ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XIV Fasc. 1-2 Szczecin 1984

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Hematology

EFFECT OF PHYSIOLOGICAL FACTORS, STRESS, AND DISEASE ON HEMATOLOGICAL PARAMETERS OF CARP, WITH A PARTICULAR REFERENCE TO LEUKOCYTE PATTERN. II. HEMATOLOGICAL RESULTS OF STRESS IN CARP

WPŁYW CZYNNIKÓW FIZJOLOGICZNYCH, STRESSOWYCH I CHOROBOWYCH NA PARAMETRY HEMATOLOGICZNE KARPIA ZE SZCZEGÓLNYM UWZGLĘDNIENIEM OBRAZU BIAŁOKRWINKOWEGO. II. HEMATOLOGICZNY EFEKT STRESSU U KARPIA

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Effects of three stress-producing factors: capture, transportation, and starving on hematological parameters of carp blood were studied.

Oxygen deficiency and crowding of fishes occurring on capture and transportation as well as a 21-week starving were found to considerably affect the hematological parameters examined, the effect being confirmed by statistical tests.

INTRODUCTION

Adverse environmental conditions, mechanical damage, or a wrong diet affect markedly the fish health. Those factors are particularly operative in fish cultures as, over

the production cycle in ponds, the fishes are being several times captured and transported. Moreover, the keeping of carp in storage ponds prior to marketing and in wintering reservoirs is associated with a long term food deficiency. All those factors act as stressors for fish.

Effects of stress on the teleost physiology were studied by numerous authors, some of them (Klontz and Smith, 1968) pointing out to a remarkable sensitivity of a fish organism to variations in environmental parameters. The following external stressors were dealt with: oxygen depletion (Benditt et al., 1941; Holeton and Randall, 1967; Eddy and Morgan, 1969; Soivio et al., 1973; 1974; 1976); temperature (Houston, 1962; Wedemeyer, 1973); transportation (Black and Berret, 1957; Fraser and Beamish, 1969); fish handling in laboratory (Houston et al., 1971a, 1971b). The above papers concerned salmonids and the changes followed not always included those in hematological parameters. The literature review shows additionally that a few papers only have so far dealt with the carp blood.

The objective of supplementing those data and providing new ones was to gain an insight into effects of three stressors: capture, transportation, and starving on carp hematological parameters.

MATERIALS AND METHODS

Effect of capture on hematological parameters was studied on 40 individuals of C_1 carp and 40 individuals of C_2 carp. The blood was collected from the fish while in pond (water temperature 6–8°C; oxygen content) 7–8 mg/dm^3) just before the onset of autumnal capture. After removal of water from the pond the fish gathered in a so-called fishing ground, i.e., in the lowest part of the pond situated by the water outflow. The entire stock remained in that place for several hours in a water depleted of oxygen (water temperature 6–9°C; oxygen content 3–4 mg/dm^3).

The effect of stress was assessed from comparing data obtained from the fish in pond before capture with those collected from the fish remaining in the pond after removal of water.

To study effects of transportation, the blood was collected directly after capture and – from the same individuals – after transportation. The fish were transported in 20 l containers, 40 C_1 carp or 10 C_2 carp each.

Each time before and after transportation the blood was collected from 20 individuals of C_1 and 20 individuals of C_2 carp. The transportation in a van took usually 2 hours (the distance of about 80 km) and was effected in June, July, and August. The water temperature and oxygen content as measured in the pond before transportation were 24–26°C and 5–6 mg/dm^3, respectively.

Effects of transportation were assessed from hematological data obtained prior to and after transportation.

Effects of starving on hematological parameters were studied on C_1 carp. The

experimental group and control contained 80 individuals each. They were kept in 100 l constantly aerated flow-through aquaria holding 20 individuals each. The water temperature in experimental and control aquaria was 7–10°C. The experimental group was deprived of food for 21 weeks, while the control fish were fed barley oilmeal twice a week.

The blood both from the experimental and control fish was taken 8 times at 1-week intervals, from the 14th week fo starving onwards.

The blood parameters examined were: erythrocyte and leukocyte counts, hematocrit, hemoglobin content, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC). Additionally, the leukogram (leukocyte pattern) was determined and morphology of erythrocytes and leukocytes observed. Leukocytes were identified as in Ivanova (1970).

The numerical results were treated statistically. Two-way analysis of variance (Elandt, 1964) was employed to compare results on effects of starving on hematological parameters; significance of the remaining effects was assessed by Student's t.

RESULTS

A. Capture

In many cases, the comparison of hematological parameters in C_1 and C_2 carp before capture and after a few hours at the „fishing ground" (high concentration of fish) reveals differences between the values obtained (Table 1).

Hematocrit in C_1 and C_2 increased very highly significantly ($p < 0.001$).

Increasing values were recorded in the leukocyte count, neutrophil percentage in C_1 and C_2, and basophil percentage in C_2.

The erythrocyte count in C_1 increased as well, and remained unchanged in C_2. The eosinophil and lymphocyte percentages increased. No significant differences were found in C_1 and C_2 hemoglobin contents, C_1 basophil percentage, and monocyte percentage in C_1 and C_2.

B. Transportation

The values of hematological parameters shown by C_1 and C_2 carp before and after transportation (Table 2) differed in most cases. The erythrocyte count, hematocrit, and hemoglobin content increased significantly in C_1 and C_2. The leukocyte count and neutrophil percentage increased markedly. The eosinophil and lymphocyte percentages decreased significantly. Differences in the remaining parameters (basophils and monocytes) are less significant ($p < 0.01$ or $p < 0.05$).

Table 1

Effect of capture on hematologic indices of C_1 and C_2 carp blood

Hematologic indices	Carp age group	Before capture		During capture		p
		$\bar{x}$	SD	$\bar{x}$	SD	
Erythrocyte count ($x10^6/mm^3$)	C_1	1.32	0.13	1.63	0.16	+
	C_2	1.61	0.15	1.59	0.15	−−
Hematocrit (%)	C_1	27.15	2.52	44.50	4.01	+++
	C_2	35.80	4.48	49.80	5.16	+++
Hemoglobin (g%)	C_1	5.71	1.26	6.13	1.05	−
	C_2	7.83	1.19	8.01	1.35	−
MCV (μm^3)	C_1	202.65	24.31	271.40	34.42	+++
	C_2	220.36	23.46	311.20	29.66	+++
MCH (pg)	C_1	43.25	7.29	43.70	6.91	−
	C_2	48.50	6.91	51.58	5.29	−
MCHC (%)	C_1	20.53	2.27	16.50	2.27	++
	C_2	21.64	3.58	16.74	4.08	++
Leukocyte count ($x10^3/mm^3$)	C_1	33.75	7.85	64.50	7.35	+++
	C_2	45.01	7.56	82.16	7.94	+++
Neutrophils (%)	C_1	8.70	1.89	27.30	3.84	+++
	C_2	7.95	2.26	24.70	4.31	+++
Eosinophils (%)	C_1	2.05	1,28	1.35	0.81	+
	C_2	0.70	0.80	0.00	−	++
Basophils (%)	C_1	0.70	0.86	0.85	0.99	−
	C_2	0.85	0.81	3.86	0.69	+++
Monocytes (%)	C_1	6.35	2.35	5.90	2.25	−
	C_2	3.20	1.40	3.95	1.43	−
Lymphocytes (%)	C_1	73.75	3.61	62.60	4.65	+++
	C_2	82.60	3.13	66.80	4.70	+++

C_1 = carp fry (mean values for 20 individuals)

C_2 = juvenile carp (mean values for 20 individuals) for the remaining explanations see Table 2.

Table 2

Effects of transportation on hematologic indices of C_1 and C_2 carp blood

Hematologic indices	Carp age group	Before transportation		After transportation		p
		$\bar{x}$	SD	$\bar{x}$	SD	
Erythrocyte count ($x10^6$/mm)	K_1	1.17	0.19	1.35	0.14	+++
	K_2	1.43	0.14	1.53	0.27	+
Hematocrit (%)	K_1	26.05	6.14	34.05	6.14	+++
	K_2	30.40	5.00	38.95	4.43	+++
Hemoglobin (g%)	K_1	5.80	1.26	7.53	1.39	++
	K_2	6.28	1.25	8.49	1.36	+++
Leukocyte ($x10^3$/mm)	K_1	37.64	5.89	54.00	8.26	+++
	K_2	40.51	10.65	72.16	7.48	+++
Neutrophils (%)	K_1	4.25	1.16	28.90	1.41	+++
	K_2	8.85	2.46	23.05	2.58	+++
Eosinophils (%)	K_1	1.55	0.60	0.00	0.30	+++
	K_2	1.10	0.91	0.25		
Basophils (%)	K_1	1.75	0.72	1.25	1.12	–
	K_2	0.80	0.41	3.50	0.51	++
Monocytes (%)	K_1	7.15	1.04	8.25	0.85	+
	K_2	8.85	2.08	6.05	1.19	+
Lymphocytes (%)	K_1	79.20	1.63	57.15	3.03	+++
	K_2	75.60	2.74	62.85	4.32	+++

C_1 = carp fry (mean values for 60 individuals)
C_2 = carp juveniles (mean values for 60 individuals)
$\bar{x}$ = arithmetic mean
SD = standard deviation
p = statistical significance of differences
+++ = $p < 0.001$
++ = $p < 0.01$
+ = $p < 0.05$
– = non-significant

Table 3

Effects of period of starving on leukocyte pattern of C_1 carp blood

Period of starving (wk)	Blood sampling date (day, month)	Rod neutrophils (%)				Segmented neutrophils (%)				Neutrophils (%)				Lymphocytes (%)			
		C		E		C		E		C		E		C		E	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
14	11.01	1.80	0.97	5.25	0.80	1.60	0.66	4.00	0.77	3.00	1.35	10.50	1.28	79.40	2.61	62.20	1.00
15	18.01	1.90	0.22	4.00	0.64	1.90	1.04	5.30	0.64	3.20	1.48	11.30	1.10	81.70	4.90	68.40	1.91
16	25.01	1.75	0.26	7.35	0.78	0.90	0.50	5.20	0.54	2.20	0.87	14.00	1.79	82.00	1.84	62.00	2.05
17	02.02	1.80	0.27	6.25	0.90	0.70	0.66	6.60	0.66	3.20	2.22	15.20	1.04	78.00	4.90	63.60	1.74
18	09.02	1.20	0.18	3.75	1.62	0.90	0.70	4.80	0.89	3.30	1.00	14.40	2.69	81.80	3.10	67.80	5.98
19	16.02	1.50	0.29	4.00	1.61	1.40	1.02	6.60	0.48	5.90	0.83	13.00	2.07	82.50	3.55	65.10	4.02
20	23.02	1.50	0.29	5.30	1.00	1.40	0.66	5.10	1.34	5.90	1.70	14.65	1.84	80.80	4.33	63.80	2.72
21	02.03	2.50	0.38	6.75	0.87	2.10	0.70	7.40	1.04	6.10	1.81	17.45	1.26	80.31	5.70	68.40	2.40
Mean		1.74	–	5.33	–	1.36	–	5.62	–	4.10	–	13.81	–	80.81	=	63.91	–
p		$p_a < 0.001$ $p_b > 0.1$ $p_{ab} < 0.001$				$p_a < 0.001$ $p_b > 0.2$ $p_{ab} < 0.001$				$p_a < 0.001$ $p_b > 0.2$ $p_{ab} < 0.01$				$p_a < 0.001$ $p_b > 0.1$ $p_{ab} < 0.001$			

C = control fish
E = experimental fish
$\bar{x}$ = arithmetic mean
SD = standard deviation

p = significance level
p_a = significance level for differences between control and experimental fish

p_b = significance level for effects of period starving
p_{ab} = significance level of interaction

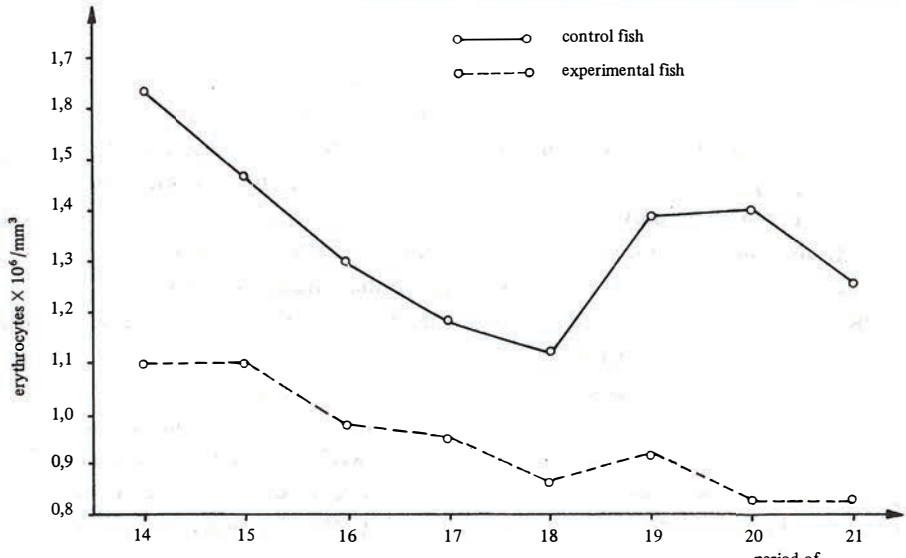


Fig. 1. Effect of period of starving on erythrocyte count in C_1 carp bllod

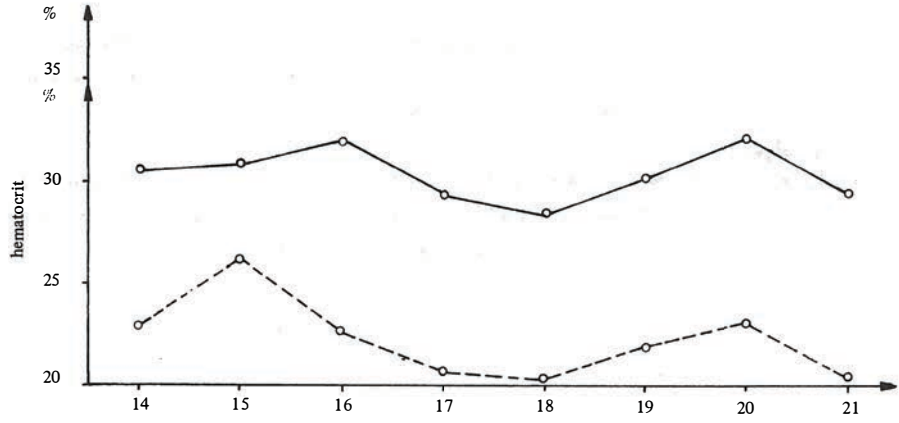


Fig. 2. Effect of period of starving on hematocrit in C_1 carp blood

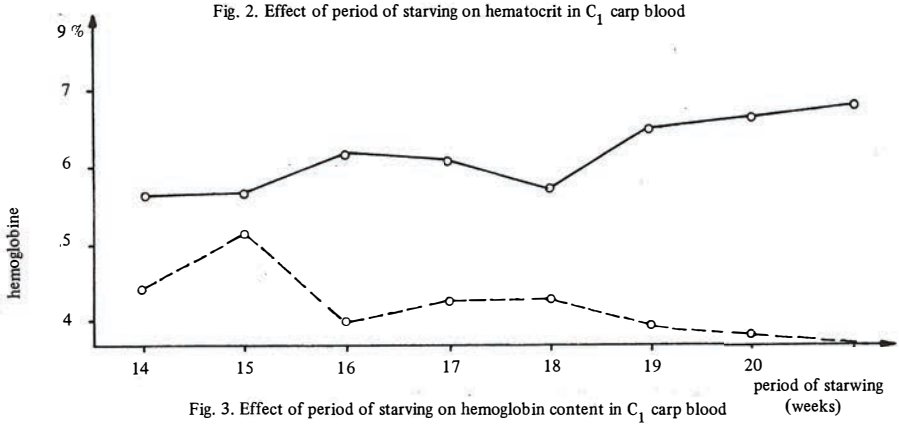


Fig. 3. Effect of period of starving on hemoglobin content in C_1 carp blood

C. Starving

Changes in blood composition versus the length of period of starving are presented in Table 3 and Figs . 1, 2, 3, 4, 5, and 6. Starving was found to have affected very highly significantly ($p < 0.001$) the erythrocyte count, hemoglobin content, and leukocyte count (Figs. 1, 3). The starved fish had lower leukocyte counts in each of the 8 periods of starving; also the difference in erythrocyte count between the starved and fed fish was very highly significant ($p_{ab} < 0.001$) as related to the duration of starving.

The effect of starving on hematocrit in the starved fish was somewhat less significant ($p_a < 0.01$). The hematocrit values for the starved and fed fish in function of the duration of starving are distributed randomly ($p_{ab} > 0.7$) (Fig. 2).

The mean erythrocyte volume was higher in the starved fish both in the initial and final phases of starving, while in the 3rd and 4th weeks the control individuals showed higher MCV's. The control-experimental differences are very highly significantly ($p_{ab} < 0.001$) related to the duration of starving. On the other hand, the effect of starving on MCH (mean hemoglobin content in an erythrocyte) was non-significant ($p_a > 0.6$).

Starving affected the mean hemoglobin percentage in an erythrocyte (MCHC) in a very highly significant way ($p_a < 0.001$). The difference with respect to that parameter between the starved and fed fish relative to the duration of starving are distributed randomly ($p_{ab} < 0.2$).

In all the eight phases of starving, the percentage of myelocytes, metamyelocytes, and particularly rod and segmented neutrophils were higher than in controls; starving affected those parameters in a very highly significant way ($p_a < 0.001$).

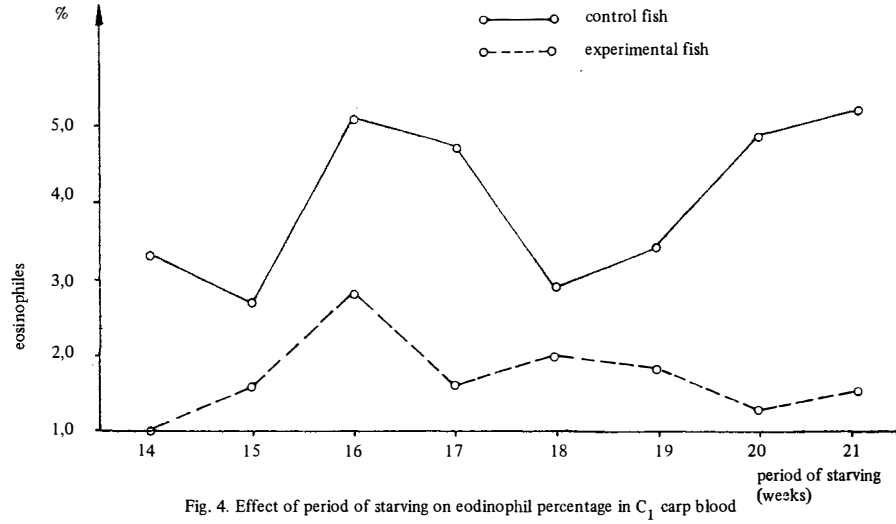


Fig. 4. Effect of period of starving on eodinophil percentage in C_1 carp blood

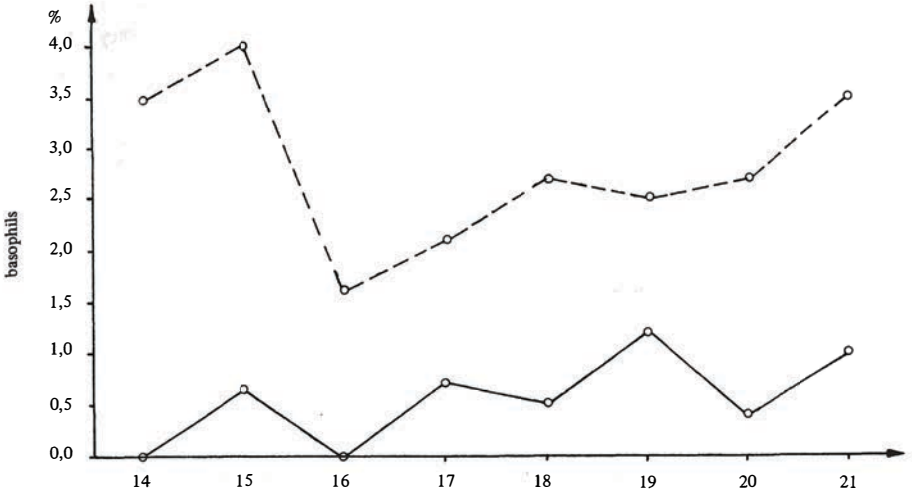


Fig. 5. Effect of period of starving on basophil percentage in C_1 carp blood

A very highly significant ($p_a < 0.001$) effect of starving on the neutrophil percentage decrease was recorded as well (Table 3).

The eosinophil percentage in the starved fish was markedly lower than that of the control. Statistical significance was revealed for the difference between eosinophil percentages of the starved and fed fish in function of the duration of starving (Fig. 4).

The basophil percentage was very highly significantly ($p < 0.001$) higher in the experimental fish (Fig. 5).

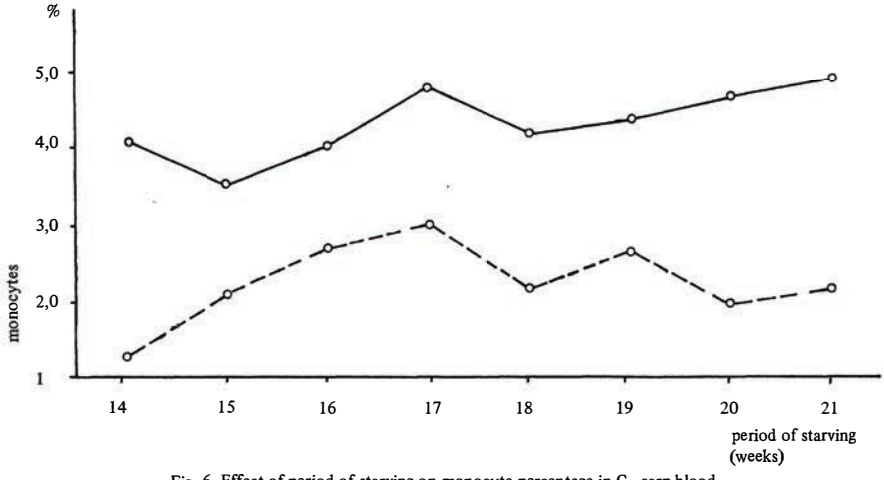


Fig. 6. Effect of period of starving on monocyte percentage in C_1 carp blood

The monocyte and lymphocyte percentages were much lower in the starved fish than in the control. The agranulocyte percentage in the starved fish was very highly significantly different from that of the fed group as related to the duration of starving ($p_{ab} < 0.001$) (Table 3, Fig. 6).

MORPHOLOGY OF PERIPHERAL BLOOD LEUKOCYTES

I. Leukocyte types and their description, following the classificatition of Ivanova (1970)

Hemocytoblast is a maternal cell for all blood cells; it is large, contains a large, spherical nucleus filling almost the entire cell. The nucleus stains red-purple and is surrounded by a well-marked basophil cytoplasm.

Myeloblast is the youngest granulocyte. A very large cell with weakly basophil cytoplasm surrounding a delicately reticulated nucleus containing nucleoli (Plate 1A).

Promyelocyte is a progeny of the myeloblast; a large oval cell with an excentric red-purple nucleus containing nucleoli. Cytoplasm contains poorly visible neutrophil granulations (Plate 1B).

Granulocytes:

Neutrophils contain fine, almost colourless granulations in their cytoplasm; 4 types of neutrophils are known:

- myelocytes: contain large circular red-purple nuclei located excentrically; the nuclear chromatin is condensed;
- metamyelocytes with oval or slightly elongated nuclei; excentric, with a clearly condensed chromatin;
- rod neutrophils with elongated, often kidney-shaped nuclei with a slight indentation;
- segmented neutrophils with nuclei divided into 2 parts (Plate 1C).

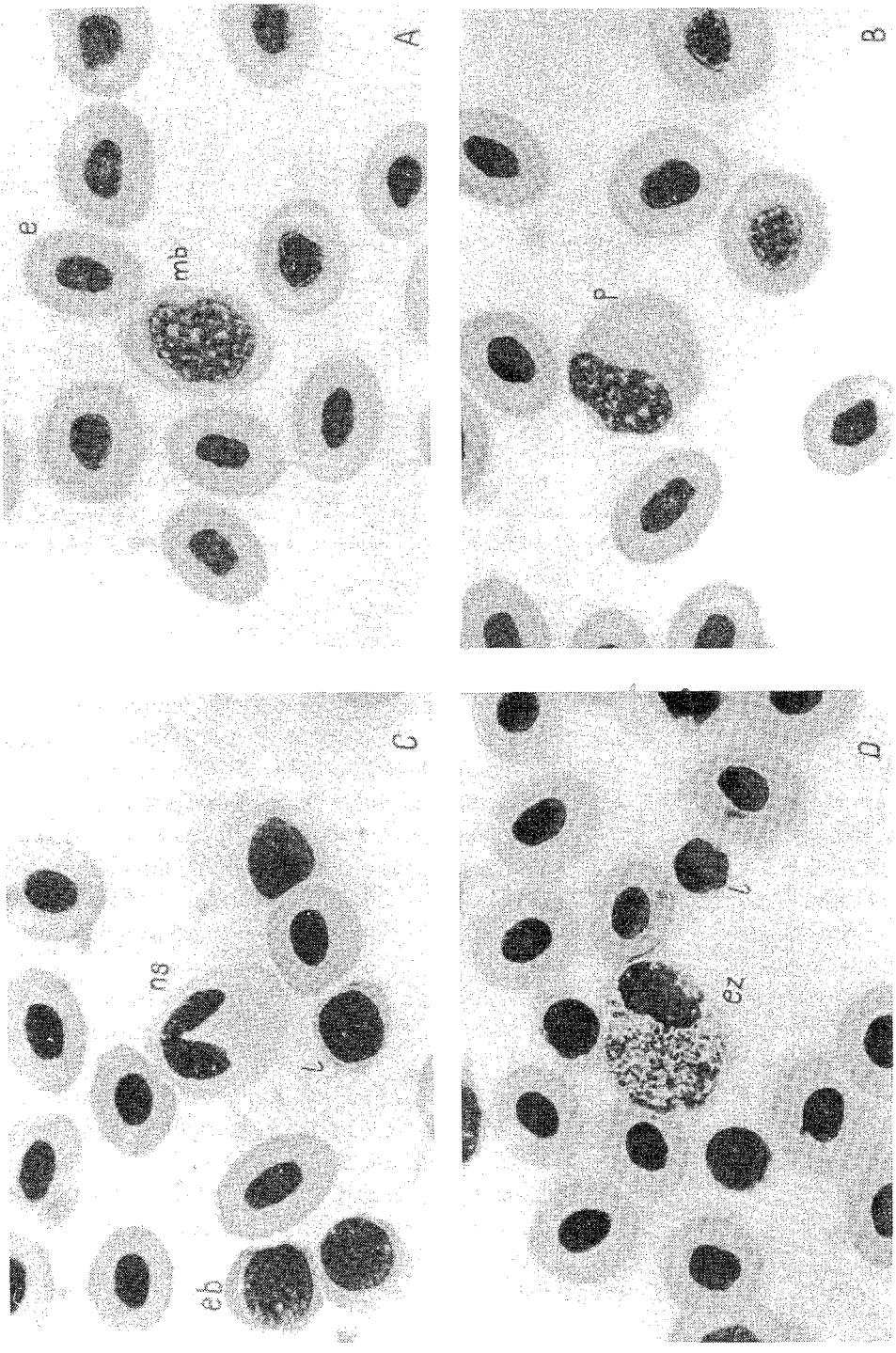
Eosinophils are circular large cells with compact nuclei, oval or circular; cytoplasm filled with densely packed eosinophil granulations (plate 1D).

Basophils have circular or oval nuclei, red-purple; cytoplasm contains a few large red granulations (Plate 1E).

Agranulocytes:

Monocytes – large, elongated or kidney-shaped nucleus with a characteristic chromatin pattern. The nucleus stains red-purple and is located excentrically. The cytoplasm is homogenous, grey-blue.

Lymphocytes – cells of various shapes: circular, oval, or elongated. Clearly basophil cytoplasm as a narrow ring surrounds the dense red-purple nucleus. The cytoplasm may be poorly discernible, in which case the nucleus seems „naked" (Plate 1C, 1D).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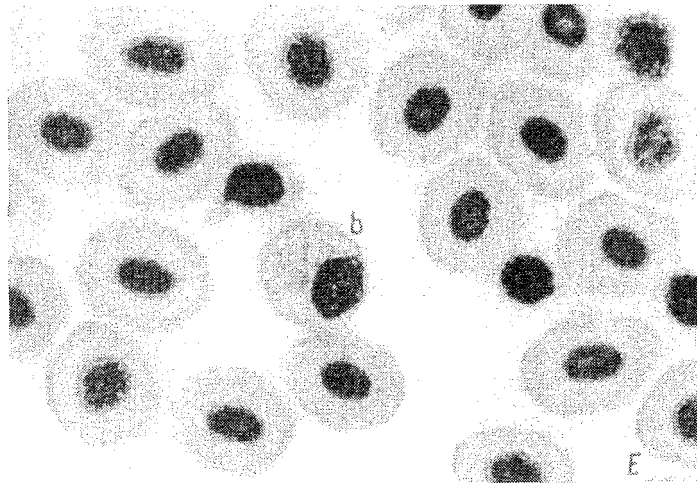


Plate 1. Morphotic elements of carp peripheral blood (1800 x) A. e = erytrocyte; mb = myeloblast B. eb = erythroblast; ns = segmented neutrophil; L = lymphocyte D. ez = eosinophil E. b = basophil

II. Changes in the peripheral blood cells in the starved fish

A long-term starvation markedly affects the morphology of carp blood cells. Changes in erythrocytes, typical of anemia, were observed: cells of various sizes (anisocytosis) and polychromasia (Plate 2). Degenerative changes in leukocytes were at their strongest in the 14th week of starving and persisted until the termination of the experiment.

Neutrophils showed the vacuolar degeneration of cytoplasm and nucleus. The latter lost its chromatin pattern and showed irregular lobes. Intensified degenerative changes are indicative of an irreversible process (Plate 2A).

Eosinophils showed a partial cell lysis, markedly enlarged nuclei, and vacuolar degeneration of cytoplasm (Plate 2B).

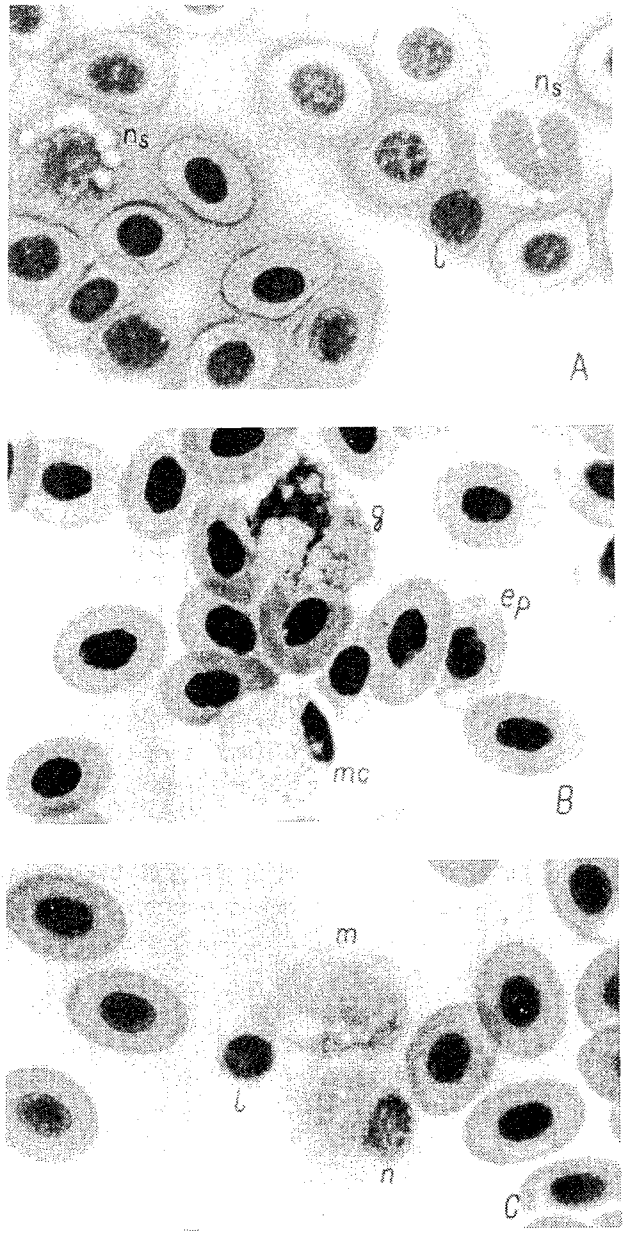
Basophils did not usually show any changes. Some cells were slightly enlarged; no signs of degeneration were observed in the cytoplasm. Occasionally unstained spots were visible as remains of lost granulations.

Monocytes showed obliterated cellular structure and numerous vacuoles in the cytoplasm; the nucleus was swollen and filled almost the entire cell space. Vacuolar degeneration was observed in the nuclear plasm (Plate 2C, 2D).

Lymphocytes occasionally increased slightly in size. In some of them the cytoplasm became invisible, thus indicating an increased amount of forms with „naked" nucleus.

No degenerative changes in the blood cells were observed in the control group.

No morphological changes in the blood cells examined were found as a result of capture and transportation.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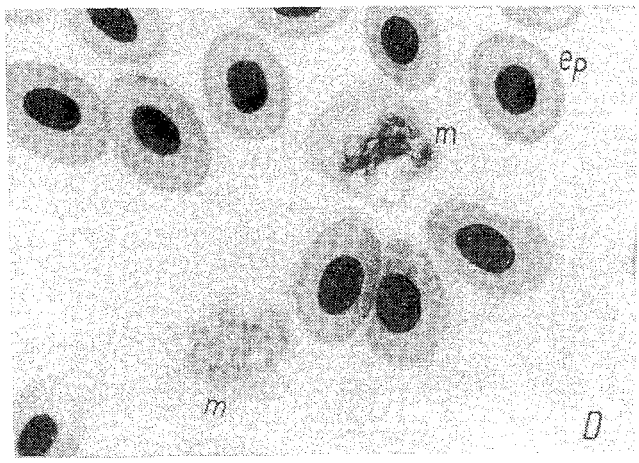

Plate 2. Degenerative changes in C_1 carp blood after 14 weeks of starving (1800X) A. ns = segmented neutrophil with vacuolar degeneration of cytoplasm; l = lymphocyte B. me = myelocyte; g = disintegration of degenerated granulocyte; ep = polychromatic erythrocyte C. m = monocyte affected by vacuolar degeneration; n = disintegrating neutrophil; l= lymphocyte with „naked" nucleus D. m = monocyte with vacuolar degeneration of cytoplasm; ep = polychromatic erythrocyte

DISCUSSION

A. Capture

A long-term oxygen deficiency resulting in fatigue and difficult breathing during capture affected some hematological parameters of the fish tested.

The present study showed a slight increase in the C_1 erythrocyte count and increased nautrophil character of these cells. The hematocrit increase found might have resulted either from an increased number or volume of blood cells (mainly erythrocytes). The results obtained indicate the hematocrit increase to result from increased volume of erythrocytes (MCV) (Table 1). This finding is in agreement with those reported by Benditt et all. (1941), Ferguson and Black (1941), Irving et al. (1941), Holeton and Randal (1967), Eddy and Morgan (1969). Soivio and Nyholm (1973), Soivio et al. (1974, 1976). Those authors are of the opinion that the blood cell swelling is the cause of an increased hematocrit in salmonids affected by oxygen deficiency. The swelling is associated with an increased CO_2 partial pressure occurring on hypoxia.

Hall et al. (1926) found increased hemoglobin level and erythrocyte count in marine fish subjected to asphyxia. The present study does not confirm this finding.

A fish response to a stressor is presumably similar to that exhibited by higher vertebrates. The conditions on capture acting as a stressor for fish presumably induce, like in mammals, an increased secretion of ACTH stimulating the adrenal cortex activity (Fryer, 1975). The result is the mobilisation of protective mechanisms of the body, the leukocyte system included. Increased leukocyte count and percentages of neutrophils and

basophils seem to be the result of an alarm reaction occurring during stress, which is confirmed by results obtained by Weinreb (1958) who observed changes in the rainbow trout blood following the application of ACTH.

The increased ACTH secretion during stress is instrumental in lymphocyto-, eosino-, and neutrophilopenia. By virtue of stimulating the adrenal cortex, the hormone causes an intensified cortisone secretion, which in turn affects the blood cell count via the ribonuclease-deoxyribonuclease complex. The deoxyribonuclease activation results in the destruction of lymphocytes and eosinocytes as DNA-rich cells (Aleksandrowicz, 1955).

Cortisone deactivates the ribonuclease complex. Granulocytes as the cells containing mostly RNA (Aleksandrowicz, 1955) are thus not subjected to destruction, which is why the leukogram shows an increased percentage of granulocytes, neutrophils in particular.

B. Transportation

Adverse conditions on transportation, mainly an increased concentration of fish and associated oxygen depletion (2.3–2.7 mg/dm^3) as well as a marked temperature increase (from 18°C to 24°C) were a plausible cause of the increased leukocyte count, hematocrit, and hemoglobin level in C_1 and C_2 carp. Such a reaction may have facilitated the oxygen deficiency compensation. Some authors (e.g., Puczkow, 1962) hold that oxygen deficiency triggers a reflectional spleen contraction and the resultant release of erythrocyte reserves into the blood stream.

The increase in leukocyte count and neutrophil percentage as well as the declined eosinophil and lymphocyte percentages in fish after transportation were presumably a typical response triggered by a strong stressor, the response being similar to that described above for the fish subjected to adverse conditions on capture.

C. Starving

The present study showed marked decreases in the erythrocyte count, hematocrit, and hemoglobin content in the C_1 carp subjected to long-term starving. The response is typical, under the circumstances, not only of fish but also of higher vertebrates. The phenomenon was observed in carp Pučkov Fedorova (1951). A 5-month starving, as shown by the present study, led to progressing anemia: the erythrocyte count, hematocrit, and hemoglobin content dropped down to $0.83 \times 10^3/mm^3$ blood, 20.3%, and 3.78 g%, respectively.

Observations on erythrocyte morphology confirm the occurrence of anemia as evidenced by anisocytosis and polychromasia. The changes point out to an increased demand for erythrocytes and migration of immature erythrocytes to the peripheral blood.

The decrease in mean hemoglobin percentage in cell (MCHC) found in the present study in the starving fish evidences the hypochromasia of erythrocytes and points to the intensity of hunger as a stressor.

The starved fish leukocyte count was decreasing considerably with time, which is a typical response found not only in fish.

Starving results both in a marked increase in the rod and segmented neutrophils and clear degenerative changes in these cells (Table 3, Plates 2A, 2C), which seems to have led to disintegration of some of them. The disintegration products, particularly proteins released from the cells, may stimulate the neutrophil production, as pointed out by Aleksandrowicz (1955).

The effects of starving on the percentage and morphology of eosinophils and on the morphology of monocytes are well-marked, the percentage of the cells decreasing considerably with time, whereas changed nuclei and cytoplasm evidence the irreversible degenerative processes leading often to cell disintegration (Plate 2C, 2D).

A long-lasting starvation results in mammals in a typical increase in the basophil percentage in the peripheral blood (Pinkiewicz, 1971). An identical response was encountered in the present study (Fig. 9).

Lymphocytes seem to be less sensitive to damaging factors; although the lymphocyte percentage decreased markedly in the starved fish, their morphology changed less than that of eosinophils.

Conclusions

1. Capture as a stressor in C_1 and C_2 carp results in changes of some hematological parameters: the erythrocyte volume (MCV) increases, leading to an increased hematocrit. The leukocyte count and percentage of neutrophils and basophils increase as well, while the percentages of eosinophils and lymphocytes decrease markedly.
2. Transportation, a strong stressor for fish, affects C_1 and C_2 carp by increasing the erythrocyte count, hematocrit, hemoglobin content, leukocyte count, and neutrophil percentage; the percentages of eosinophils and lymphocytes decrease.
3. Long-lasting deprivation of food results in a decreased erythrocyte count, hematocrit, and hemoglobin content in C_1 carp. As a consequence of anemia in the starved fish, the peripheral blood contains erythrocytes showing anisocytosis and polychromasia. A further consequence is a decrease in the percentage of lymphocytes, eosinophils, and monocytes. The two latter cell types develop degenerative changes that may lead to disintegration. The percentage of rod and segmented neutrophils and basophils increase. The netrophils may develop degenerative changes.

REFERENCES

Aleksandrowicz J., 1955: Hematologia kliniczna. PZWL. Warszawa.

Benditt E., Morrison P., Irving L., 1941: The blood of the Atlantic salmon during migration.-Biol. Bull. 80: 429–440.

Black E.C., Barret I., 1957: Increase in levels of lactic acid in the blood of cutthroat and steelhead trout following handling and live transportation.-Can. Fish. Cult. **20**: 13–24.

Eddy F.B., Morgan R.I., 1969: Some effects of carbon dioxide on the blood of rainbow trout Salmo gairdneri Richardson.-J. Fish. Biol. **1**: 361–373.

Elandt R., 1964: Statystyka matematyczna w zastosowaniu do doświadczalnictwa rolniczego. [Mathematical statistics applied to agricultural experiments]. PWN Warszawa.

Ferguson J.K.W., Black E.C., 1941: The transport of CO_2 in the blood of certain freshwater fishes.-Biol. Bull., **80**: 139–152.

Fraser J.M., Beamish F.W.H., 1969: Blood lactic acid concentrations in brook trout, Salvelinus fontinalis, planted by air drop.-Trans.Am. Fish.Soc. **98**: 263–267.

Fryer J.N., 1975: Stress and adrenocorticosteroid dynamics in the goldfish, Carassius auratus.-Canad.J.Zool., **53**: 1012–1020.

Holeton G.R., Randall D.J., 1967: The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout.-J.Exp. Biol., **46**: 317–327.

Houston A.H., 1962: Some observations on water balance in the goldfish Carassius auratus L.during cold death.-Can.J.Zool., **40**: 1169–1174.

Houston A.H., Madden J.A., Woods R.J., Miles H.M., 1971a: Some physiological effects of handling and tricaine methansulphonate anaesthetization upon the brook trout.-J.Fish. Res.Bd.Can. **28**: 625–633.

Houston A.H., Madden J.A., Woods R.J., Miles H.M., 1971b: Variations in the blood and tissue chemistry og brook trout, Salvelinus fontinalis, subsequent to handling, anaesthesia, and surgery. – J.Fish. Res.Bd.Can **28**: 635–642.

Irving L., Black E.C. Safford V., 1941: The influence of temperature upon the combination of oxygen with the blood of trout.-Biol. Bull. **80**: 1–17.

Ivanova N.T., 1970: Materiały k'morfologii krovi ryb. Rostov-n-Donu. s. 136. (in Russian)

Klontz G.W., Smith L.S., 1968: Methods of using fish as biological research subjects. Methods of animal experimentation. vol. 3. Academic Press, New York, London.

Pinkiewicz E., 1971: Podstawowe badania laboratoryjne w chorobach zwierząt. [Basic laboratory tests in animal diseases]. PWRiL. Warszawa.

Pučkov N.W., Fedorova A.L., 1951: Isledovanie izmenenija sostava krovi karpa pod vlijanem gołodanija i ochłazdenija. -Trudy Mosrybvtuza, **4**: 157–162. (in Russian)

Puczkow N.W., 1962: Fizjologia ryb. [**Fish physiology**]. PWN, Warszawa.

Soivio A., Nyholm K., 1973: Notes on haematocrit determinations on rainbow trout, Salmo gairdneri. Aquaculture, **2**: 31–35.

Soivio A., Mälkönen M., Tuurala O., 1974: Efects of asphyxia and MS-222 anaesthesia on the circulation of the kidney in Salmo gairdneri Richardson. A microscopical study.-Ann. Zool. Fenn., **11**: 271–275.

Soivio A., Westman K., Nyholm K., 1974: The influence of changes in oxygen tension on the haematocrit value of blood samples from asphyxic rainbow trout (Salmo gairdneri). -Aquaculture, **3**: 395–401.

Soivio A., Oikari A., 1976: Haematological effects of stress on a teleost. Esox lucius L.-J.Fish. Biol., **8**: 397–411.

Wedemeyer G. 1973: Some physiological aspects of sublethal heat stress in the juvenile steeelhead trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch).-J.Fish. Res. Bd. Can. **30**: 831–834.

Weinreb E.L., 1958: Studies on the histology and histopathology of the rainbow trout Salmo gairdneri irideus. I. Hematology: Under normal and experimental conditions of inflammation.-Zoologica, N.Y., **43**: 145–154.

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WPŁYW CZYNNIKÓW FIZJOLOGICZNYCH STRESSOWYCH I CHOROBOWYCH NA PARAMETRY HEMATOLOGICZNE KARPIA ZE SZCZEGÓLNYM UWZGLĘDNIENIEM OBRAZU BIAŁOKRWINKOWEGO. II. HEMATOLOGICZNY EFEKT STRESSU U KARPIA

STRESZCZENIE

Materiałem do badań nad wpływem odłowu na parametry hematologiczne było 40 sztuk karpi K_1 i 40 sztuk karpi K_2, nad wpływem transportu 60 sztuk K_1 i 60 sztuk K_2 (po 20 sztuk w miesiącach: czerwcu, lipcu i sierpniu). Do badań nad wpływem głodzenia użyto 160 sztuk ryb.

Badania krwi obejmowały: liczbę krwinek czerwonych i białych, wartość hematokrytową, poziom hemoglobiny, MCV, MCH, MCHC. Określono również leukogram oraz obserwowano morfologię krwinek czerwonych i białych. Wyniki opracowano statystycznie.

Trudne warunki odłowu będące u ryb niekorzystnym stresorem powodują zwiększenie objętości krwinek czerwonych (MCV), oraz wartości hematokrytowej. Następstwem uaktywnienia sił obronnych ustroju jest zwiększenie liczby leukocytów oraz procentu neutrofili i bazofili.

Transport będący silnym stresorem u ryb powoduje zwiększenie liczby erytrocytów, wartości hematokrytowej i poziomu hemoglobiny a także wzrost liczby leukocytów oraz procentu neutrofili. Zmniejsza się procent eozynofili i limfocytów.

Długotrwałe głodzenie trwające 21 tygodni prowadziło do postępującej niedokrwistości o czym świadczy obniżenie liczby erytrocytów, wartości hematokrytowej i poziomu hemoglobiny oraz anizocytoza i polichromazja erytrocytów. Następstwem głodzenia była obserwowana leukocytopenia i limfopenia oraz eozynopenia. W tych ostatnich krwinkach występują wyraźne zmiany degeneracyjne. Zwiększeniu ulega procent neutrofili i bazofili. W neutrofilach mogą pojawić się również zmiany degeneracyjne.

Сопиньска А.

ВЛИЯНИЕ ФИЗИОЛОГИЧЕСКИХ ФАКТОРОВ СТРЕССА И БОЛЕЗНИ НА ГЕМАТОЛОГИЧЕСКИЕ ПАРАМЕТРЫ У КАРПА, С ОСОБЫМ УЧЁТОМ КАРТИНЫ ЛЕЙКОЦИТОВ. II. ГЕМАТОЛОГИЧЕСКИЙ ЭФФЕКТ СТРЕССА У КАРПА

Р е з ю м е

Материалом для исследования влияния улова на гематологические параметры служили: 40 особей карпа $К_1$ и 40 особей карпа $К_2$. Для установления влияния транспорта исследовали 60 особей $К_1$ и 60 особей $К_2$ (по 20 особей в месяце июне, июле и августе). Влияние голодания испытывалось с употреблением 160 особей рыбы.

В крови определяли: числа красных и белых кровяных телец, гематокритную величину, уровень гемоглобина, определялась также лейкограмма и и велись

наблюдения за морфологией красных и белых кровяных телец. Результаты подвергались статистической обработке.

Затрудненные условия ловли способствовали отрицательным стрессам у рыбы, что проявлялось в увеличении объёма красных кровяных телец (MCV), а также гематокритной величины. Последствием увеличения активности защитных сил организма являлось повышение количества лейкоцитов, а также процента нейтрофилов и базофилов.

Транспорт, представлявший собой мощный стрессовый фактор у рыбы, способствует увеличению числа эритроцитов, гематокритной величины и уровня гемоглобина, а также повышению числа лейкоцитов и процента нейтрофилов и уменьшению процента эозинофилов и лимфоцитов.

Длительное голодание (21 неделя) вызвало прогрессирующую анемию, проявлявшуюся уменьшением числа эритроцитов, гематокритной вкличины и уровня гемоглобина, а также анизоцитозом и полихромазией эритроцитов.

Последействием голодания являлись лейкоцитопения и лимфопения, а также эозинопения с резкими деградационными изменениями этих кровяных шариков. Увеличивался процент нейтрофилов и базофилов. Могли также присутствовать деградационные изменения нейтрофилов.

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Received: 25 January 1983