A C T A I C H T H Y O L O G I C A E T P I S C A T O R I A
Vol. VI, Fasc. 2 Szczecin 1976

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Biochemistry

APPLICATION OF DRIP TO FISH SARCOPLASMATIC PROTEIN CHARACTERISTICS BY STARCH GEL ELECTROPHORESIS

WYKORZYSTANIE WYCIEKU DO CHARAKTERYSTYKI BIAŁEK SARKOPLAZMATYCZNYCH RYB METODĄ ELEKTROFOREZY NA ŻELU SKROBIOWYM

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The studies were aimed at checking the possibility of applying the drip to fish species' identification from their sarcoplasmatic protein fraction determined by the starch gel electrophoresis. 5 Baltic and 14 freshwater fish species were examined. The studies proved an excellent applicability of the method to the biochemical identification of fish. The results obtained are presented on photographs and drawings.

INTRODUCTION

New fish species are more and more widely used in the fish processing industry; they are also technologically improved on processing so that the specific organoleptic properties of the flesh are no longer perceived. Thus only the flesh proteins can form a basis for a specific diagnosis of fish raw materials. Those identification procedures are particularly applicable after the starch- and polyacrylic gel electrophoresis has been developed. These allow the fish protein diagnosis to be performed not only for the technologic purposes but also in genetic studies (Bioch. gen. 1973).

Thomson (1960) was one of the first workers to make use of the starch gel in electrophoretic separation of proteins of gadids and marine fishes in order to identify them. He used aquaeous muscle extracts which he thought would have turn out wieldy in

specific diagnosis. Then Yamaka et al. (1963) applied the starch gel electrophoresis of blood haemoglobin proteins to identify 12 salmonid species. The authors studied effects of a species, sex, place of capture and age on a picture of protein fractions. Only an effect of a species was found to be relevant to the protein fraction assessment.

Tsuyuki and Roberts (1963) attempted to identify the salmonids from their myogenic protein fractions separated also on the starch gel. During the next years, Tsuyuki et al. (1966) separated myogenic, blood serum and haemoglobin proteins of five salmonids by starch and polyacrylic gel electrophoresis. The blood serum proteins characteristics obtained on starch and polyacrylic gels were used by Uthe et al. (1966) in their specific diagnosis of *Petromyzontidae, Esocidae, Centrarchidae* and *Percidae* of American lakes. The results obtained allowed some different fenotypes to be established within the representatives of a particular family from various lakes. Carrying on their observations, Uthe and Tsuyuki (1967) attempted, through studies of myogenic and haemoglobin fractions by a protein gel electrophoresis to determine the polymorphism of three American lampreys. Basing on the electrophoretic separation of extracts of muscle, blood serum and haemoglobin proteins, Tsuyuki et al. (1967) characterized the family *Catostomidae* comprising about 80 fish species. They used starch and polyacrylic gels. Their studies showed the protein polymorphism to occur within *Catostomus catostomus*. Brouk and Ball (1968) applied lactate dehydrogenase separated on a polyacrylic gel to their assessment of trout species and hybrids. In order to detect some genetic alterations, Gray and Kenzie (1970) separated, on a starch gel, a muscle myogen of *Salmo trutta* and *S. gairdneri* caught in different geographic areas. Chen and Tsuyuki (1970) used a starch gel to separate myogens of muscles, haemoglobin, transferases and blood serum esterases, aiming at the identification of *Tilapia sp.* and other fish species of an economic importance for African countries.

When identifying fishes of the family *Scorpaenidae*, Westerheim and Tsuyuki (1971) found the reliability of the starch gel protein separation method for muscle proteins and haemoglobin. The studies carried out by Nyman and Pippy (1972) confirmed these findings; using the starch gel separation of blood serum proteins they found the North-American and European salmons to belong to different subspecies.

The electrophoretic picture of fish proteins separated on gels not only allows to determine the specific affinity of fish but also provides valuable information on genetic problems within the species. The gel electrophoresis can prove extremely helpful in cases when morphologic and meristic characters examined cast little light on a particular biological material and/or when they cannot be applied at all, e.g., in the assessment of skinned fillets or fish mince meat.

When embarking on studies of this kind, the right method of electrophoresis as well as the protein group are very relevant to the species' identification. The biochemical assessment of fishes reviewed so far indicates the sarcoplasmatic proteins of flesh to be the group of proteins electrophoreograms of which supply a wide array of information. Thus applying the gel electrophoresis to this group of proteins we can obtain an aid in the

determination of a species as well as an insight into the physicochemical changes in proteins occurring both posthumously and during technological processes.

If we consider then the albumin-type sarcoplasmatic fractions of fish flesh as the basic component of the tissue fluid, in the fish processing technology referred to as the "drip", the conclusion emerges that they can prove a valuable material for the electrophoretic assessment of protein fractions of fish raw materials and products. The possibility of a complete utilization of the drip in the determination and quality control of fish raw materials (1969) is thus confirmed. Therefore our studies were conceived as an attempt to utilize the drip in the fish flesh specific assessment carried out by the starch gel electrophoresis.

MATERIAL AND METHODS

The raw materials derived from the following fish species were used:

- marine Baltic fish:
  Baltic herring *Clupea harengus membras* L., Baltic sprat *Sprattus sprattus* (L.), Baltic cod *Gadus morrhua calarias* L., plaice *Platessa platessa* (L.), grey gurnard *Trigla gurnardus* L.;
- freshwater fish:
  salmon *Salmo salar* L., trout *Salmo trutta* L., rainbow trout *Salmo gairdneri* R., ablen *Coregonus albula* (L.), pike *Esox lucius* L., eel *Anguilla anguilla* (L.), carp *Cyprinus carpio* L., bream *Abramis brama* (L.), *Blicca bjoercna* (L.), *Aspius aspius* (L.), roach *Rutilus rutilus* (L.), tench *Tinca tinca* (L.), perch *Perca fluviatilis* L., pikeperch *Lucioperca lucioperca* (L.).

The species listed were caught from the Pomeranian Bay, lake Miedwie, and a trout hatchery.

Immediately after capture the fishes were placed in ice and brought to the laboratory. The *rigor mortis* being gone, the protein fraction characteristics was performed: samples of flesh were cut out from the anterior dorsal part of a fish individual and the drip was produced by centrifuging. The drip obtained was subject to the electrophoretic separation on a starch gel appropriately prepared (Podeszewski, 1975). A 13% starch gel in Tris – citric acid buffer of 7.6 pH was used in the electrophoresis, while an 8.6 pH borate acid buffer for electrode vessels. The separation was performed at 320V during 4.5 hrs till the beginning of borate line was reached at a distance of 11 cm from the start. Protein fractions were stained with 0.1% 10B amide black in 5:1:1 water : methanol : acetic acid. A Jounan (France) densitometer was used to draw the protein fractions obtained.

RESULTS AND DISCUSSION

The specific determination of fishes investigated started from choosing a suitable material on which the muscle proteins could be characterized using the starch gel electrophoresis. The separation procedure was carried out on muscle proteins extracted

by water, phosphate buffer (0.4539 g KH_2PO_4 + 5.3440 g Na_2HPO_4), low and high ionic strength sodium chloride solutions (0.15 and 0.5 μ, respectively), and on the drip.

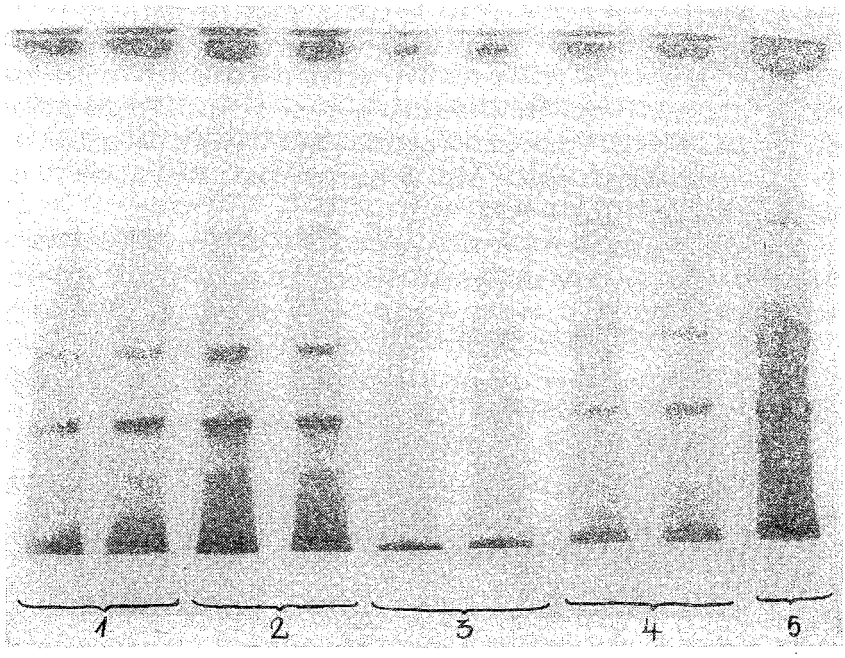


Fig. 1. Sarcoplasmatic protein fractions in cod (*Gadus morrhua*)
1. water extract, 2. phosphate buffer extract, 3. 0.5M NaCl extract, 4. 0.15M NaCl extract, 5. drip

The results obtained are presented on Fig. 1.

Basing on the protein separation intensity, the drip was found to contain the highest, among all the extracts, density of proteins mobile in the electric field per a volume unit. This is a result mainly of a protein extracting technique used, in which dilutions obtained by using particular muscle tissue/extracting medium ratios cannot be avoided. The protein content of the drip ranging within 4–6%/cm^3 is found to be essential in optimal separation intensity of each fraction. The myofibril proteins moving to the high ionic strength solutions as a result of using low ionic strength electrophoretic buffers fall out at the start during the first phase of electrophoresis (Fig. 1(3)) taking no part in the electrophoretic picture.

In view of the fact that no differences were found in protein electropherogram of the drip, water and low ionic strength extracts, the drip as a natural physiologic fluid containing sarcoplasmatic proteins was regarded as the most suitable material for the studies of the kind described.

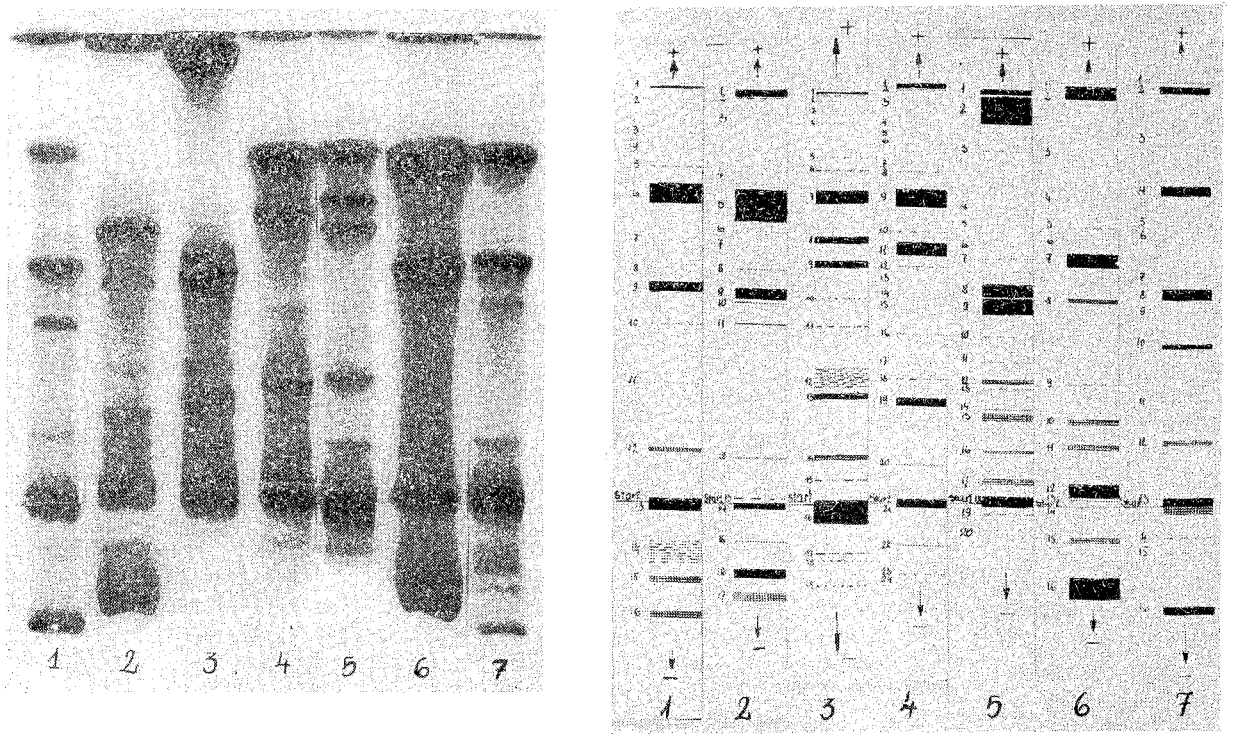


Fig. 2. Electropherograms of freshwater fish protein fractions, 2a. electropherograms of drip proteins, 2b. graphs of protein fractions 1. perch (*Perca fluviatilis*), 2, pikeperch (*Lucioperca lucioperca*), 3. pike (*Esox lucius*), 4. *Blicca bjoercna*, 5. bream (*Abramis brama*), 6. *Aspius aspius*, 7. roach (*Rutilus rutilus*)

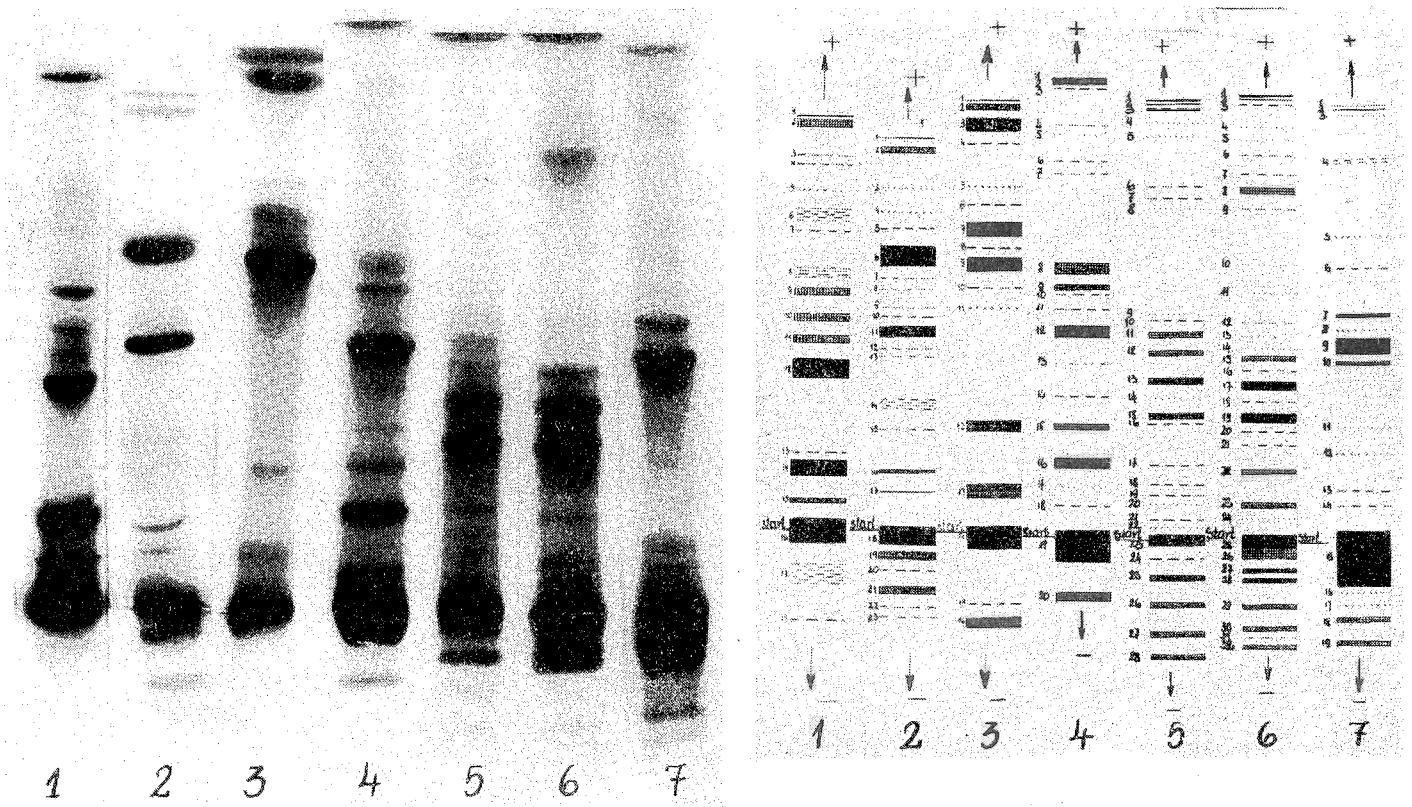
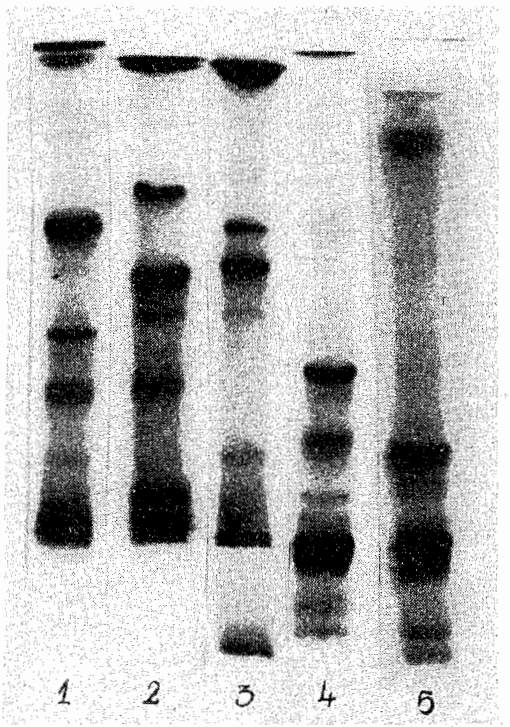


Fig. 3. Electropherograms of freshwater fish protein fractions, 3a. electropherograms of drip proteins, 3b. graphs of protein fractions 1. eal (*Anguilla anguilla*), 2. tench (*Tinca tinca*), 3. carp (*Cyprinus carpio*), 4. ablen (*Coregonus albula*), 5; rainbow trout (*Salmo gairdneri*), 6. trout (*Salmo trutta*), 7. salmon (*Salmo salar*)



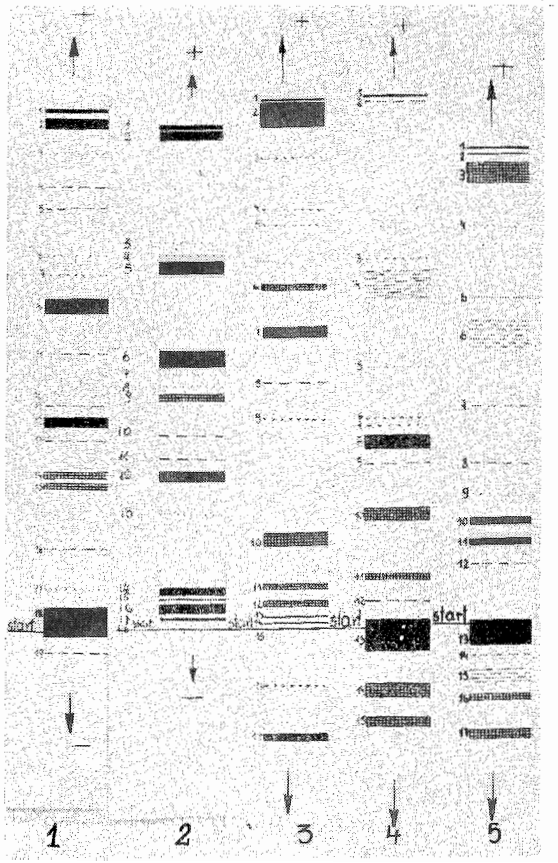


Fig. 4. Electropherograms of Baltic fish protein fractions, 4a. electropherograms of drip proteins, 4b. graphs of protein fractions
1.plaice (*Platessa platessa*), 2. cod (*Gadus morrhua*), 3. grey gurnard (*Trigla gurnardus*), 4. sprat (*Sprattus sprattus*), 5. herring (*Clupea harengus*)

Figs. 2, 3, and 4 show the original electrophoreograms and graphs of protein fractions in the drip of fish examined. The qualitative and quantitative results obtained for each species are fully documented in Figs. 5–23.

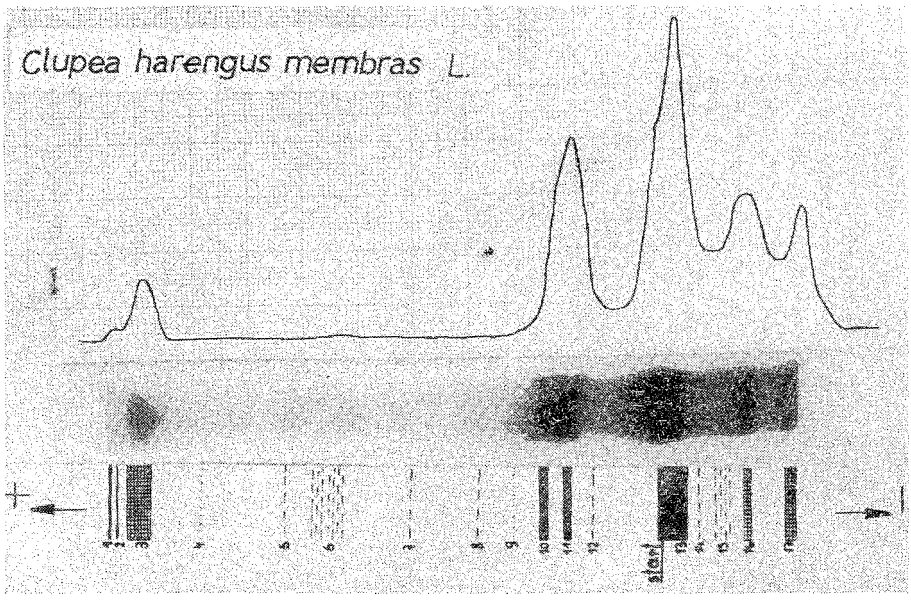


Fig. 5. Electropherograms of drip sarcoplasmatic proteins in Baltic herring *Clupea harengus membras* L.

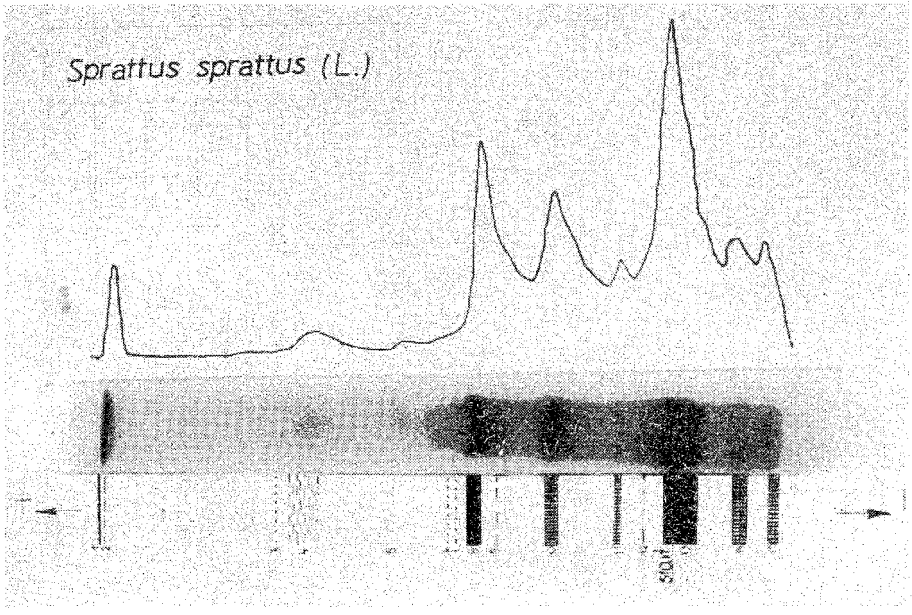


Fig. 6. Electropherograms of drip sarcoplasmatic proteins in Baltic sprat *Sprattus sprattus* (L.)

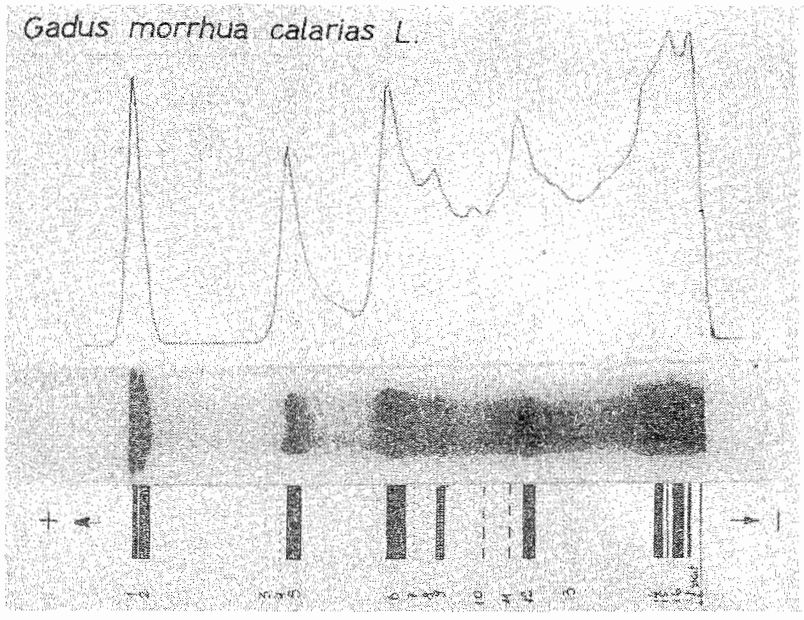


Fig. 7. Electropherograms of drip sarcoplasmatic proteins in salmon *Salmo salar* L.

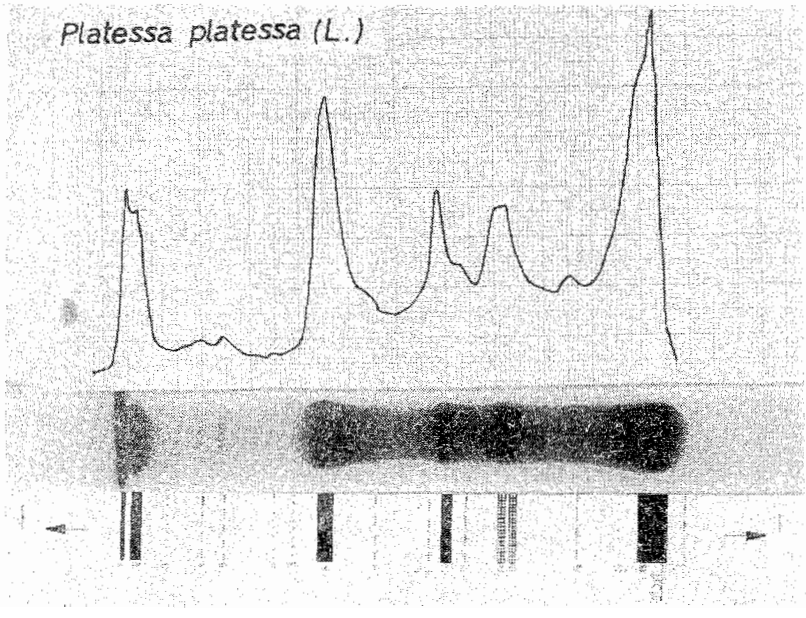


Fig. 8. Electropherograms of drip sarcoplasmatic proteins in trout *Salmo trutta* L.

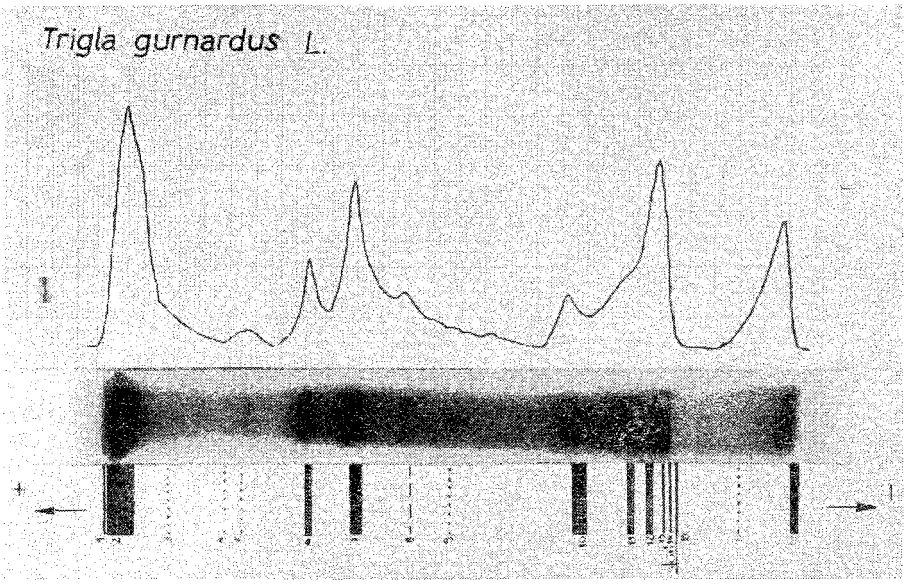


Fig. 9. Electropherograms of drip sarcoplasmatic proteins in ablen *Coregonus albula* (L.)

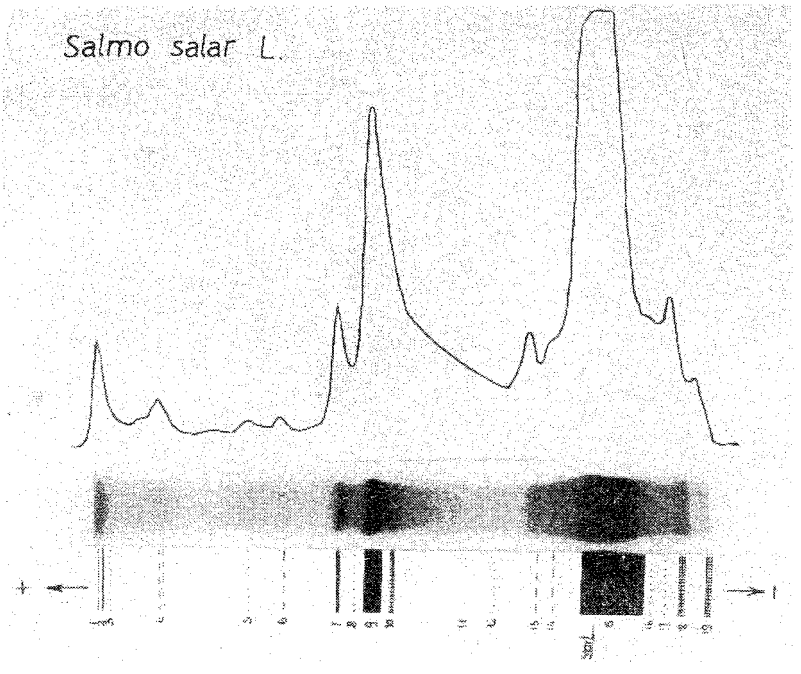


Fig. 10. Electropherograms of drip sarcoplasmatic proteins in eel *Anguilla anguilla* (L.)

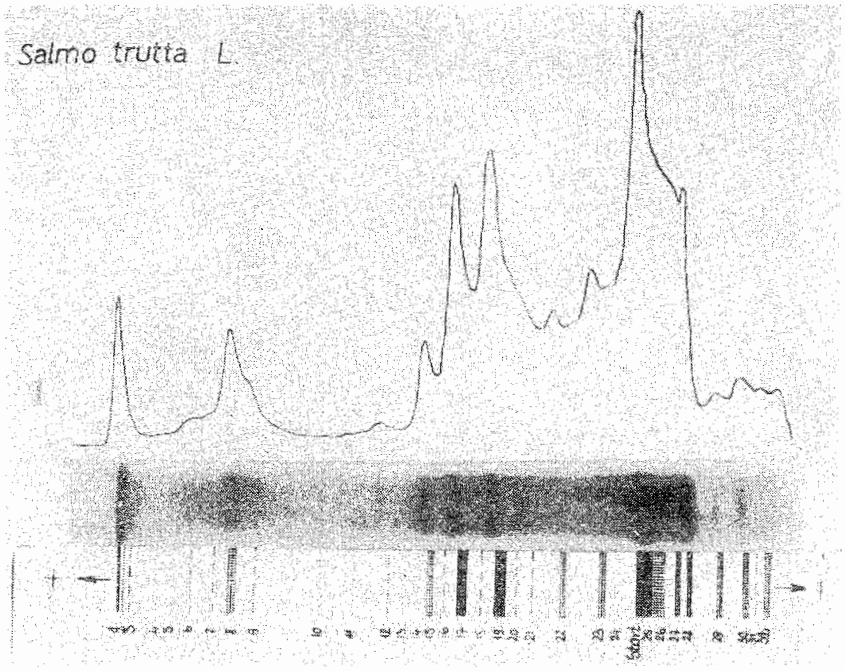


Fig. 11. Electropherograms of drip sarcoplasmatic proteins in Baltic cod *Gadus morrhua callarias* L.

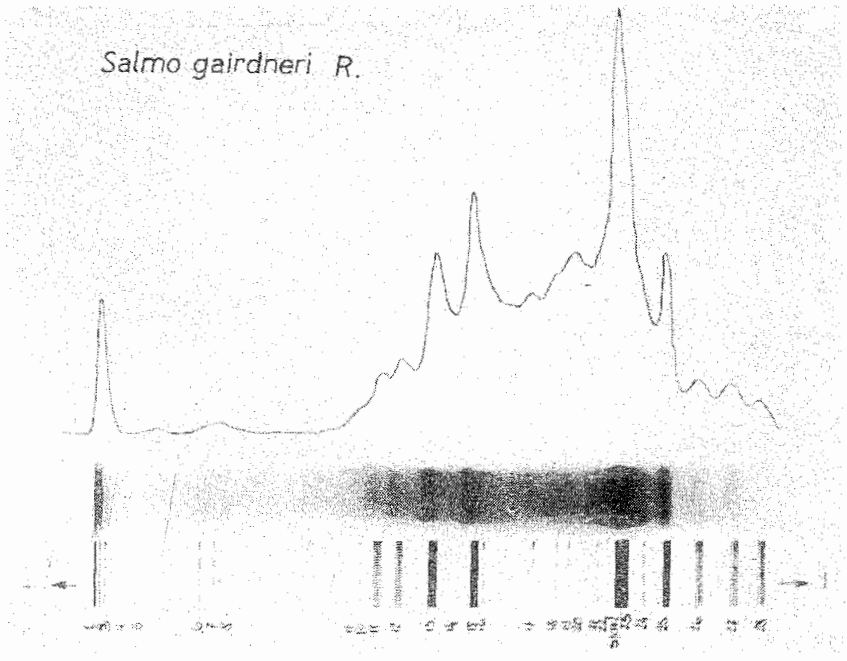


Fig. 12. Electropherograms of drip sarcoplasmatic proteins in grey gurnard *Trigla gurnardus* L.

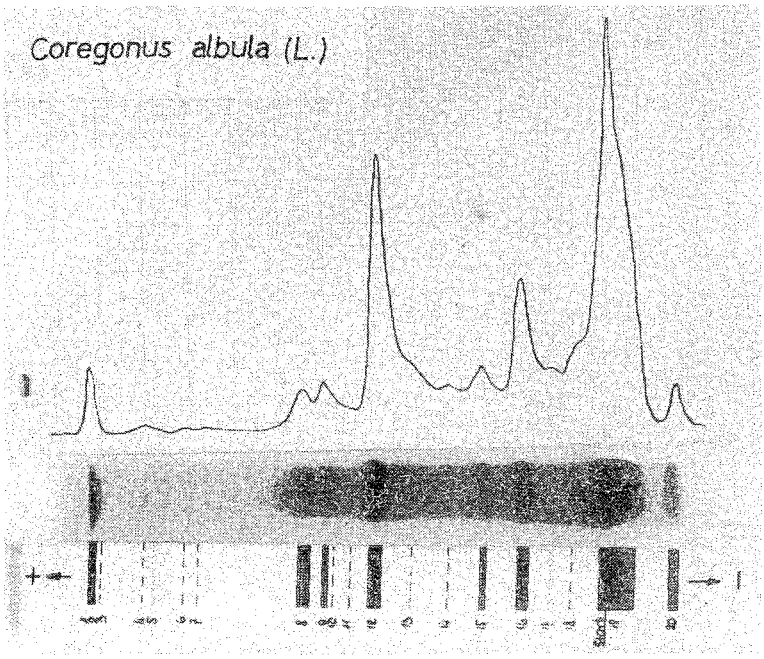


Fig. 13. Electropherograms of drip sarcoplasmatic proteins in plaice *Platessa platessa* (L.)

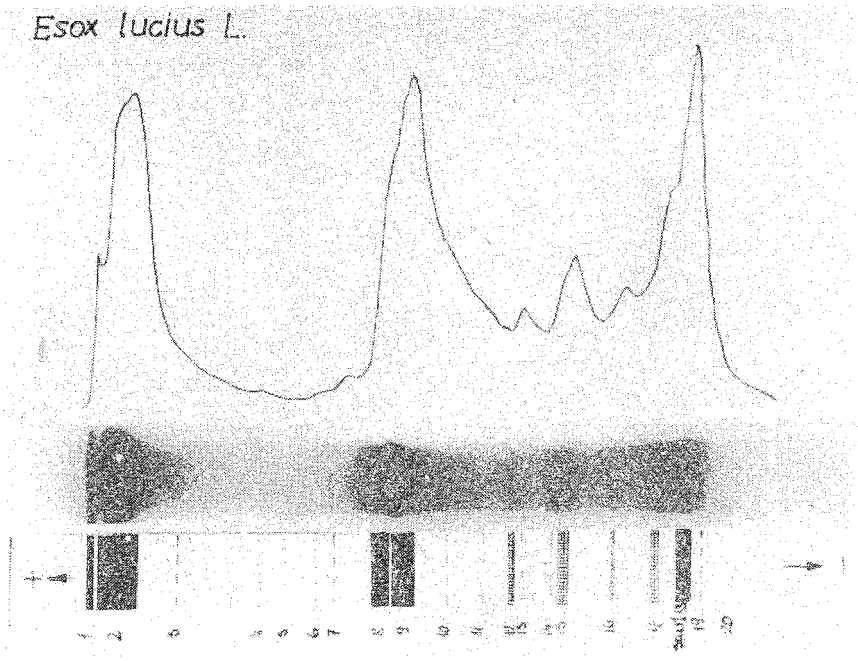


Fig. 14. Electropherograms of drip sarcoplasmatic proteins in rainbow trout *Salmo gairdneri* R.

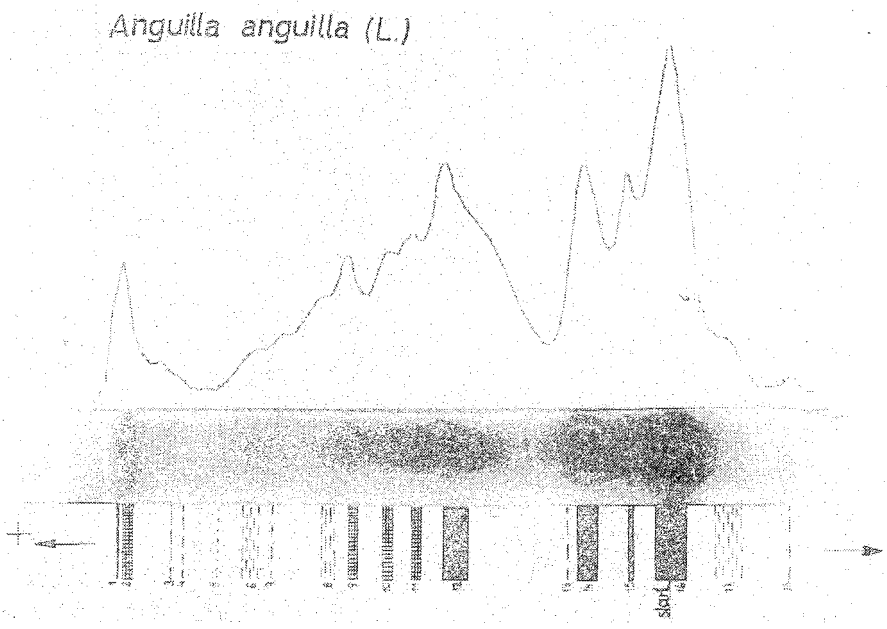


Fig. 15. Electropherograms of drip sarcoplasmatic proteins in pike *Esox lucius* L.

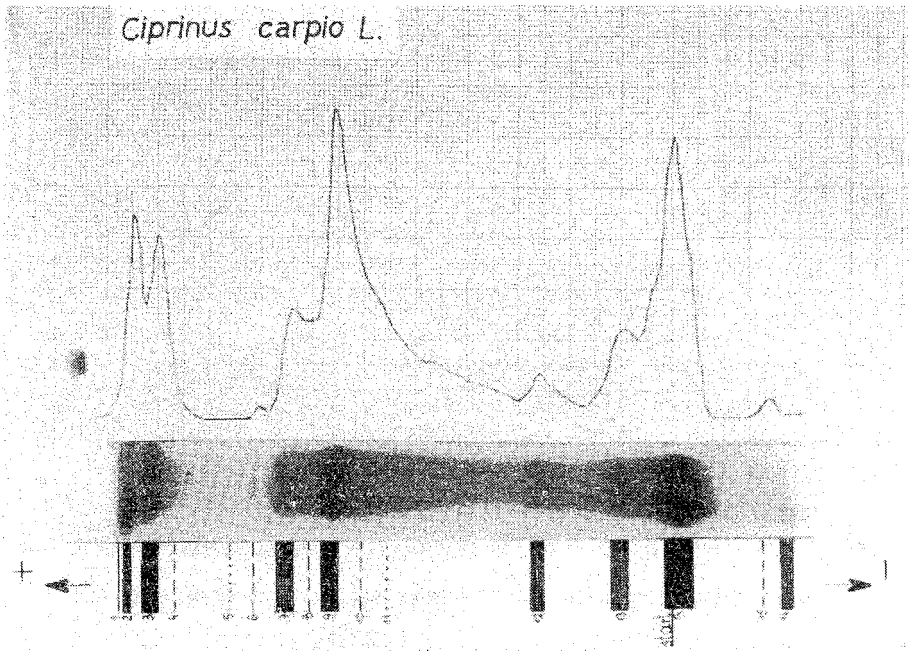


Fig. 16. Electropherograms of drip sarcoplasmatic proteins in carp *Cyprinus carpio* L.

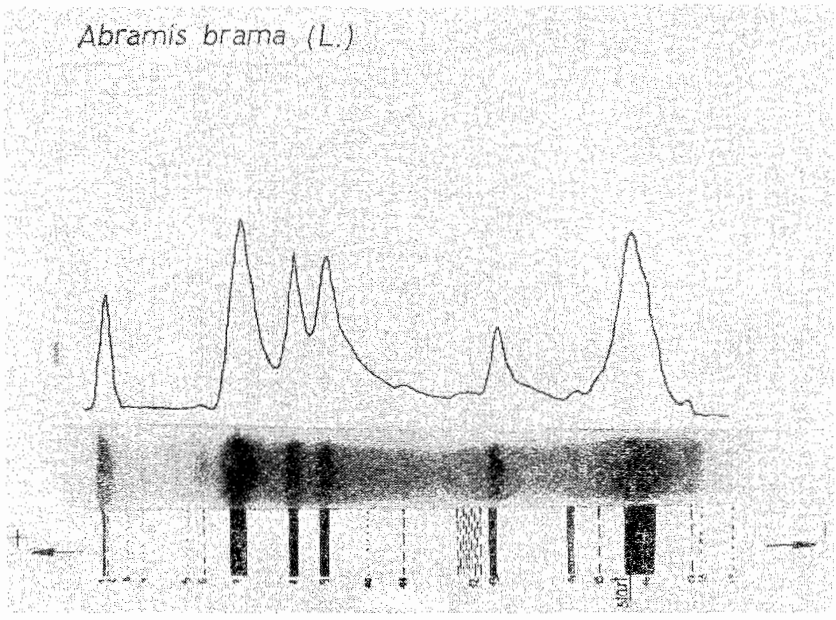


Fig. 17. Electropherograms of drip sarcoplasmatic proteins in bream *Abramis brama* (L.)

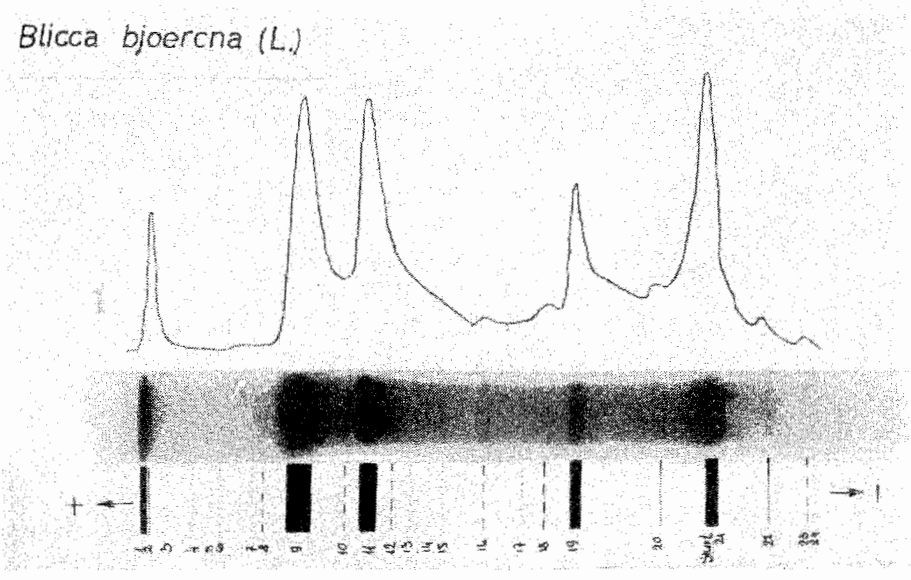


Fig. 18. Electropherograms of drip sarcoplasmatic proteins in *Aspius aspius* (L.)

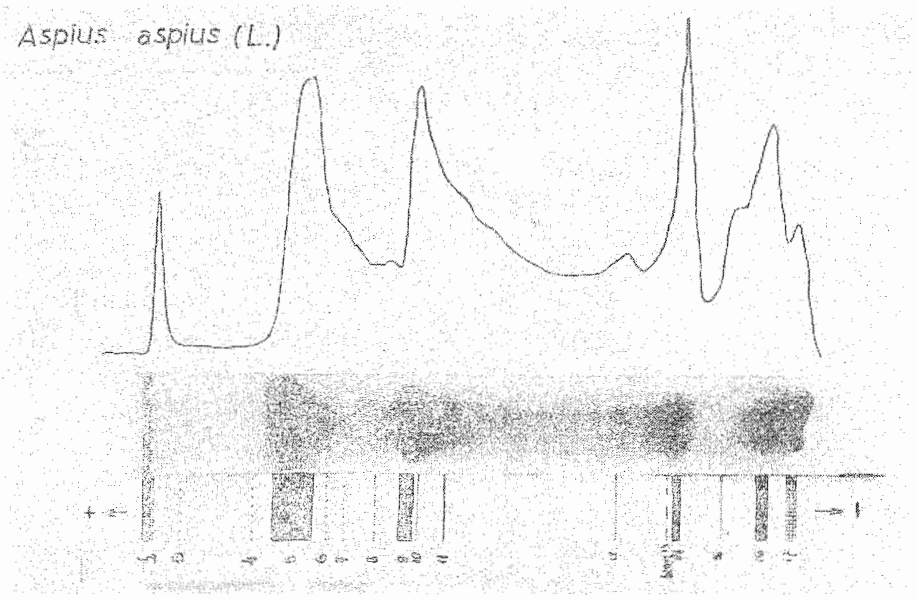


Fig. 19. Electropherograms of drip sarcoplasmatic proteins in *Blicca bjoercna* (L.)

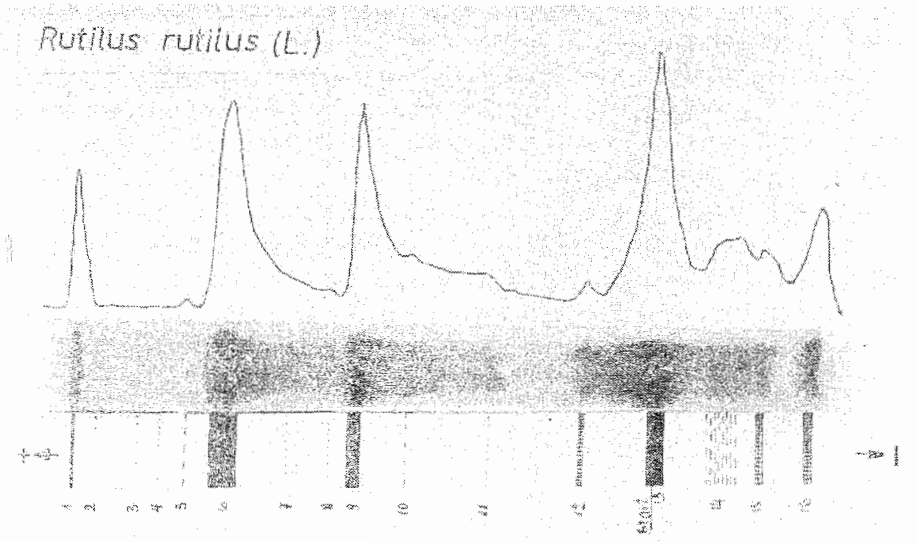


Fig. 20. Electropherograms of drip sarcoplasmatic proteins in tench *Tinca tinca* (L.)

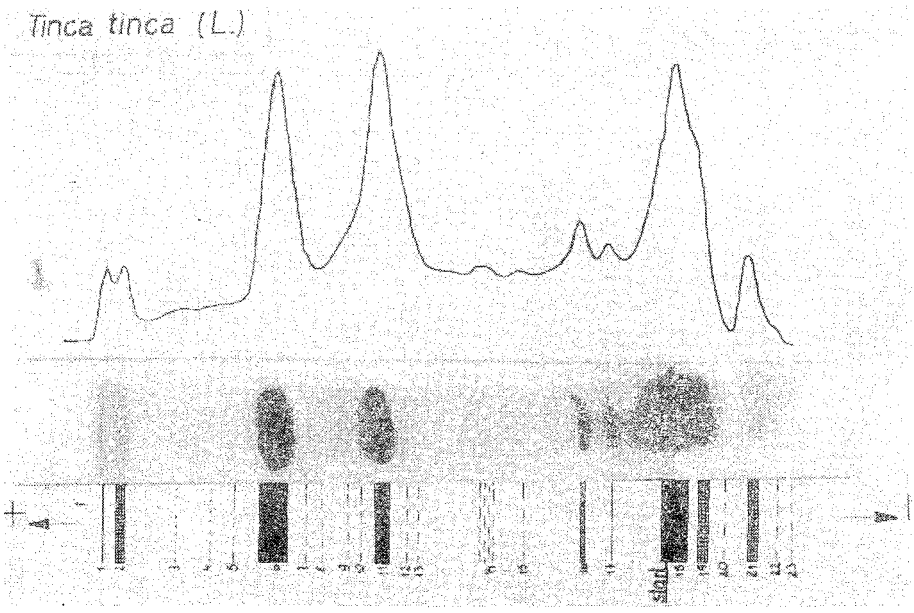


Fig. 21. Electropherograms of drip sarcoplasmatic proteins in roach *Rutilus rutilus* (L.)

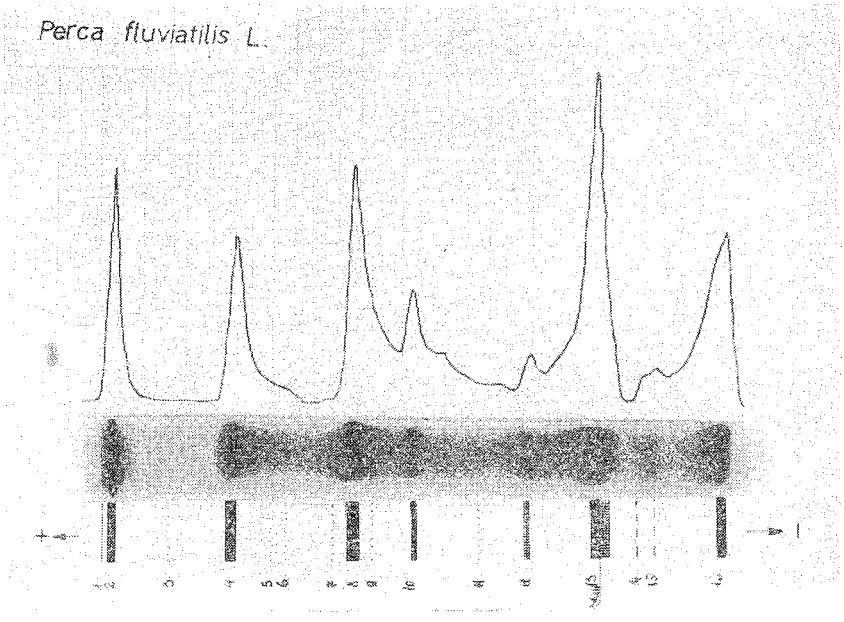


Fig. 22. Electropherograms of drip sarcoplasmatic proteins in perch *Perca fluviatilis* L.

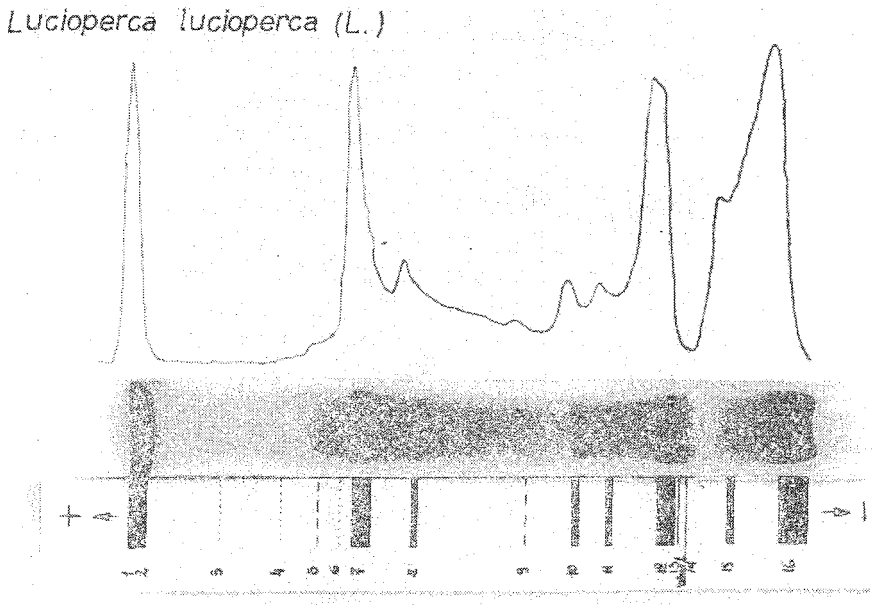


Fig. 23. Electropherograms of drip sarcoplasmatic proteins in pikeperch *Lucioperca lucioperca* (L.)

The drip protein electrophoreograms analyzed showed an excellent separating properties of the starch gel which guaranteed the replicability of the differentiation of the fractions for every fish species. Two basic groups of protein fractions can be found on electrophoreograms: major ones, intensively staining and appearing in high concentrations visible as dark fields and minor ones, poorly stained.

The major fractions can serve as an essential clue in the sarcoplasmatic proteins characteristics. They occur in varying numbers and in different places on electrophoreograms of each fish species. The minor fractions, scattered over the whole separation band, play an ancillary role.

The starch gel allowed to separate 16–32 and 15–19 fractions of sarcoplasmatic proteins, depending on a species, in freshwater and Baltic fishes, respectively. This would point to a more complex system of protein fractions in freshwater fishes, salmonids in particular, which in general results from different genetic features of both groups of fishes that had been formed during the evolution.

With regard to the arrangement of protein fractions, four zones of localization can be differentiated on the electrophoreograms (Fig. 24).

Within the anode area a frontal zone (I) can be separated, containing the fractions most quickly migrating within the electric field. Most frequently 2–3 major fractions of various species-dependent concentrations occur, e.g., this zone is very well developed in pike and also, though in a different way, in carp. The zone is typical of all the species, the proteins found there belonging to albumins and myoglobulins. The other zone of the area, the middle one (II) contains a varying number of fractions depending on a species,

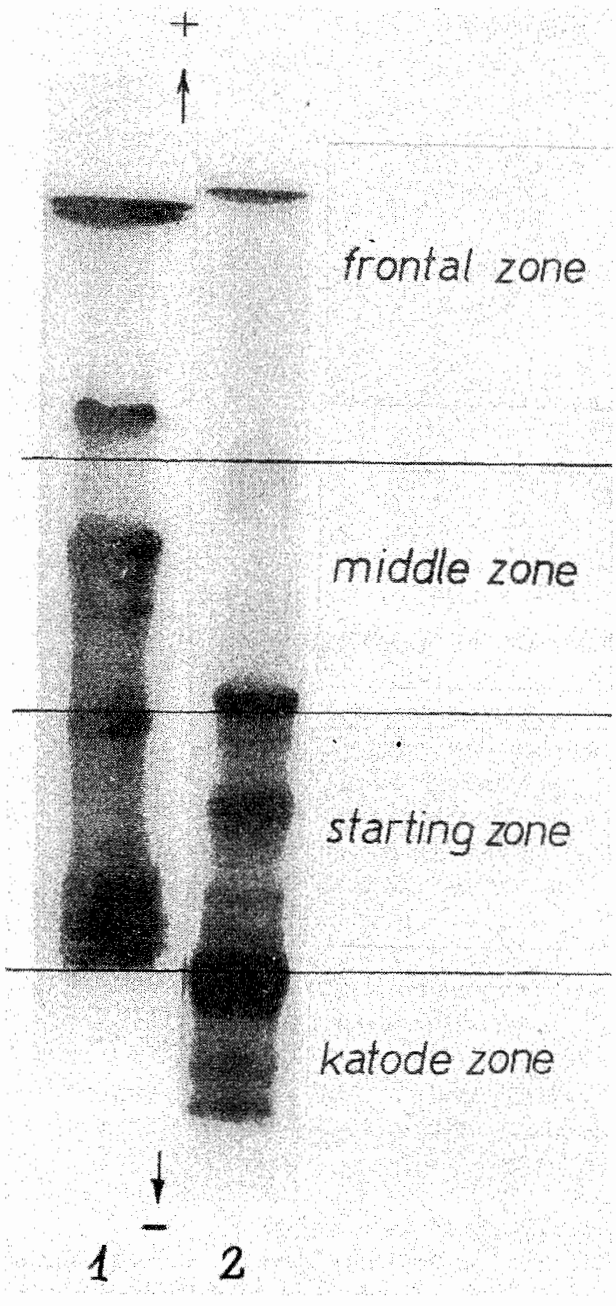


Fig. 24. Electropherogram divided into zones
1. cod (*Gadus morrhua callarias*)
2. sprat (*Sprattus sprattus*)

of intensity and localization differing too. The starting zone (III) contains the least-mobile proteins, densely adsorbed on the gel and making up the start fractions. The zone II and III proteins belong mainly to enzymatic (glycolytic) ones as well as to those showing some globulin-like properties.

Within the katode area there is one zone (IV) to be found. The proteins of this zone do not occur in all the fish species and have a different electric charge than the anode area proteins; their number varies and is typical mainly of freshwater fish. Of the Baltic fishes, cod and plaice contained no protein of this kind.

Scopies (1966) applied the protein fraction zones to his studies on beef cattle; he used as much as six zones. Our investigations showed the three anode and one katode zones to be sufficient in a correct identification of protein fractions as applied to comparative studies of various fish species.

The studies presented allowed some typical biochemical characters to be revealed in fishes occurring within the Polish water areas. These characters are shown through a differentiated picture of fractions of sarcoplasmatic proteins. A possibility of comparative studies on the fish species determination as well as of following the dynamics of posthumous changes resulting from various methods of preservation is also introduced. Additionally, the applicability of the method is increased through using the drip to the sarcoplasmatic protein fraction assessment. At the same time a quantitative method of measurement of the drip (Podeszewski et al., 1971) enables us to determine the water-binding properties and follow the decomposition of protein fractions. Thus the utilization of the drip as a diagnostic element proved fully warranted.

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WYKORZYSTANIE WYCIEKU DO CHARAKTERYSTYKI BIAŁEK SARKOPLAZMATYCZNYCH RYB METODĄ ELEKTROFOREZY NA ŻELU SKROBIOWYM

Streszczenie

Celem przeprowadzonych badań było wykorzystanie wycieku do identyfikacji gatunków ryb na podstawie frakcji białek sarkoplazmatycznych oznaczonych metodą elektroforezy na żelu skrobiowym. Przebadano 5 gatunków ryb morskich – bałtyckich oraz 14 gatunków ryb słodkowodnych. W wyniku przeprowadzonych badań stwierdzono doskonałą przydatność metody do biochemicznej diagnostyki ryb. Uzyskane wyniki przedstawiono na zdjęciach i schematach.

ИСПОЛЬЗОВАНИЕ ТКАНЕВОГО СОКА ДЛЯ ХАРАКТЕРИСТИКИ САРКОПЛАЗМАТИЧЕСКИХ БЕЛКОВ РЫБ МЕТОДОМ ЭЛЕКТРОФОРЕЗА НА КРАХМАЛЬНОМ ГЕЛЕ

Резюме

Целю проведенных исследований было использование тканевого сока для видовой идентификации рыб на основе фракций саркоплазматических белков, определённых методом электрофореза на крахмальном геле. Исследовано 5 видов морских (балтийских) рыб и 14 видов пресноводных рыб. В результате

проведенных исследований установлена полная пригодность метода для биохимической диагностики рыб. Полученные данные представлены на снимках и в схемах.

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Received: 5 II 1976 r.