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APPLIED METHODS FOR EARLY DIAGNOSIS OF DISEASES ON SEVERAL FISH SPECIES IN SKADAR-LAKE

INTRODUCTION

Since December 1977 physical condition of fishes in Skadar-Lake has been investigated for the ministry of environment at the request of the German—Jugoslavian cooperation. Results of these annual investigations, which were done in spring as well as in autumn, were presented during the International Symposium in Titograd 1980. These examinations involved hematological investigations as performed by Halsband (1979) on German rivers.

The increasing of pollution in oceans and rivers caused by the growing industries without sufficient purification installations make investigations on the health state of fishes living in polluted waters necessary.

MATERIAL AND METHODS

The investigations specified below were conducted on four fish species from Skadar-Lake:

1. Leuciscus cephalus albus
2. Anguilla anguilla
3. Carassius auratus
4. Cyprinus carpio

In order to obtain plasma blood was taken from the heart directly (bulbus arteriosus) with heparinisated syringes. The following parameters have been measured:

1. Quantity of erythrocytes (Mio/mm^3)
2. Content of hemoglobin (g/100 ml)
3. Hematocrit in %
4. Cholesterin (mmol/l)
5. Total protein concentration (g/l)
6. Glucose concentration (mmol/l).

Tests were conducted using an equipment of Compur Electronic GmbH, which allowed fast and exact measurements. All given tests were supplied with cuvettes containing the reagents and the required concentrations.

Test principles specified by Compur:

1. Erythrocytes: By adding Gowers' solution to blood the erythrocytes become spherical and are analysed photometrically by measuring the turbidity. Because of the factelvet the size of erythrocyte in fish is different from human one, it is required to divide the read-out by a factor.

2. Hemoglobin: Hemoglobin is oxidized by potassium hexocyanoferrate (III) to form methemoglobin. This in turn reacts with cyanide to produce cyanmethemoglobin which is particularly stable. The absorbance of the cyanmethemoglobin is directly proportional to the hemoglobin concentration and is measured at 540 nm. The read-out gives the direct value expressed as the concentration in g/l.

3. Hematocrit: Hematocrit has been determined using a centrifuge. The values can be read out the centrifuge-disc directly.

4. Cholesterin: Cholesterin and its esters are released from lipoproteins by the action of detergents. Cholesterin esterase hydrolyses the esters. H_2O_2 is formed during the subsequent enzymatic oxidation by means of cholesterin oxidase. The resultant hydrogen peroxidase reacts with 4-aminophenazone and phenol under the catalytic effect of the peroxidase to yield a red colour complex.

5. Total protein: Protein forms a blue-violet colour complex with copper ions in a basic solution. The intensity of this colour complex is proportional to the protein concentration.

6. Glucose: Glucose oxidase catalyses the oxidation of glucose. The hydrogen peroxide produced during this reaction react with 4-aminophenazone and phenol under the catalytic effect of the peroxidase to form a red colour complex.

External changes at skin, gills and vertebral column were investigated. The condition of internal organs like liver, kidney and stomach were checked.

RESULTS AND DISCUSSION

The quantity of erythrocytes, hemoglobin concentration and hematocrit characterize the condition and nature of a single erythrocyte:

1. MCV: Mean corpuscular volume in μ^3 =

$$\frac{\text{hematocrit} \times 10}{\text{number of erythrocytes}}$$

2. MCH: Mean corpuscular hemoglobin in $\gamma\gamma$ =

$$\frac{\text{hemoglobin concentration} \times 10}{\text{number of erythrocytes}}$$

3. MCHC: Mean corpuscular hemoglobin concentration in $g^0/_0/100$ ml =

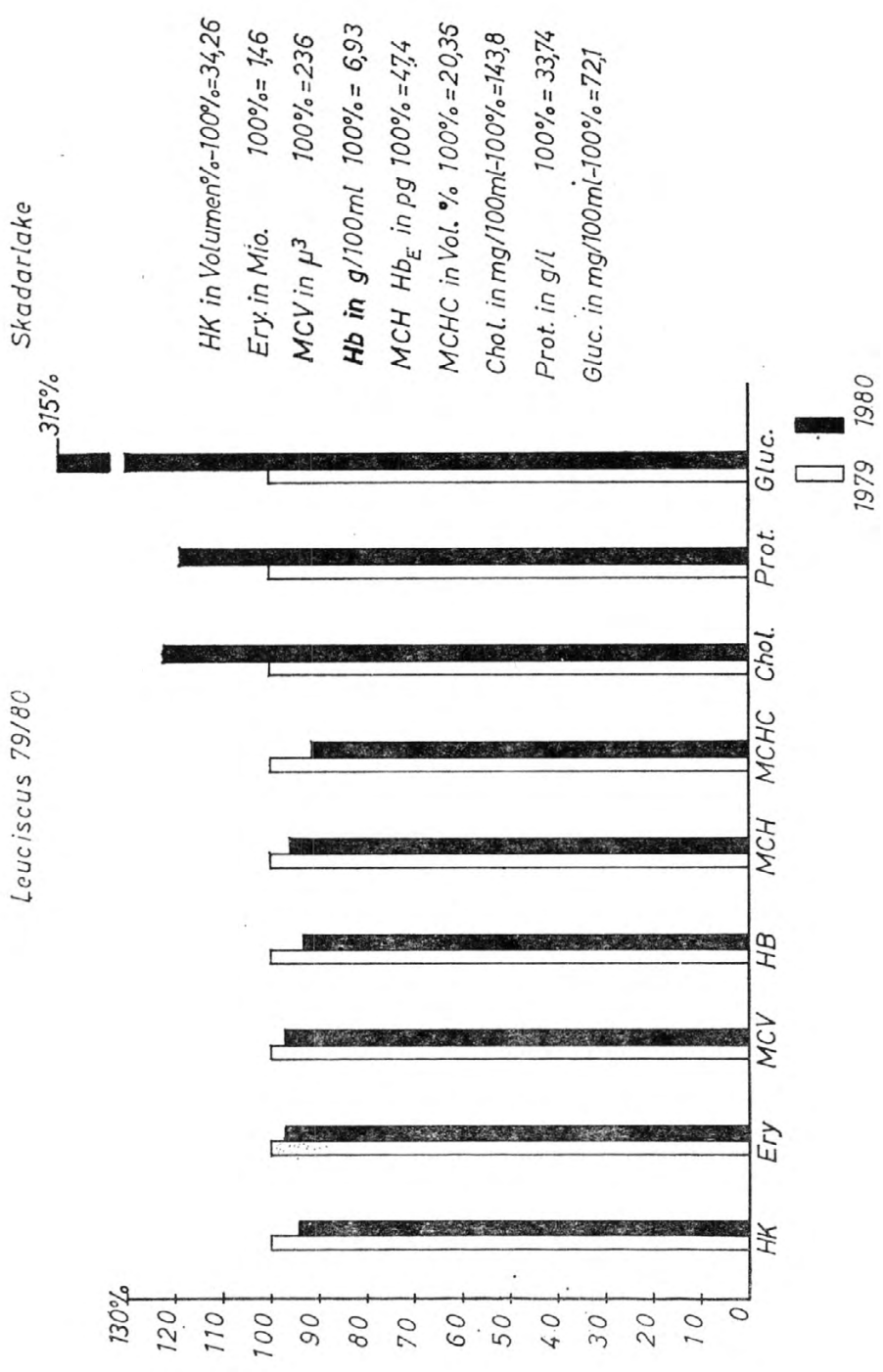
$$\frac{\text{hemoglobin concentration} \times 100}{\text{hematocrit.}}$$

The pictures 1 to 4 point out the mean values of the single measurements as conducted in 1979 (August-investigations) and in 1980 (July-investigations).

Leuciscus cephalus albus (Fig. 1):

Small fluctuations of 5—8 $^0/_0$ within the blood values are natural and represent no great variations. Very significant are the enormous high values of glucose, which indicate the intensive disturbance of the metabolism.

The carbohydrates are the most important foundations of the cells to produce energy. Normally the carbohydrates are oxidized to CO_2 and H_2O. The cells also have the capability to decamp glucose anaerob to lactic acid, a process, which one calls glycolysis. The erythrocytes produce the necessary energy only by an anaerob decomposition of carbohydrates. An important function of the liver is the storage of glycogen concerning of the maintaining the blood sugar constant. The decomposition of sugar in the liver only serves for the own need of energy of this organ. Henceforth the process of modifying are located in the liver, which lead from the glucose to the other important substances. The muscle cell does not possess any phosphatases for the splitting of glucose-6-phosphates. A glucose-molecule esterified by a muscle cell is not able to give this cell outward again, this is an arrangement of security, to preserve the muscle against an early state of exhaustion of the reserves of carbohydrates. The glycogen of the liver and of the muscle can only


Leuciscus 79/80
Skadarlake
315%
HK in Volumen%-100%=34,26
Ery. in Mio. 100%= 1,46
MCV in µ³ 100%=236
Hb in g/100ml 100%= 6,93
MCH Hb_E in pg 100%=4,74
MCHC in Vol. % 100%=20,35
Chol. in mg/100ml-100%=143,8
Prot. in g/l 100%= 33,74
Gluc. in mg/100ml-100%=72,1
130%
120
110
100
90
80
70
60
50
40
30
20
10
0
HK
Ery
MCV
HB
MCH
MCHC
Chol.
Prot.
Gluc.
1979
1980

merge into each other over a larger reaction chain. A dislocation of glycogen from the muscle to the liver, as it can be observed by an extra giving of adrenaline, presumes a preceding decomposition of the muscle-glycogen into lactic acid, which is delivered to the blood and changed into glycogen again by the liver.

The increased values of cholesterin and glucose might be produced by toxical damages of the liver as f.i. in the frequent happening of yellow liver dystrophy, which is connected with a »hepato-renales Syndrom«, which signifies an increasing of cholesterin. By liverdamages we see usually an increasing of the content of protein, as it also can be registered at Leuciscus. The total protein content is significantly increased at a »lupoid« hepatitis (hepatitis with granulation tumors).

Anguilla anguilla (Fig. 2):

We can see the fluctuations which are natural on the grounds of the specifically given food.

Carassius auratus (Fig. 3):

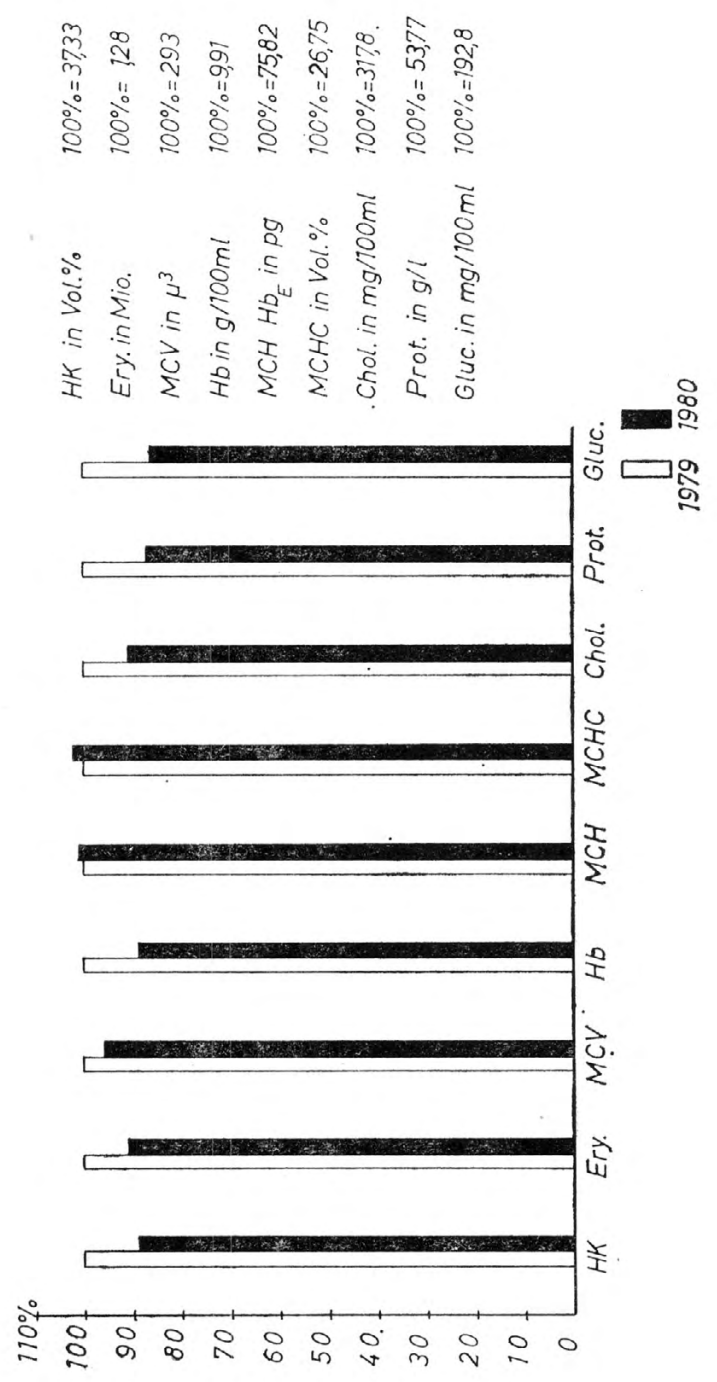
Excepted of the level of glucose all values are remained approximately the sames, from which one can conclude that here are no diseases, but an effect of shocking by the capture.

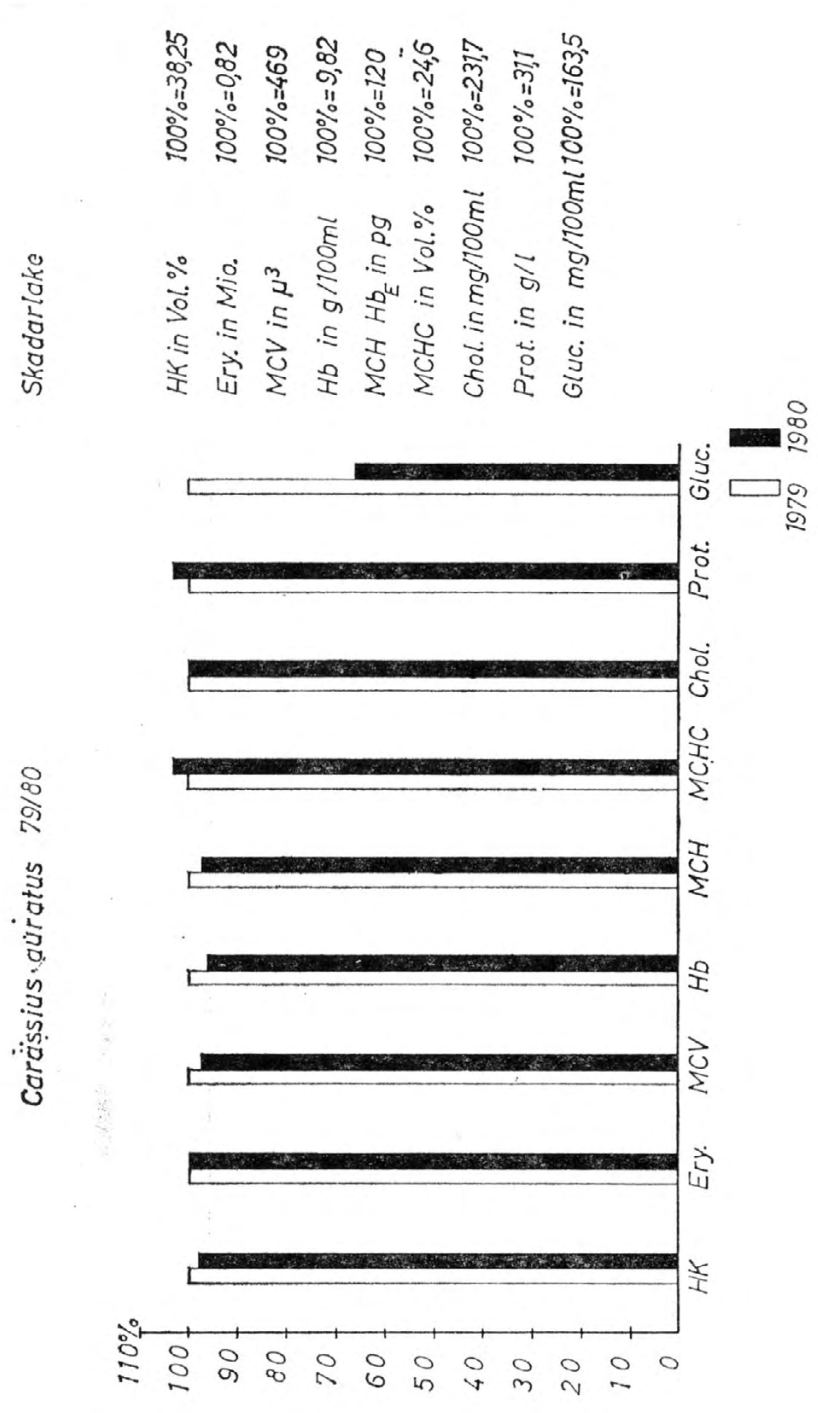
Cyprinus carpio (Fig. 4):

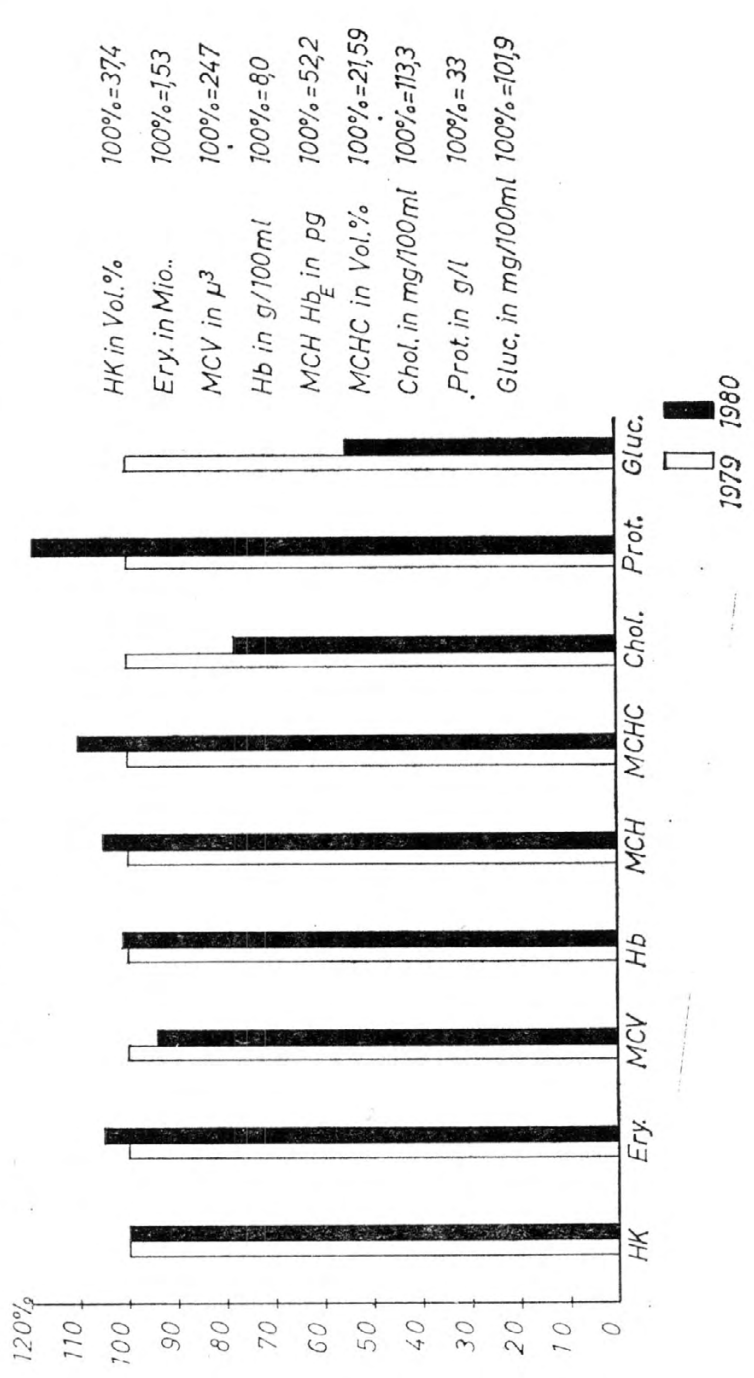
Also here the dates of hematocrit, erythrocytes, MCV, hemoglobin, MCH and MCHC are approximately remained the sames, all fluctuations are under 10 %.

The cholesterin is however diminished about 20 %. From the decomposition of the fat the metabolism of the cholesterin is dependent. The synthesis of the cholesterinesters takes place in the liver. It can be a sign for decontamination, as it can be observed as »ester-fall« at persons, whose liver is sick. At certain disease of the liver parenchym the synthesis of glycogen is disturbed. In the liver also the deamination of the amino acids to carbamide (urea) takes place. The liver is also responsible for the production of albumins and globulins, therefore at a disfunction of the liver significant changes in the fractionation of the proteins of the serum can be measured, especially a decrease of the albumins and an increase of the »gammaglobulins«. Intensive investigations at the liver of Cyprinus have to demonstrate to what extend hypoproteinanaemia exists.

The space of two years seems to be too short for final declarations about changes. Also seasonal changes of metabolism are to take into consideration. It is remarkable that we did not find essential changes in number of erythrocytes, hemoglobin concentration and hematocrit although cholesterin, total protein and glucose concentration were changed. To point out the deviated liver values


Anguilla 79/80
Skadarlake
HK in Vol.% 100%=37,33
Ery. in Mio. 100%= 1,28
MCV in µ³ 100%=293
Hb in g/100ml 100%=9,91
MCH Hb_E in pg 100%=75,82
MCHC in Vol.% 100%=26,75
Chol. in mg/100ml 100%=317,8
Prot. in g/l 100%= 5377
Gluc. in mg/100ml 100%=192,8
110%
100
90
80
70
60
50
40
30
20
10
0
HK
Ery.
MCV
Hb
MCH
MCHC
Chol.
Prot.
Gluc.
1979
1980


Carassius auratus 79/80
Skadarlake
HK in Vol.% 100%=38,25
Ery. in Mio. 100%=0,82
MCV in µ³ 100%=469
Hb in g/100ml 100%=9,82
MCH Hb_E in pg 100%=120
MCHC in Vol.% 100%=24,6
Chol. in mg/100ml 100%=231,7
Prot. in g/l 100%=3,1
Gluc. in mg/100ml 100%=163,5
110%
100
90
80
70
60
50
40
30
20
10
0
HK
Ery.
MCV
Hb
MCH
MCHC
Chol.
Prot.
Gluc.
1979
1980


Skadarlake
Cyprinus carpio L. 79/80
HK in Vol.% 100%=37,4
Ery. in Mio.. 100%=1,53
MCV in µ³ 100%=247
Hb in g/100ml 100%=8,0
MCH Hb_E in pg 100%=52,2
MCHC in Vol.% 100%=21,59
Chol. in mg/100ml 100%=113,3
Prot. in g/l 100%= 33
Gluc. in mg/100ml 100%=101,9
120%
110
100
90
80
70
60
50
40
30
20
10
0
HK
Ery.
MCV
Hb
MCH
MCHC
Chol.
Prot.
Gluc.
1979
1980

and to characterize the action of pollutants on bone system more exactly, it seems to be useful that more investigations should be made to measure the sodium, potassium and calcium in plasma and organs on the one side and determinations of the activity of alkaline phosphatase on the other side.

SUMMARY

In this paper results of blood analysis are presented. The investigations are performed at Skadar-Lake (Yugoslavia) in 1979 and 1980 with following species:

1. Leuciscus cephalus albus
2. Anguilla anguilla
3. Carassius auratus
4. Cyprinus carpio.

Blood is taken from bulbus arteriosus and is checked employing a new method with an equipment of Compur Electronic GmbH. The parameters being measured are:

1. number of erythrocytes
2. hemoglobin concentration
3. hematocrit
4. total protein concentration
5. glucose concentration.

From number of erythrocytes, hemoglobin concentration and hematocrit are obtained by calculation:

1. MCV (mean corpuscular volume)
2. MCH (mean corpuscular hemoglobin)
3. MCHC (mean corpuscular hemoglobin concentration)

Results are shown graphically and are discussed. While hematocrit, number of erythrocytes and hemoglobin concentration are not essential changed within two years, we found significant variations in cholesterin, total protein and glucose concentrations. These values are increased in L. cephalus albus and decreased in A. anguilla. C. auratus shows a very low glucose concentration. C. carpio has an increased total protein concentration although cholesterin and glucose concentrations were decreased. To point out these changes in metabolism further studies are proposed.

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PROUČAVANJE ZDRAVSTVENOG STANJA RIBA POMOĆU NOVOG METODA ZA HEMATOLOŠKO ISTRAŽIVANJE

Rezime

Ova ispitivanja su vršena radi istraživanja sadržaja krvi kod različitih vrsta riba Skadarskog jezera u Jugoslaviji i rijeke Weser u Njemačkoj.

Dokazano je da se krvni parametri mogu uzeti kao indikatori da li su ribe imale dobro zdravstveno stanje ili ne, pošto krv ima direktan uticaj na sve organe.

Krv je uzimana iz bulbus arteriosusa riba i pojedine vrijednosti su mjerene veoma modernim metodom »Compur«.

Prednost ovog novog metoda je u tome što je potrebno veoma malo vremena za dobijanje krvnih parametara na tačan i brz način.

Istraživane su sljedeće vrijednosti:

1. Vrijednost hematokrita u vol %
2. Sadržaj hemoglobina u g Hb/100 ml = g%
3. Broj eritrocita u mio/mm³
4. Prosječan volumen pojedinog eritrocita μ u³ (MCV = srednji korpuskularni volumen)
5. Apsolutni sadržaj hemoglobina pojedinog eritrocita u g% za 100 ml (MCH = srednji korpuskularni hemoglobin)
6. Prosječna koncentracija pojedinog eritrocita u g% za 100 ml (MCHC = srednja korpuskularna koncentracija hemoglobina)
7. Vrijednosti glukoze u plazmi u m mol/1
8. Vrijednosti proteina u plazmi u g/1
9. Vrijednosti holesterina u m mol/1 u plazmi

Vršili smo ispitivanja na sljedećim ribama:

U Skadarskom jezeru: Anguilla anguilla
Leuciscus cephalus albus
Cyprinus carpio
Carassius auratus
Scardinius erythrophthalmus

U rijeci Weser:

Anguilla anguilla
Salmo gairdneri
Leuciscus cephalus
Squalius cephalus
Abramus brama
Acerina cernua.

Takođe smo ispitivali stanje spoljašnjih i unutrašnjih organa kao što su jetra, bubreg, kičma, koža, stomak i škrge da bi eventualno utvrdili postojanje abscesa, ulcera ili parazita.

Određene su srednje vrijednosti i pojedinačni načini ispitivanja su detaljno opisani.

Možemo upoređivati vrijednosti različitih vrsta riba na osnovu rezultata, a i simptomi bolesti mogu biti registrovani.