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A study of the thermostability of isolated muscles of the marinus and mentella types of redfish in connection with the problem of their taxonomical relation

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ABSTRACT

The thermostability of isolated muscles was studied in more than 1000 speciemens of the 'marinus' and 'mentella' types of redfish from the North-West Atlantic. These forms were found to differ in the examined feature, the higher level of which is characteristic for the 'mentella' type.

Thermostability of isolated muscles is species-specific criterion for poikilothermal animals; in this connection is possible to conolude on taxonomical range of the differences between the 'marinus' and 'mentella' types of redfish.

The problem of the taxonomical relation of the marinus and mentella types of redfish from North Atlantic remains open up to now. V.I.Travin (1951), for example, had described, according to morphological characteristics, the mentella as an independent species Sebastes mentella Travin, whereas A.P.Andriyashev (1954) classified it as a subspecies S.marinus infraspecies mentella Travin. Other researchers also prefer to speak of different subspecies and even "types" of redfish (see, for example, Kotthaus, 1961).

At the same time the essential peculiarities of the marinus and mentella types of redfish can be traced down not only in their morphological characteristics, but also when we subject them to biochemical and immunological studies (Schaeffer, 1961; O'Rourke, 1961). Hence it seems expedient to seek for divergencies in North Atlantic redfish also according to a

number of other features, which are of a diagnostical value in the systematics of species. This report deals with the results of the analysis of the differentiation of the marinus and mentella types according to the degree of thermostability of isolated muscle tissue, which has been established as the species-specific criterion for the poikilothermal animals (Ushakov, 1959 b; Ushakov, 1964).

Material and Methods

The study was done at the research vessel, the "Sevastopol", in September-October 1964, and repeated in August 1965 in North-West Atlantic. The places where the samples were taken are shown in Fig.1. The experimental pattern boiled down to determining and comparing, according to Ushakov's method (1959b), of thermostability of isolated muscles (m.genio-hyoideus) of the redfish lifted in trawls.

The muscle, of specimens were attached to thin glass rods and inserted into Dewar vessels, filled to one-third with Ringer's solution heated within $\pm$ 0.2° precision. From time to time the muscles were taken out for several seconds and tested for the excitability of tissues to induced electric current from a transistorised stimulator with a maximum voltage of 130V (Arzumanov and Kusakina, 1960). The time for which the tissue preserved excitability, since the moment it had been immersed in the heated solution up to the complete absence of contraction even in the filaments the most resistant to the action of heat, served as the measure for thermostability. Research was done at a number of experimental temperatures (24,26,28 and 30°) and at one experimental temperature of 28°C, which provided the opportunity to analyse the inter- and intra-population variability of the feature. 1,023 experiments on determining the thermostability of the isolated muscles of redfish have been carried out.

Table 1.

Retention time of excitability (in minutes) of the isolated muscle tissue of redfishes from N.-W.Atlantic.

Type of redfish	Temperature (°C)											
	24			26			28			30		
	n	M±m	P	n	M ± m	P	n	M ± m	P	n	M ± m	P
'mentella'	-	-		17	59,9±3,4		20	28,3±2,1		7	11,9±1,6	
			-			0,001			0,01			0,42
'intermediary'	18	61,1±3,8		10	39,2±1,4		17	21,6±0,9		13	9,5±1,6	
			0,60			0,01			0,001			-
'marinus'	9	55,4±7,6		15	28,8±2,7		25	15,4±0,9		-	-	

Note: n - number of tests; M ± m - the arithmetic mean and its error in the second power; P - probability of difference unreliability

Results

The results of the determination of the thermostability of the muscles of the redfish from the area of Iceland at several experimental temperatures are related in Table 1 and Fig.2. As the graph shows, the muscle tissues of the mentella - type (straight line 2) is approximately 2^{o} more thermally stable than the muscle tissue of the marinus - type (line 1). The revealed difference is statistically significant (Table 1) and is quite in accordance to the differentiation which can usually be observed when we compare the thermostability of the cells of the taxonomically close species of fish and other poikilothermal animals, (Kusakina, 1959; 1960; Altukhov, 1962 a, b, 1967; Altukhov and Glushankova, 1966; Ushakov, 1964; Kaufman, 1965).

It must be taken into consideration, however, that nearly in all the areas of joint habitation by the redfish in North-West Atlantic there are caught considerable quantities of fish with mixed morphological characteristics of the marinus and mentella - types, which are probably their hybrids (Kotthaus, 1961 b; Travin and Pechenik, 1962). In conformity with thise data, the results of the comparison of the thermostability of the muscle tissue both in the initial forms and in redfish with mixed characteristics (for convenience sake we'll be calling this latter type the "intermediary" type) confirm that, this latter type is actually characterised by an intermediary level of thermostability (Fig.2, line 3). The statistical data on the material does not distinguish any actual difference, in two cases, between the intermediane type and the marinus (temp.24^{o}) and the mentella (temp.30^{o}) type of redfish (Table 1).

Let us now consider the results of determination of the thermostability of the isolated muscle tissue of redfish at one experimental temperature of 28^{o}. The characteristics of the material studied is given in Table 2.

Table 2.

Thermostability (in min.) of the isolated muscles of redfishes from N.-W. Atlantic (in experimental 28°C).

Type of redfish	The places and time of sampling	The statistical data		
		n	M ± m	P
'marinus'	Iceland, August,1965	109	13,6±0,69	-
	East Greenland, October, 1964	71	14,25±0,78	0,001
	West Greenland, September, 1964; August,1965	166	20,05±0,58	0,001
'mentella'	Iceland, October,1964; August, 1965	95	27,8±1,07	-
	East Greenland, September, 1964; August,1965	82	27,9±1,13	-
	West Greenland, September, 1964; August,1965	80	28,4±1,09	-
	Labrador, August, 1965	142	28,5±0,78	0.001
'intermediary'	Labrador, Greenland, Iceland; September-October, 1964; August,1965	189	22,7±0,75	

Note: Statistical symbols - see table 1.

As we see from Table 2 all the studied samples of the mentella type are characterised by a pattern which coincides with the average. The amplitude of variability was the same also. This speaks of the lack of inter-populatory variability according to the given feature and, hence, of the wholeness of the population of mentella - type redfish that habitates in the areas studied. A similar picture is observed also in the case of the Icelandic and East Greenland populations of the marinus - type redfish, which, being cytophysiologically identical, differ tangibly from the mentella - type in the thermostability of muscles. At the same time the marinus - type redfish from West Greenland is characterised by a specific level of thermostability of muscles, differing in this respect both from the Icelandic-East Greenland marinus, and from mentella - type redfish, holding an intermediary position between them.

The Fig.3 shows the sum-up curves of the distribution of the thermostability of muscles in the marinus and mentella redfish, and the curve for the intermediary redfish[x]. This data determines that each of the studied kinds of redfish has its own peculiar distribution, and that for the marinus and mentella types it is characterised by one-peak curves (curves 1 and 2 respectively), whereas under cytophysiological analysis of the intermediary redfish it is bimodal (curve 4). And this means that in the waters of Iceland, Greenland and Labrador, where natural hybridisation takes place among the redfish forms, there is observed either the splitting up according to the studied feature into F_2, or else, the intermediary fish appear on account of the reverse crossings between the partially fertile hybrids F_1 with the initial forms.

Of great interest is the curve of the distribution of the thermostability of muscles of the marinus - type redfish from the West Greenland waters (Fig.3, curve 3), which is a one-peaked one and takes, as already mentioned, an intermediary position, between the corresponding curves for the mentella

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x/ The problem an the right-hand assymetry of the curves of this kind is considered by Ushakov and Chernokozheva (1963).

and marinus - type of redfish from Iceland and East Greenland. As we see from Table 2, the average life time of the muscles of the West Greenland redfish (20.05 ± 0.58 min) differs from the actually identical figures, obtained from the marinus type from waters of Iceland (13.6 ± 0.69 min) and East Greenland (14.25 ± 0.78 min.), tangibly nearing the level of thermostability of the muscles of the intermediary redfish (22.7±0.75min).

Thus, the considered experimental material revealed the existence in the explored area of North West Atlantic of four cytophysiologically different populations of redfish: mentella, intermediary and marinus, represented, on the one hand, by the West Greenland, and, on the other — by Icelandic and East Greenland redfish. The hybrid nature of the intermediary type causes no doubt at all. As for the West Greenland marinus type, the results of cytophysiological analysis also speak in favour of its being a hybrid. Apparently this type of redfish is a marinus only in appearance, but genotypically is a hybrid of first generation between homozygous fishes of the marinus and mentella type.

The biological data obtained by G.P.Zakharov (1962), which speak of the juvenile nature of a considerable part of the population of the marinus type in the waters of West Greenland, are also in favour for such an assumption. The author in the course of the last 6-7 years, had analysed about 5,000 adult females and arrived at the conclusion that as to the condition of their gonads they are no different from the immature marinus type from the Sea of Barents.

According to the cytophysiological data, cited above, the marinus and mentella types of redfish have diverged adequately far apart. Hence the possibility cannot be excluded that the juvenile nature of the population of females of the West Greenland marinus type is the consequence of their hybrid origin from initial types, between which there exists a certain degree of physiological isolation. This leads to the underdevelopment of the reproductive system of the considerable part of the F_1, type hybrids, which cannot be outwardly

discerned from the marinus - type. This violation, probably, mostly concerns the females and, to a lesser degree, the males, if we assume that they are homogamous.

This data allows to think that the so-called intermediary redfish come into life as the result of mating between the initial types of redfish with the sexually mature males, and, partially, with the hybrid females of the F_1. The pairing and spawning take place in the area of Iceland, and redfish is concentrated in West Greenland by means of the bringing of the fry there by the Irminger, East-Greenland and West-Greenland currents (Taning, 1949; Templeman, 1959; Zakharov, 1962). We seem it to be highly probable that the F_1 hybrids, because of their un-specific risen resistance, and, hence, their frequency in West Greenland waters is especially high. It isn't hard to imagime the natural selective factor [x/] - suffice it to compare the temperature conditions of the drift of the fry in the waters of the warm Irminger current (4-6°) and in the fjords where the fry settles down, in the latter case the water temperature is clearly outside the toleration level for the redfish, going down to 2°, and sometimes to 0° and even lower (Külerich, 1934; Taning, 1949; Dunbar, 1951; Templeman, 1959; Herman, 1963).

All the above-mentioned may be summed up as follows: On the basis of the results of the cytophysiological analysis and the available data on the biological peculiarities of the population of the marinus - type redfish from West Greenland, we suppose that this population is, to a considerable degree, represented by sterile hybrids of F_1, and that it is formed as the result of the elimination of the fry of the initial types of redfish which are brought from the spawning grounds in Iceland and the Danish Straits and which encounter the unfavourable low temperatures on some parts of their drift and in the fjords. It goes without saying that the assumptions laid down here are only in the nature of a hypothesis which

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x/ Templeman in his substantial report (1959) relates facts of the discovery of dead fry and even adult redfish in the cold waters in West Greenland.

is in need of further experimental research. In our other report we shall supply the biochemical proof in favour of such an assumption.

Discussion

In the conclusive part of the work let us consider the problem of the taxonomic status of the differentiation between the marinus and mentella types of redfish. In this connection we must mention - besides the already quoted conclusions made by Travin (on the species type) and of Andriyashev (on the sub-species type) of the forms - also the point of view of two other authors - O'Rourke (1961) and Kotthaus (1961), based on biochemical-immunological and morphological data, respectively.

The opinion of O'Rourke, proposing the analysed type are sibling-species, is doubtable, since in their appearance these fishes tangibly differ from one another in most parts of their areal.

Kotthaus (1961) puts forward three possible explanations for the taxonomic and philogenetic relations among the redfish - 1) marinus and mentella types are independent species, connected by intermediary fish of hybrid origin; 2) S.marinus is a philogenetically more ancient type, from which the mentella - type had detached itself. The process of the speciation has not been yet terminated, hence the emergence of intermediary forms; 3) the intermediary type is the initial one, and the marinus and mentella - types are formed depending - on what depth-less or more - does the fry settle down. Out of the three assumptions, the author considers the first two to be the most probable.

As our research has established, there has been found one more differentiation between the two types - according to the thermostability of isolated muscle tissues, which is substantiated as the cytophysiological criterion of the species. The discovery of a difference in the thermostability of the cells of any taxonomically near forms of poikilothermic animals can be considered as an important proof of their being

an independent species, or as an indication that the speciation going under our own eyes (Ushakov, 1964). Hence, the first two suggestions made by Kotthaus have now been confirmed experimentally.

Since evolution is an adaptive process it is interesting to compare the discovered difference in the thermal stability of the muscles of redfish forms with their relation to the water temperature. According to Travin (1951) among the fish in the Barents Sea, the marinus - type should be considered as a more warmth-loving one than the mentella-type. At the same time, the data discussed at the 1959 Symposium in Copenhagen give evidence to the contrary. The researchers at the Polar Institute, engaged in the study on the biology of the Barents Sea redfish are also of the opinion that the mentella-type redfish is the more warmth-loving ones (T.S.Berger, G.P.Zakharov, V.P.Sorokin - personal communications), since the temperature at the greater depths where it mostly lives, is as a rule, 1.5-2° higher than at the comparatively shallow habitats of the marinus - type redfish. Templeman also cites similar data (1959).

So we may suppose that the cytophysiological differences between the marinus and mentella types result from their adaptation to temperature, which can be found also in the cells level. This differentiation seems to be adequately great, and, combined with the data of other authors, who had demonstrated essential differences in the marinus and mentella type redfish, according to a whole number of features - can, in our opinion, be regarded as an argument in favour of the concept of the taxonomic range of divergence of these types.

Refferences

Altukhov J.P.,1962a. Study of the Thermostability of the Isolated Muscles of the "large" and the "small" Black sea horse mackerel (Trachurus mediterraneus Stdr.). Cytology U.S.S.R.,4, 1:72-73.

Altukhov Ju.P.,1962b. Study of Thermostability of Isolated Muscles and Serological Analysis of "large" and "small" Black Sea horse mackerel (Trachurus mediterraneus Stdr.). Trudi Karadagskoj Biologitcheskoj Stancii, 18:3-16

Altukhov Ju.P., 1967. Cytophysiological and serological analysis of intraspecific differentiation of the Black Sea horse mackerel. In: The Cell and Environmental Temperature", Oxford:356-360.

Altukhov J.P. & M.A.Glushankova, 1966. Thermostability of the Isolated Muscles and Actomyosin in some Species of the Gobius from the Azov Sea. Cytology U.S.S.R.,8, 4:516-519.

Andrijashev A.P., 1954. Fishes of the Northern Seas of U.S.S.R. (Moscow-Leningrad)

Arzumanov V.I. & A.A.Kusakina, 1960. The Pertable Stimulator for Work in Field Conditions. Cytology, U.S.S.R.,2,4:501-503.

Dietrich G.,Aurich H. & Kotthaus A.,1961. On the Relationship between the Distribution of Redfish larvae and the Hydrographical Conditions in the Irminger Sea. ICNAF/ICES, Redfish Symposium, Sp.Publ., 3:124-139.

Dunbar M.I., 1951. Eastern Artic Waters. Fish.Res.Bd.Canada, Bull.,88,Ottawa.

Hermann F., 1963. Hydrographic conditions of West Greenland, 1961. Cons.perm.int.expl.mer, Ann.Biol., 18:16-17.

Kaufman Z.S., 1965. Thermostability of Muscular Tissue of Some Fish from the White Sea in Connection with the Temperature Conditions of Their Existence. Cytology, U.S.S.R., 7,5:655-657.

Kotthaus A., 1961. Contribution to the Rase Problem in Redfish. Rapp.Cons.Int.Expl.Mer, 150:42-44.

Külerich A., 1943. The Hydrography of the West Greenland Fishing Banks Medd. fra Komm. for Danmarks Fiskeri-og Havunder-sogelser, 3,3,Cöpenhague.

Kusakina A.A., 1959. Cytophysiological analysis of the Muscle Tissue at the Heterosis for some interspecific Hybrids of Fishes. Cytology, U.S.S.R., 1,1:111-120.

Kusakina A.A., 1960. Thermostability of the muscles of some Atlantic Salmon and taimen Trout. In: Voprosi cytologii i Protistologii, Moscow-Leningrad; 107-111.

O'Rourke F.J., 1961. An Immunological and Chromotogrpaphic Study of Sebastes marinus (Linne) and S.mentella (Travin). ICNAF,Sp.Publ., 3:100-103.

Schaefer H., 1961. A Biochemical Contribution to the Redfish Problem. Rapp.Cons.perm.int.expl.mer, 150:104.110.

Taning A.V., 1949. On the Breeding Places and Abundance of the Redfish (Sebastes) in the North Atlantic. J.Cons.perm.int.expl.mer, 16:85-95.

Templeman W., 1959. Redfish distribution in the North Atlantic. Bull.Fish.Res.Bd.Canada, 120:1-173.

Travin V.I., 1951. A New Species of Redfish in the Barentz Sea (Sebastes mentella Travin sp.nov.). Dokl. Akad.Nauk U.S.S.R, 77:741-744.

Travin V.I. & Pechenik L.N., 1962. Soviet Researches on Fisheries in the N.-W.Atlantic.In:Sov.ribokhozajstv.i issled.v S.-Z.Atlantike., Moscow; 5-55.

Ushakov B.P., 1959. Thermostability of Tissues-a Specific Criterion in Poikilothermal Animals.Zoology, U.S.S.R,38, 9:1292-1302.

Ushakov B.P., 1964, Thermostability of Cells and Proteins of Poikilotherms and its significance in Speciation. Physiol.Rev.,44,3:518-560.

Ushakov B.P.&I.S.Chernokozheva, 1963. The Changes in Thermostability of Muscle Tissues of Frog's Tadpoles as the Result of thermal action upon the spermatozoa. Cytology U.S.S.R., 5, 2:240-243.

Zakharov G.P., 1962. On the Biology of the marinus - type redfish in West Greenland. In: Sov.ribokhozajstv issled. v S.-Z.Atlantike., Mosoow: 319-330.

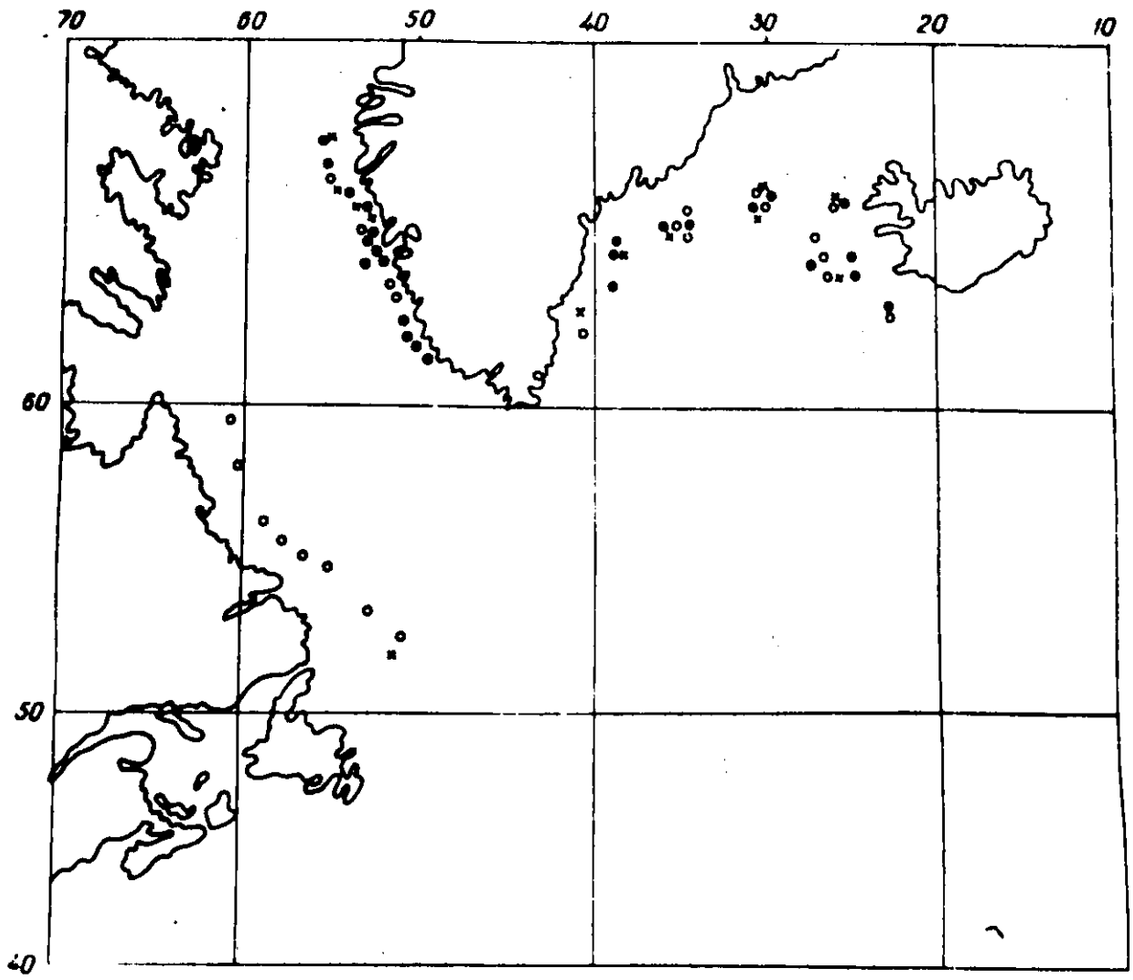

Fig. 1. Places where fish were caught for cytophysiological experiments.
Light circles = mentella type; black circles = marinus type; crosses = "intermediary" redfish

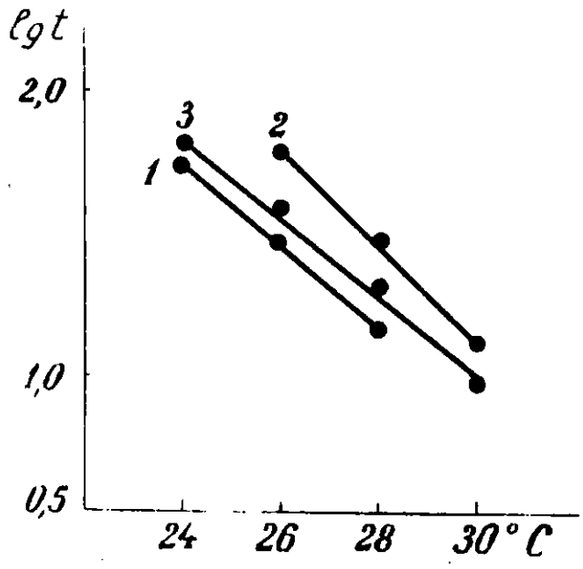

Fig. 2. Thermostability of isolated muscles of redfish from Northwest Atlantic.
1 - marinus type; 2 - mentella type; 3 - intermediary redfish.
The abscissa gives the temperature in °C.
The ordinate - logarithm of the lifetime of muscles (in min.)

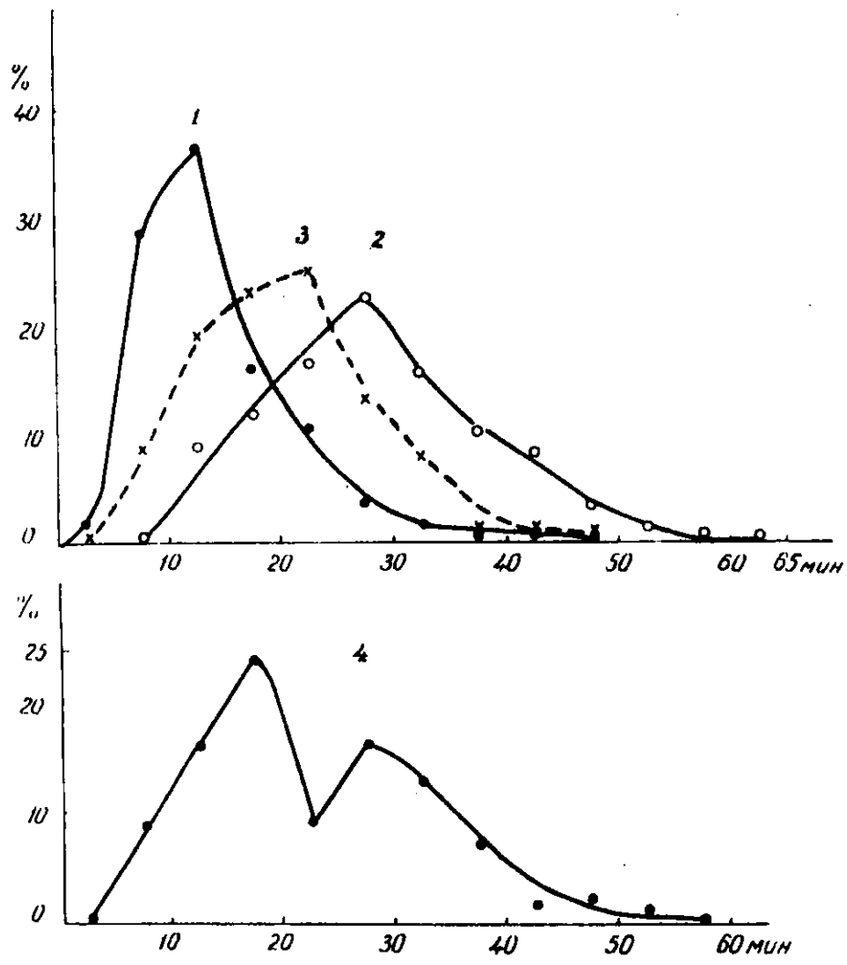

Fig. 3. Variation curves, giving the quantitative characteristics of the distribution of thermostability of isolated muscle tissue of the types of redfish studied.
1 - marinus type from Iceland and East Greenland;
2 - mentella type; 3 - marinus type from West Greenland;
4 - intermediary redfish.