biochem. American	B Biochem. European	Total (A + B)	Tagged American
1966	...	...	54.6 (97)	100 (4)
1967	...	...	31.8 (88)	100 (1)
1968	...	...	52.9 (157)	62.5 (8)
1969	22.4 (76)	11.8 (102)	16.3 (178)	17.2 (93)

Table 6. Continent of origin of Atlantic salmon in samples collected in the Labrador Sea, April 1970.

Station (Fig. 1)	Date (1970)	No. of fish analysed	Geographic composition A = North America E = Europe		Percentage of North American origin
F	13/4	2	A = 2	E = ...	100
G	14/4	3	...	3	...
H	15/4	12	7	5	58
H	16/4	2	1	1	50
I	19/4	3	...	3	...
J	21/4	3	2	1	67
		—	—	—	—
	Total:	25	12	13	48

Table 7. Comparison of abundance of *Anisakis*, incidence of *Eubothrium*, mean fork length and mean smolt age of biochemically identified salmon of American (A) and European (E) origin from the southern Labrador Sea (April 1970). For calculation of 't' and 'P' values for abundance of *Anisakis* transformed data were employed.

	Mean no. of *Anisakis*/fish	Incidence of *Eubothrium*	Mean fork length	Mean smolt age
A(n = 11)	1.73	82%	69.9 cm	2.73 yr
	t = 1.31	t = 0.49	t = 0.01	t = 0.07
E(n = 12)	5.58	50%	71.3 cm	2.75 yr
	(0.2 < P < 0.3)	(0.6 < P < 0.7)	(0.3 < P < 0.4)	P >> 0.9