EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. 20. KÖIDE
BIOLOOGIA. 1971, NR. 2

ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. ТОМ 20
БИОЛОГИЯ. 1971, № 2

https://doi.org/10.3176/biol.1971.2.07

УДК 591.111.05+591.151.1:597.554

AARE KIRSIPUU

SEASONAL CYCLE OF CHANGES IN THE BLOOD SERUM PROTEIN FRACTIONS IN BREAM

Up to now the investigation of protein composition in fishes has, in general, been carried out in two directions: 1) the ascertaining of the genetic structure of several systematic groups, and 2) settling the questions of comparative biochemistry. The physiological aspect of the changeability in the blood serum proteins in fishes has only very briefly been touched upon or has even not been taken into account in most of the concerning works. But, if taking into consideration the lability of the inner medium of fishes as poikilotherm organisms, the investigation of the changes in their blood serum protein system in connection with the changes in the surrounding medium and physiological processes may, undoubtedly, contribute to the research of metabolic processes in fishes. We assume that in this sense the primary task is the ascertaining of the seasonal cycles of changes in the blood serum proteins in fishes, since the knowledge of these cycles is a necessary prerequisite for raising other connected questions.

If to take into account the exceptional variety of the blood serum protein composition in fishes and the sensibility of the system to several ecological conditions, the necessity of ascertaining the seasonal cycle of changes in the blood serum proteins in several fishes of different systematic groups in nearly the same ecological conditions, on the one hand, and in the same fish in different ecological conditions, on the other hand, becomes evident. Only when having such materials can we make general conclusions on the significance of the genetic origin and the influence of environmental conditions on the blood serum protein system in fishes.

The first attempt to elucidate the seasonal cycle of the changes in the blood serum protein system in some freshwater fishes was made by us some years ago (Кирсипуу, 1964b). As a shortcoming of this work we can notice the relatively great intermissions between analyses (2—3 months). However, such a shortcoming is characteristic of most of the works of similar profile: in most cases investigations are carried out only 4—6 times a year. The data received in such a way enable to draw very general conclusions only. Our investigations have shown that for obtaining a real picture of the seasonal changes in the blood serum protein system of fishes it is necessary to carry out analyses not more rarely than once a month.

Materials and methods

In the present work we have used the data of 765 analyses of the blood of bream, which were collected from 1961 to 1968. In 1961—1965 analyses were made 4—8 times a year, in 1966—1968 20—30 times a year with intervals not longer than one month.

The length of the fishes investigated was from 15 to 50 cm, the weight ranging from

150 to 2800 g. The biological analysis was in most cases made by H. H. Haberman. Herewith the author expresses his gratitude to him. In other cases the biological analysis was made by the author. The length and weight, sex, sexual maturity (by the 6-number scale), filling of the intestines, fatness of the interior, age, rate of growth and some morphological characters were determined.

The electrophoresis of the blood serum proteins was carried out on filter paper strips by the methods described by us earlier (Кирсипуу, 1964a).

At the interpretation of the data all the individuals were divided into three sexual groups: females (sexually mature), males (sexually mature) and immature individuals.

Results

The blood serum proteins of the bream were usually separated into 5—6 fractions by paper electrophoresis: albumins, α_1-, α_2-, β- (in very many cases β_1- and β_2-)and γ-globulins. Sometimes the separation of β-globulins into 3 subfractions was detected. In such a case it was difficult to decide on whether the quickest fraction of the group belongs to α-globulins (α_3) or to β-globulins (β_1). The separation of β-globulins into subfractions was not connected with the sex of fish or the season of the year. We assume that first and foremost it depends on the genotype of the fish (Хаберман et al., 1969).

Seasonal changes in the percentages of the fractions (mean values of many years) are represented by Figs 1—9: Figs 1—6 represent the changes of the fractions in different sexual groups, while Figs 7—9 represent those in all fractions in each sexual group.

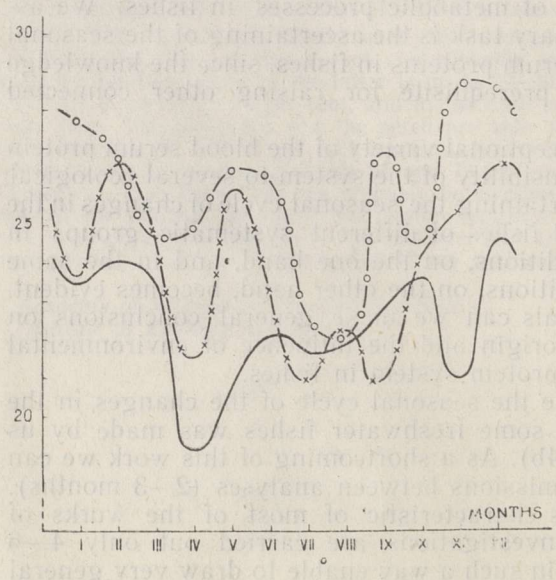

Fig. 1. Seasonal changes in the percentage of albumins.
——female; —o— male; —×— immature individuals (the same symbols apply to Figs 2—6).

When observing the levels of the fractions in Figs 1—6 one may notice some parallelism in the curves of seasonal changes of fractions in all sexual groups, except for albumins and α_2-globulins in the females. Some deviations in time and differences in the amplitude of the swing can, obviously, be explained by the unequal distribution of the material during several years, when the general level or the amplitude of the swing of the fractions were different. Besides that, the seasonal cycle of the changes during different years may be shifted.

The percentage of albumins on the whole changes according to the scheme given by us earlier (Кирсипуу, 1964b; Кирсипуу, 1965): in winter it is high, in spring it falls, in summer it is low and in autumn it rises again. But monthly analyses have shown that the decrease in the level of albumins does not proceed gradually through the whole winter, but begins in March only and then proceeds very quickly. In females the decrease, as shown by us earlier (Кирсипуу, 1964b), is more noticeable. In May or June

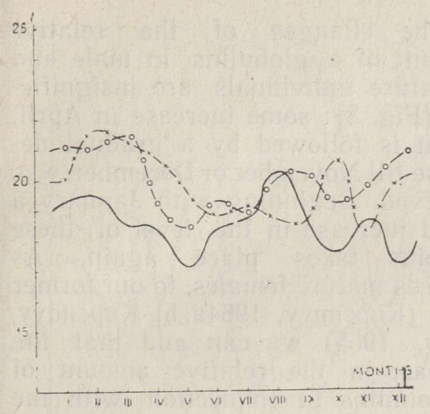

Fig. 2. Seasonal changes in the percentage of α_1-globulins.

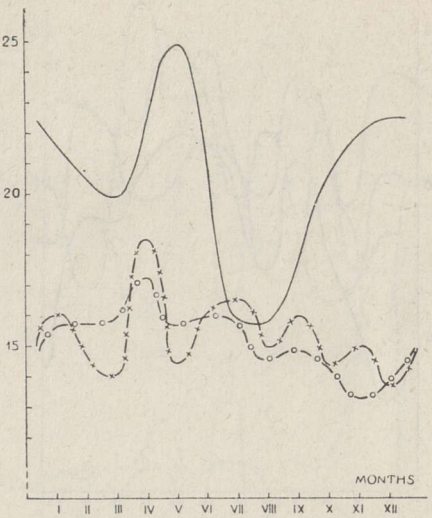

Fig. 3. Seasonal changes in the percentage of γ-globulins.

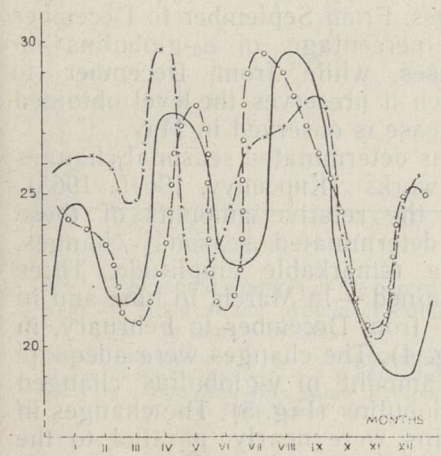

Fig. 4. Seasonal changes in the percentage of β-globulins.

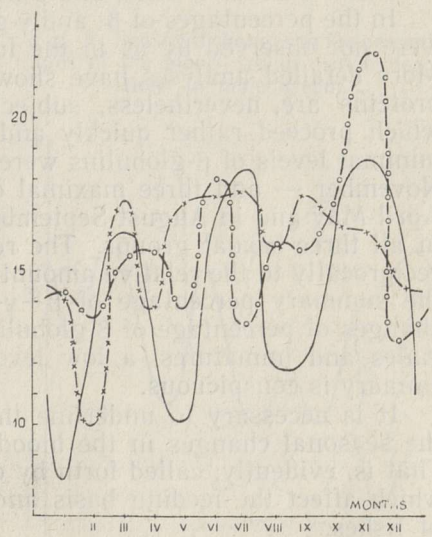

Fig. 5. Seasonal changes in the percentage of γ-globulins.

a small increase may be observed, but in July and August the lowest level of albumins was found (except for the mature females, in which the lowest level is observed in April). The increase of the percentage of albumins begins in September or October and proceeds rather quickly. In winter (from November to March) the percentage of albumins does not change noticeably (Fig. 1). The range of the annual changes is 6—7%.

Seasonal changes in the percentage of α_1-globulins were not detected by us earlier (Кирсипуу, 1964b, 1965). Additional investigations have, however, shown that in winter months (December-March) the relative amount of these globulins is on the average by 2—2,5% greater than in summer (Fig. 2). An insignificant increase in the level of α_1-globulins was also observed in females in August and in males and immature individuals in September or October.

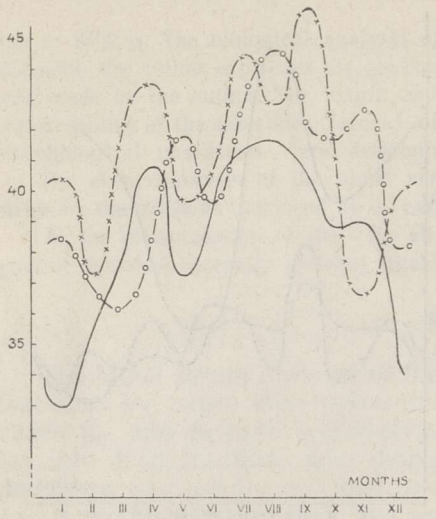

Fig. 6. Seasonal changes in the percentage β+γ-globulins.

The changes of the relative amount of α_2-globulins in male and immature individuals are insignificant (Fig. 3): some increase in April, which is followed by a gradual decrease till November or December, was observed. Beginning with January a small increase in the level of these proteins takes place again. As regards mature females, to our former data (Кирсипуу, 1964a, b; Кирсипуу, Пиху, 1965) we can add that the increase in the relative amount of α_2-globulins in connection with the ripening of the hard roe, which begins in September, does not proceed evenly during the whole winter as it was assumed by us but it happens in two stages. From September to December the percentage of α_2-globulins increases, while from December to March it preserves the level obtained or even decreases a little, and a new increase is observed in May.

In the percentages of β- and γ-globulins determinated seasonal changes were not observed by us in the former works (Кирсипуу, 1964b, 1965). More detailed analyses have shown that the relative amounts of these proteins are, nevertheless, subjected to determinated seasonal changes, which proceed rather quickly and with a remarkable amplitude. Three minimal levels of β-globulins were ascertained — in March, in June and in November — and three maximal ones — from December to February, in April-May and in August-September (Fig. 4). The changes were adequate in all three sexual groups. The relative amount of γ-globulins changed reciprocally to the relative amount of β-globulins (Fig. 5). The changes in the summary percentage of β+γ-globulins were nearly parallel to the changes of percentage of β-globulins (Fig. 5), but in females, contrary to males and immatures, a low level of these proteins in December and January is conspicuous.

It is necessary to underline that during different years the range of the seasonal changes in the blood serum protein fractions is different. That is, evidently, called forth by differences in meteorological conditions, which affect the feeding basis and also the intensity of the metabolism of fishes.

Discussion

In the investigations on the changes in the percentages of the blood serum protein fractions in fishes it is necessary to take into account the fact that every change in the percentage of any fraction calls forth a reciprocal change in the percentages of all other fractions. Thus we can call it a change in the amount of the protein in any fraction only in such a case when the change is reciprocally reflected in all the other fractions. In our investigations such a state was observed rather rarely. A change in the percentage of one of the fractions is usually accompanied by changes in only one or two fractions, in others the changes were parallel to it or were not observed. Evidently, it is necessary to discuss the changes in any fraction in an interdependence with all other fractions.

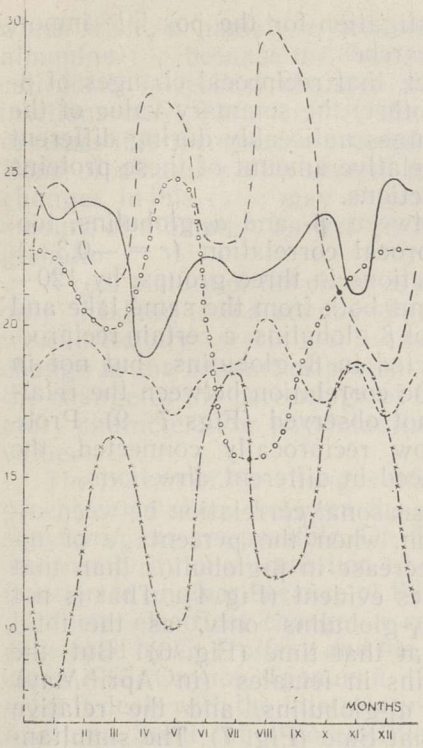

Fig. 7. Seasonal changes in the percentages of blood serum protein fractions in female bream.
——— albumins; - - - - - α_1-globulins; –o– α_2-globulins; — — — β-globulins; — × — γ-globulins (the same symbols apply to Figs 8 and 9).

A very close interdependence was observed by us between β- and γ-globulins: any change in the percentage of one of them was accompanied by a reciprocal change in the percentage of the other. Such an interdependence is detectable all the year round in all sexual groups (Figs 7—9). Taking into account the heterogeneity of β-globulins, which becomes evident in the frequent dividing of them into subfractions, we may assume that the electrophoretically slowest proteins of that fraction may change their electrophoretic mobility in connection with physiological causes, which call forth changes in their charge or conformation, and may combine, therefore, with β- or γ-globulins or separate as an independent fraction. The causes of such behavior are quite ob-

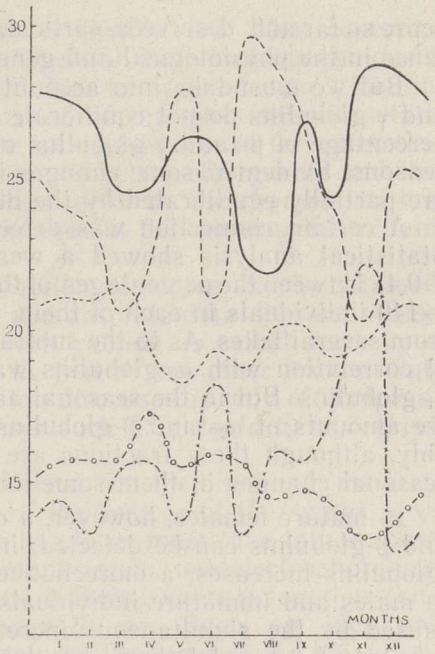

Fig. 8. Seasonal changes in the percentages of the blood serum protein fractions in male bream.

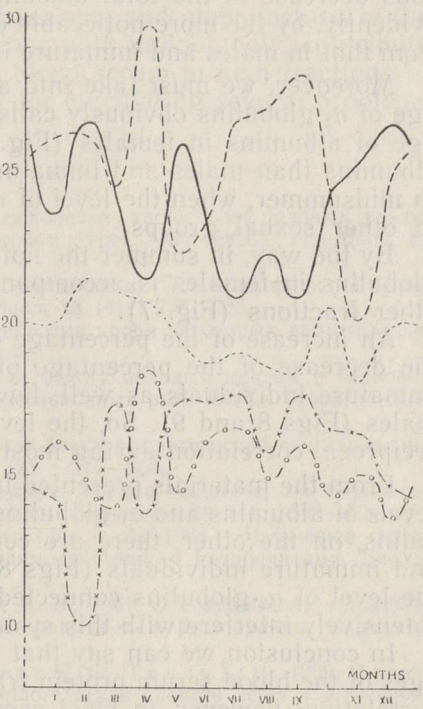

Fig. 9. Seasonal changes in the percentages of the blood serum protein fractions in immature bream.

scure so far and deserve a particular investigation for the possible importance in the physiological and genetic researches.

But we must take into account the fact that reciprocal changes of β- and γ-globulins do not equilibrate one another: the summary value of the percentage of β- and γ-globulins also changes noticeably during different seasons. Evidently, some changes in the relative amount of these proteins are partially equilibrated by the other fractions.

A certain connection was detected between β- and α_2-globulins, too. Statistical analysis showed a weak reciprocal correlation ($r = -0{,}3 \ldots -0{,}4$) between the percentages of these fractions in three groups, by 120—140 individuals in each of them, of breams both from the same lake and from several lakes. As to the subfractions of β-globulins, a certain reciprocal correlation with α_2-globulins was detected in β_2-globulins, but not in β_1-globulins. But in the seasonal aspect, the correlation between the relative amounts of α_2- and β-globulins was not observed (Figs 7—9). Probably, although these fractions are somehow reciprocally connected, the seasonal changes in them sometimes proceed in different directions.

In mature females, however, a certain seasonal correlation between α_2- and β-globulins can be detected: in autumn, when the percentage of α_2-globulins increases, a more noticeable decrease in β-globulins than that in males and immature individuals becomes evident (Fig. 4). That is not caused by the simultaneous increase in γ-globulins only, as the total amount of β+γ-globulins also decreases at that time (Fig. 6). But the second stage of the increase in α_2-globulins in females (in April-May) happens on account of the albumins and α_1-globulins, and the relative amount of β-globulins even increases at that time (Fig. 7). The simultaneous decrease in the total amount of β+γ-globulins (Fig. 6) is caused, evidently, by the more noticeable decrease in the percentage of γ-globulins than that in males and immature individuals (Fig. 5).

Moreover, we must take into account that the increase in the percentage of α_2-globulins obviously calls forth a lasting decrease in the percentage of albumins in females (Fig. 1): they always have a lower level of albumins than males and immature individuals, except for a short period in midsummer, when the level of α_2-globulins in females is as low as that in other sexual groups.

By the way, in summer the noticeable decrease in the percentage of α_2-globulins in females is accompanied by the increase in the levels of all other fractions (Fig. 7).

An increase of the percentage of α_2-globulins, which is connected with the decrease of the percentage of albumins, takes place in males and immature individuals as well; however, it is not so remarkable as in females (Figs 8 and 9). So, the levels of α_2-globulins and albumins are in reciprocal correlation during most of the periods of the year.

From the materials presented here it becomes evident that between the levels of albumins and α_1-globulins, on the one hand, and α_2- and β+γ-globulins, on the other, there are certain reciprocal correlations in males and immature individuals (Figs 8 and 9), but in females the changes of the level of α_2-globulins connected with the ripening of the hard roe very intensively interfere with this system (Fig. 7).

In conclusion we can say that the quantitative changes in the percentages of the blood serum protein fractions in fishes are mostly in very close correlation; however, in most cases the change in one fraction is not connected with all fractions, but only with some of them. These reciprocal relations change in different seasons. Therefore, the changes in one fraction cannot be set off against all the others, but it is necessary to make sure by

which fraction namely a detected change is equilibrated. That concerns albumins, too, because the changes in the levels of these proteins obviously call forth reciprocal changes not in all, but in one or two globulin fractions only. Therefore, by the appreciation of the physiological state of fishes it is incorrect to proceed from the ratio A/G because the interrelations of albumins with several globulin fractions are changeable and the changes in this ratio may as well be called forth by the changes in the amount of albumins, as well as by the changes in the amount of a certain globulin.

A real picture of the changes in any fraction, of course, can be obtained only if taking into account both relative and absolute data. In such a case we can exactly ascertain if a change in any fraction is called forth by a change in the amount of protein in it or by that in any other fraction and the change in the percentage of the fraction discussed is only of compensatory character. For physiological purposes such data could have a great value.

The reason of the changes may be different. On the one hand, the reciprocal correlations between the activity of the centres of the synthesis of some proteins are possible. On the other hand, we must take into account the possibility of a transition of some proteins from one electrophoretical fraction to another because of the changes in their transport function (and in their electrical charge in connection with it). Such an idea was developed by G. Troitsky and his collaborators (Троицкий et al., 1961; Соркина, 1963; Окулов, Троицкий, 1963; Соркина et al., 1968). First and foremost it deals with the interrelations of albumins and γ-globulins with other fractions. Some other possibilities are not excluded, either. Therefore, for physiological investigations, the study of the transport functions and their changes caused by the alteration of seasons and other factors, is the primary task. The settling of these questions will, undoubtedly, contribute to the researches on the interrelations of blood serum protein fractions in fishes. That, in its turn, is very important for elucidating several problems of metabolism.

REFERENCES

Кирсипуу А., 1964a. О белковых фракциях сыворотки крови и их половых различиях у некоторых промысловых рыб Эстонии. Изв. АН ЭССР, сер. биол. **13** (1) : 45—54.

Кирсипуу А., 1964b. О сезонных изменениях соотношений белковых фракций сыворотки крови рыб. Изв. АН ЭССР, сер. биол. **13** (4) : 278—283.

Кирсипуу А., 1965. О белковых фракциях сыворотки крови некоторых промысловых рыб Эстонии. Автореф. канд. дисс. Тарту.

Кирсипуу А. Й., Пиху Э. Р., 1965. О связи между половым циклом и белковой системой сыворотки крови некоторых пресноводных рыб. В сб.: Теоретические основы рыбоводства. М. : 49—52.

Окулов В. И., Троицкий Г. В., 1963. Тепловая электрофоретическая гомогенизация белков сыворотки крови. Биохимия **28** (2) : 277—284.

Соркина Д. А., 1963. О содержании в перфузате сердца веществ, вызывающих «альфа, бета-глобулинизацию» белков крови. Биохимия **28** (4) : 589—594.

Соркина Д. А., Троицкий Г. В., Каминская Г. А., 1968. Изменение структуры сывороточного альбумина при взаимодействии с метаболитами. Биохимия **33** (2) : 220—227.

Троицкий Г. В., Окулов В. И., Соркина Д. А., 1961. О возможности трансформации альбумина и γ-глобулина плазмы крови в α- и β-глобулины. Биохимия **26** (1) : 44—56.

Хаберман Х. Х., Кирсипуу А. Й., Таммерт М. Ф., Телль Х. И., 1969. О связи породных признаков и продукционно-биологических свойств леща в различных озерах. Докл. на XV конф. по изуч. внутр. водоемов Прибалтики в Минске 21/X—25/X 1969 (In press).

Academy of Sciences of the Estonian SSR,
Institute of Zoology and Botany

Received
Jan. 29, 1970

AARE KIRSIPUU

LATIKA VERESEERUMI VALGUFRAKTSIOONIDE SESOONNE MUUTUSTE TSÜKKEL

Resümee

Paberelektroforeesi abil uuriti aastail 1961—1968 Võrtsjärve latika vereseerumi valgufraktsioonide sesoonseid muutusi. Ajavahemikus 1961—1965 tehti analüüse 4—8 korda, 1966—1968 20—30 korda aastas vaheaegadega mitte üle ühe kuu. Kokku uuriti 765 latikat.

Selgus, et sesoonsed muutused latika vereseerumi valgufraktsioonide protsentuaalsetes suhetes haaravad kõiki paberelektroforeesil eraldunud fraktsioone (albumiinid, α_1-, α_2-, β- ja γ-globuliinid). Mõnedes fraktsioonides (β- ja γ-globuliinid, α_2-globuliinid sugukõpsetel emastel) toimuvad nihked üsna järsku ja on tähelepandava ulatusega. Muutused kulgevad samas suunas kõikidel sugurühmadel (suguküpsed isased ja emased ning mittesuguküpsed indiviidid), välja arvatud muutused, mis seoses marja valmimisega esinevad emastel latikatel α_2-globuliinide suhtelises hulgas. Viimased mõjustavad tugevasti muutusi ka teistes fraktsioonides. Erinevatel aastatel esinevad samad muutused, kuid nende amplituud võib olla erinev. Samuti võib ilmastikust sõltuvalt erinevatel aastatel nihkuda muutuste toimumise aeg.

Enamasti ei mõjusta ühe fraktsiooni protsentuaalse sisalduse muutus mitte kõigi teiste, vaid ainult mõnede fraktsioonide taset. Seepärast tuleb kõiki antud fraktsiooni muutusi käsitleda tihedas seoses kõigis teistes fraktsioonides toimuvate muutustega, selgitades igal üksikjuhul, millised neist ühe või teise nihke tasakaalustavad.

Seejuures tuleb silmas pidada, et eri aastaaegadel muutuvad fraktsioonide omavahelised seosed. Täieliku ülevaate saamiseks tuleb kõrvutada andmed nii kõikide fraktsioonide suhtelise kui ka absoluutse valgusisalduse sesoonsete muutuste kohta. Kalade füsioloogia seisukohalt omab aga erilist tähtsust iga fraktsiooni transportfunktsioonide ja nende sesoonsete muutuste väljaselgitamine.

Eesti NSV Teaduste Akadeemia
Zooloogia ja Botaanika Instituut

Toimetusse saabunud
29. I 1970

ААРЕ КИРСИПУУ

СЕЗОННЫЙ ЦИКЛ ИЗМЕНЕНИЙ БЕЛКОВЫХ ФРАКЦИЙ СЫВОРОТКИ КРОВИ У ЛЕЩА

Резюме

При помощи электрофореза на бумаге изучены сезонные изменения в количественных соотношениях белковых фракций сыворотки крови леща оз. Выртсъярв с 1961—1968 гг. С 1961—1965 гг. анализы проводились 4—8 раз в год, а с 1966—1968 гг. — 20—30 раз в год с перерывами не более одного месяца. Проанализировано 765 лещей.

Сезонные изменения наблюдались в процентном содержании всех белковых фракций, разделяющихся при электрофорезе на бумаге (альбумины, α_1-, α_2-, β- и γ-глобулины). У всех половых групп (половозрелые самцы и самки, неполовозрелые особи) сезонные изменения происходят в основном в одном направлении. Исключение составляют связанные с созреванием икры изменения относительного содержания α_2-глобулинов, в свою очередь влияющие на ход изменений в других фракциях у самок. В разные годы ход изменений одинаков, но амплитуда колебаний может несколько различаться. Кроме того, в зависимости от метеорологических условий возможны сдвиги во времени происхождения изменений.

В большинстве случаев изменения в процентном содержании одной фракции не влияют на уровень всех остальных фракций, а только некоторых из них. Поэтому любое изменение необходимо рассматривать в тесной взаимосвязи с остальными фракциями, выяснив в каждом случае, какая именно фракция уравновешивает данное изменение. Это тем более, что в разные сезоны года взаимосвязи фракций меняются. А если мы хотим получить о динамике белковой системы сыворотки крови рыб полное представление, то необходимо сопоставить данные об изменениях как в относительном, так и в абсолютном количестве белка в них. С точки зрения физиологии особое значение имеет также выяснение транспортных функций фракций и их сезонных изменений.

Институт зоологии и ботаники
Академии наук Эстонской ССР

Поступила в редакцию
29/I 1970