Bulgarian Journal of Agricultural Science, 14 (No 2) 2008, 244-250
National Centre for Agrarian Sciences

SELECTED HEMATOLOGICAL INDICES OF FRESHWATER FISH FROM STUDEN KLADENETSH RESERVOIR

D. ARNAUDOVA[1], A. ARNAUDOV[2] and E. TOMOVA[2]
[1]*University of Plovdiv "P. Hilendarski", Branch "L. Karavelov", BG-6600 Kardzhali, Bulgaria*
[2]*University of Plovdiv "P. Hilendarski", Faculty of Biology, BG - 4000 Plovdiv, Bulgaria*

Abstract

ARNAUDOVA, D., A. ARNAUDOV and E. TOMOVA, 2008. Selected hematological indices of freshwater fish from Studen Kladenetsh Reservoir. *Bulg. J. Agric. Sci.,* 14: 244-250

Investigations on the toxic effect of heavy metals upon fish is accompanied by the investigation of changes in some hematological and biochemical blood indices. However, very few publications can be found regarding the combined impact of metals and this effect has been studied particularly by means of *ex situ* analysis and in lesser amount using freshwater basins analysis. The present article provides information for some red blood cell indices of three freshwater fish - bleak (*Alburnus alburnus* L.), rudd (*Scardinus erythrophtalmus* L.) and perch (*Perca fluviatilis* L.) from the Studen Kladentsh Reservoir (area of the Arda River). The toxic substances in Studen Kladenetsh Reservoir showed that their concentration in the water of the exceeds acceptable levels. The analysis of all three freshwater fishes inhabiting the Studen Kladenetsh Reservoir registered anaemic changes in the blood regardless of the season. However, each species developed a different type of anaemia - macrocytic hyperchromic type in the bleak, hypochromic type in the rudd and normochromic anaemia, which developed into mycrocytic normochromic anaemia in winter in the perch.The morphological examination of the erythrocytes of all three freshwater fishes demonstrated a wide range of pathological deviations as well as a large number of „amitotic" erythrocytes in peripheral blood of rudd and persh. These changes also show differences between the species.

Key words: freshwater fish, hematology, anemia, erythrocyte morphology
Abbreviations: MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, fL – femolitre, pg/cell – picograms per cell

Introduction

During recent years, considerable attention has been focussed on the fate of metals and their derivatives in the aquatic environment (Bhagwant and Bhikajee, 1999). Metal pollutants in freshwater basins tend to increase steadily thereby representing the greatest hazard to human consumers of fish (Marr and Creaser, 1983, Gutenmann et al., 1988). Investigations on the toxic effect of heavy metals upon fish is accompanied by the analysis of changes in some hematological and biochemical blood indices (Cyriak et al., 1989; Hellemans and Bailiene, 1990; Storelli and Marcotrigiano, 2001; Cavas at al., 2005; Carvalho

E-mail: arny87@abv.bg

and Fernandes, 2006, Hoyle at al. 2007). However, most examinations are concerned with the influence of each particular metal. Very few publications can be found regarding the combined impact of metals and this has been studied particularly by means of laboratory analysis. (Vosyliene, 1999). Literature sources provide scarce information about the influence of anthropogenic pollution of water basins, caused by heavy metals, upon the physiological state of freshwater fishes inhabiting these basins. Investigations performed in the area of the Arda River so far have been concerned with studies of the concentration of heavy metals in fishes inhabiting the reservoirs in close proximity to the Lead and Zinc Ore Processing Factory in Kardzhali (namely the Kardzhali Reservoir and the Studen Kladenetsh Reservoir), which is the main anthropogenic pollutant of the water in these reservoirs (Velcheva, 1995, 1998; Bachvarov and Velcheva, 1995). No information is available about the impact of heavy metals in water basins upon the haematological indices of the fishes inhabiting these basins.

The aim of this study is to analyze the influence of anthropogenic heavy metal pollution of the water in the Studen Kladenetsh Reservoir upon red blood cell indices of the organism systems in freshwater fishes: bleak (*Alburnus alburnus* L.), rudd (*Scardinus erythrophtalmus* L.) and perch (*Perca fluviatilis* L.). Therefore, the following tasks shall be fulfilled: 1. The indices of fish habitat with regard to the tested freshwater species shall be determined on seasonal basis (winter and summer):

a. Physicochemical parameters–temperature, pH, and oxygen content;

b. presence of toxic substances;

2. The red blood cell indices of the bleak, rudd and perch shall be determined on a seasonal basis (winter and summer).

Materials and Methods

This research has been conducted in the area around the Studen Kladentsh Reservoir. To obtain test results the same water indices and fish samples from the Kardzhali Reservoir have been examined. The studies have been performed in seasonal dynamic pattern, namely winter and summer. *Analysis objects.* Three species of freshwater fishes have been analyzed: bleak (*Alburnus alburnus* L.), rudd (*Scardinus erythrophtalmus* L.) and perch (*Perca fluviatilis* L.). Eight samples of each species have been analyzed. All samples were in the same size range and age group, namely: 8.0–12.0 cm for the bleak, 12.5–18.5 cm for the rudd and 9.0–15.0 cm for the perch.

Laboratory analysis

A. Physicochemical analysis of water–the following parameters have been determined: temperature, pH and oxygen content.

B. Presence of toxic substances in water. The analysis demonstrated presence of the following substances: manganese, copper, zinc (per ISO 8288), lead (per Bulgarian Government Standard 15109-80), iron (per Bulgarian Government Standard 3425-85), cadmium (per MERCK) and arsenic (per Bulgarian Government Standard EN ISO 11969).

C. Determination of red blood cell indices

Blood has been collected by cardiac punction. EDTA to final concentration of 5 g/L has been used in order to avoid the occurrence of haemocoagulation. The following indices have been determined for blood samples taken for testing:

- erythrocyte count in the peripheral blood (by means of the chamber method);
- concentration of haemoglobin (by means of the cyanhaemoglobin method);
- haematocrit determination;
- erythrocyte indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration, (MCHC);
- morphological features of red blood cells (form, features of the protoplasmic structure–coloring, presence of involvements);

When determining hematological indices we used the standard methods as described in the book by Angelov et al., (1999). The blood smears for morphological and metric analysis have been colored by

means of a series of units for immediate coloring of blood smears *DKK Color–2000 (VIVA-MT).*

Mathematical analysis of the test results

The analysis of the results of investigations has been performed by the variation and statistical methods as described in the book by Sepetliev, (1986).

Results and Discussion

The results of the investigation are as follows: the investigation on toxic substances in both water basins showed that their concentration in the water of the Studen Kladenetsh Reservoir exceeds acceptable levels. The investigation showed manganese and lead had the highest concentration compared to the acceptable levels. Lower levels of water pollution caused by toxic substance were detected in the Kardzhali Reservoir where the concentration of manganese and lead exceeded the acceptable norms only slightly (Table 1)

Table 1
Content of metals in the reservoirs

Metals, $mg.l^{-1}$	„Studen kladenetsh" reservoir		„Kardzhali" reservoir	
	winter	summer	winter	summer
Manganese	0.18	0.114	<0.016	<0.016
Total iron	0.3	0.28	0.035	0.03
Cooper	<0.020	<0.018	<0.010	<0.018
Lead	0.4	0.3	<0.01	<0.01
Zinc	<0.050	0.057	0.03	<0.013
Cadmium	0.007	0.004	0.0000216	<0.00004
Arsenic	0.00227	0.00249	0.00066	0.00074

Table 2 presents the results of physicochemical indices of the water in both water basins. The table shows pH of water falls into the acceptable range of 6.5 to 9.5. However, the level of oxygen in the water of the Kardzhali Reservoir exceeds that of the Studen Kladenetsh Reservoir in both seasons. Water temperature of the Kardzhali Reservoir as whole is lower than that of the other reservoir.

Table 2
Physiochemical indices of the the reservoirs

Indices	„Studen kladenetsh" reservoir		„Kardzhali" reservoir	
	winter	summer	winter	summer
Temperature, ^0C	12-21	8-18	15-21	5-17
pH	7.83	7.18	7.52	7.35
Oxygen content, $mg.l^{-1}$	9.9	9.8	10.80	10.6

Haematological test results regarding the bleak (*Alburnus alburnus* L.) are described in Table 3. The data in the table shows that the analyzed specimens of bleak (*Alburnus alburnus* L.) taken from the Studen Kladenetsh Reservoir exhibited lower indices in terms of red blood cell count, concentration of haemoglobin and haematocrit which indicates anaemic changes in the blood of the species in winter and summer. Analysis of red blood cell indices demonstrates that anaemic changes were of macrocytic hyperchromic type although hyperchromic anaemia is characterized by low intensity during the summer season.

The results of the haematological analysis of the rudd (*Scardinus erythrophtalmus* L.) are contained in Table 4. They demonstrate that the analyzed specimens of the rudd (*Scardinus erythrophtalmus* L.) taken from the Studen Kladenetsh Reservoir exhibited lower indices in terms of red blood cell count, concentration of haemoglobin and haematocrit during both seasons.

The analysis of red blood cell indices shows that anaemic changes are of hypochromic type (reduced values of the MCH). Hypochromia tends to be more strongly expressed and the values of MCV to decrease in winter.

Table 5 presents results from the red blood cell test of the perch (*Perca fluviatilis* L.).

The data in the table shows that the analyzed specimens taken from the Studen Kladenetsh Reservoir exhibited lower indices in terms of red blood cell count, concentration of haemoglobin and haematocrit. The perch had normochromic anaemia, which developed

Table 3

Red blood cell indices of bleak from Studen kladenetsh reservoir (I) and Kardzhali reservoir (II)

Indices	Winter		Summer	
	I	II	I	II
Erythrocyte count ($x10^{12}.l^{-1}$)	0.78±0.15 *	1.15±0.08	0.87±0.09 *	1.18±0.05
Hemoglobin content, $mmol.l^{-1}$	3.88±0.53 *	4.90±0.34	4.15±0.43*	5.19±0.38
Hematocrit value, l/l	0.221±0.02*	0.266±0.02	0.220±0.01*	0.265±0.02
Erythrocyte indices:				
MCV, fL	292.5±64.1*	233.0±24.7	288.2±25.6*	224.5±17.2
MCH, $pg.cell^{-1}$	83.2±20.4	69.1±6.1	77.0±13.4	71.4±5.6
MCHC, $g.l^{-1}$	283.2±13.0	294.8±4.8	298.7±28.3	315.6±17.7

* P<0.001

Table 4

Red blood cell indices of rudd from Studen kladenetsh reservoir (I) and Kardzhali reservoir (II)

Indices	Winter		Summer	
	I	II	I	II
Erythrocyte count ($x10^{12}.l^{-1}$)	0.95±0.04 *	1.13±0.09	0.98±0.07 *	1.16±0.23
Hemoglobin content, $mmol.l^{-1}$	4.01±0.39 *	5.49±0.40	4.30±0.41*	5.74±0.42
Hematocrit value, l/l	0.218±0.02*	0.292±0.01	0.24.1±0.01*	0.275±0.02
Erythrocyte indices:				
MCV, fL	229.6±23.3	259.2±26.2	246.5±21.0	237.1±16.0
MCH, $pg.cell^{-1}$	68.2±6.9*	78.8±9.4	70.7±7.1*	79.7±6.6
MCHC, $g.l^{-1}$	30.01±1.32	30.35±0.91	28.98±1.48	33.66±1.23

* P<0.001

Table 5

Red blood cell indices of perch from Studen kladenetsh reservoir (I) and Kardzhali reservoir (II)

Indices	Winter		Summer	
	I	II	I	II
Erythrocyte count ($x10^{12}.l^{-1}$)	0.81±0.14*	1.13±0.08	0.94±0.12*	1.19±0.12
Hemoglobin content, $mmol.l^{-1}$	3.76±0.21*	5.35±0.50	4.04±0.44*	5.55±0.60
Hematocrit value, l/l	0.217±0.02*	0.279±0.02	0.224±0.02*	0.281±0.02
Erythrocyte indices:				
MCV, fL	268.6±71.7*	248.2±28.6	240.5±22.2	236.2±16.8
MCH, $pg.cell^{-1}$	75.1±21.0	76.4±8.4	74.0±12.0	75.0±2.7
MCHC, $g.l^{-1}$	279.7±17.4	305.1±6.6	290.1±15.9	317.7±14.6

* P<0.001

into mycrocytic normochromic anaemia in winter.

The morphological examination of the red blood cells of all three freshwater fishes demonstrated a wide range of pathological deviations that occurred most strongly in the specimens inhabiting the Studen Kladenetsh Reservoir. What is most interesting is that the erythrocyte pathology differs depending on the species.

The bleak exhibited the following morphological changes in erythrocytes: presence of a large number of red cells in the form of irregular polygons, which were elongated, and some of them had sharp vertex. Observations also showed the presence of round cells. Some erythrocytes had their membrane lacerated. Pathological granulation of the protoplasm of some erythrocytes was observed (Figure 1).

Examination of the specimens of the rudd proved that there were a great number of „amitotic" erythrocytes apart from the presence of red cells in the form of irregular polygons and cells with pathological granulation of the protoplasm (Figure 2).

Pathological deviations in the specimens of the perch lacked such variety: only „amitotic" erythrocytes and a great number of round cells were detected (Figure 3).

The research shows that heavy metal pollutants in the water of the Studen Kladenetsh Reservoir caused anaemia (reduced erythrocyte count, concentration of haemoglobin and haematocrit) in all three analyzed species.

Fig. 2. Erythrocytes of rudd (*Scardinus erythrophtalmus* L.). Poikilocytosis (x 400)

Therefore, we can draw the conclusion that intoxication with the heavy metals found in the Studen Kladenetsh Reservoir had a common pathological effect on the species in terms of anaemic changes. However, anaemia is not a universal pathogenic mechanism of intoxication with heavy metals that exert their influence either separately or as mixtures. There were cases when the impact of heavy metals did not cause any changes in erythrocyte indices and under certain conditions, it could even lead to increased values of the indices (Witeska and Jezierska, 1994, Witeska, 1998, Mishra and Srivasrava, 1980). Other stress effects, such as transport and handling, which

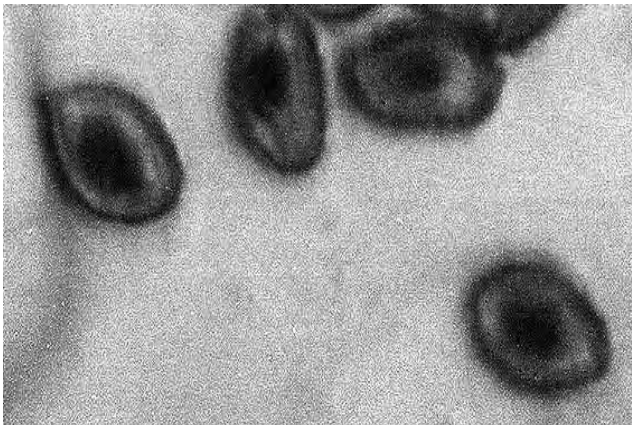

Fig. 1. Erythrocytes of bleak (*Alburnus alburnus* L.). Pathological granulation of the protoplasm (arrow)(x 400)

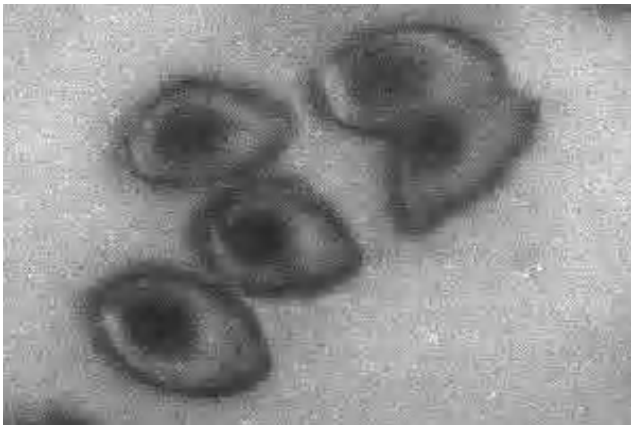

Fig. 3. Erythrocytes of perch (*Perca fluviatilis* L.) „Amitotic" erythrocyte (arrow) and some round cells (x 400)

are not related to heavy metal pollution in water basins, also appear to cause changes in hematological indices (Acerete et al., 2004).

According to Vosyliene (1999), the influence of heavy metal mixtures on hematological indices depends on the concentration of metals and the continued exposure to them. When concentration is at low level, freshwater fishes tend to adapt to the presence of metal mixtures in water after a period of time. Only morphological changes were observed: the share of old, disintegrated, and „amitotic" erythrocytes increased while the number of erythrocytes, the concentration of haemoglobin and haematocrit were within the acceptable range (Vosyliene and Svecevieius, 1997). Witeska, (2004) also provides information about similar pathological changes in erythrocytes of freshwater fishes as a result of their exposure to metal mixtures. In his opinion, morphological changes in erythrocytes are a serious indicator of heavy metal intoxication in freshwater fishes.

The morphological finding in erythrocytes we have observed showed complete coincidence with the alterations described by the authors mentioned above, including presence of „amitotic" erythrocytes. However, the fact that apart from pathological changes in erythrocytes anaemic changes in blood have been also detected shows that analyzed fish species taken from the Studen Kladenetsh Reservoir are not able to adapt effectively to the toxic substances in this reservoir because of their high concentration.

Conclusions

The results allow us to draw the following significant conclusions:

The analysis of all three freshwater fish species inhabiting the Studen Kladenetsh Reservoir registered anaemic changes in the blood regardless of the season. However, each species developed a different type of anaemia;

The analysis of all three freshwater fish species inhabiting the Studen Kladenetsh Reservoir registered various morphological changes in erythrocytes. They also show differences between the species;

Analysis of the erythrocytes in the blood of all three freshwater species inhabiting the Kardzhali Reservoir has also registered pathological changes in red blood cell morphology; however they exhibited frequency of occurrence that was much lower than that in the other reservoir.

Acknowledgements

This study was supported by Project 07B59 (Fund "Scientific investigation and mobile projects", University of Plovdiv, Plovdiv, Bulgaria).

References

Acerete, L., J. C. Balasch, E. Espinosa, A. Josa and L. Tort, 2004. Physiological responses in Eurasian perch (*Perca fluviatilis* L.) subjected to stress by transport and handling. *Aquaculture*, **237**: 167-178.

Àngelov, G., N. Ibrishimov and St. Milashki, 1999. Clinical and laboratory investigations in veterinary medicine. *Acad. Publishers "Prof. M. Drinov"*, Sofia, 367 pp. (Bg).

Bachvarov, G. and I. Velcheva, 1995. Etude du contenu de plomb (Pb), zinc (Zn), cadmium (Cd) dans *Leuciscus cephalus* L. /Pisces, Famille *Cyprinidae*/ du bassin de la riviere Arda (*Bulgarie du Sud*). Traveaux scientifiques de l'Universite de Plovdiv, *Biologie*, **6** (31): 71-75.

Bhagwant, S. and M. Bhikajee, 2000. Induction of hypochromic macrocytic anaemia in *Oreochromic* hybrid (cichlidae) exposed to 100mg/l (sublethal dose) of aluminium. *Science and Technolody*, **5**: 9-20.

Carvalho, C. S., and M. N. Fernandes, 2006. Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. *Aquaculture*, **251** (1): 109-117.

Cavas, T., N. Garanko and V. Arkhipchuk, 2005. Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride and copper sulphate. *Food and Chemical Toxicology*, **43**: 569-574.

Cyriak, P. J., A. Antoni and P. N. K. Nambisan, 1989. Hemoglobin and hematocrit values in the fish *Osteochromis mossambicus* (Peters) after short term exposure to copper and mercury. *Bull. Environ. Contam. and Toxicol*., **43** (2): 315-320.

Gutemann, W. H, C. A. Bache, J. B. McCanan and D. J.

List, 1988. Heavy metals and chlorinated hydrocarbons in marine fish products. *Nutrition Report International*, **5**: 1157-1161.

Hellemans, K and M. Bailiene, 1990. Toxicity and accumulation of copper by a fresh water fish *Rutilus rutilus* L. *Belg. J. Zool.*, **120**: 1: 37.

Hoyle, I., B. J. Shaw and R. D. Handy, 2007. Dietary copper exposure in the African walking catfish, *Clarias gariepinus*: Transient osmoregulatory disturbances and oxidative stress. *Aquatic Toxicology*, **83** (1): 62-72.

Marr, I and L., M. S. Creaser, 1983. Environmental Chemical Analysis. *Blackie & Sons Ltd.* 104 pp.

Mishra, S. and A. K. Srivastava, 1980. The acute toxic effects of copper on the blood of a teleost. *Ecotoxicol. Environ*. Saf., **4**: 191-194.

Sepetliev., D., 1986. Medical statistics. Sofia (*Publishing house of Medicine and Physical Culture*), 110 pp. (Bg).

Storelli, M. and G. Marcotrigiano, 2001. Heavy metals monitoring in Fish, Bivalve, Mollusc, Water and Sediments from Varano Lagoon, Italy. *Bulletin of Environmental Contamination and Toxicology*, **66** (3): 365- 370.

Velcheva, I., 1998. Ecological investigation on content of Càdmium (Cd), Lead (Pb) and Zink (Zn) in bleak (*Alburnus alburnus* L), carp (*Cyprinus carpio* L.) and perch (*Perca fluviatilis* L.) from the Kardzhali and Studen Kladenetsh Reservoir, PhD thesis, *University of Plovdiv Publisher*, Plovdiv, 135 pp.(Bg).

Velcheva, I., 1995. Investigation on content of Càdmium (Cd) in different origins of chub (Leuciscus cephalus L. /Pisces, Famille Cyprinide) from Arda river valley. Scientific works of University of Plovdiv "P." Êhilendarski", *Biology*, **6** (31): 86-89 (Bg).

Vosyliene, M. and G. Svecevieius, 1997. Sublethal effects of rainbow trout of chronic exposure to mixtures of heavy metals. *Fish Physiology, Toxicology and Water Quality*. EPA 600/R-97/198: 141-150.

Vosyliene, M., 1999. The effect of heavy metals on hematological indices of fish (Survey). Acta Zoologica Lituanica. *Hydrobiologia*, **9** (2): 76-82.

Witeska, M. and B. Jiezerska, 1994. The effect of cadmium and lead on selected blood parameters of common carp. *Arch. Ryb. Pol*., **2** (1): 123-132.

Witeska, M., 1998. Changes in selected blood indices of common carp after acute exposure to cadmium. *Acta vet. Brno*, **67**: 289-293.

Witeska, Ì , 2004. The effect of toxic chemicals on blood cell morphology in fish. Fresenius *Environmental Bulletin*, **13** (12A): 1379-1384**.**

Received February, 20, 2008; accepted for printing March , 17, 2008.