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SEASONAL EFFECTS ON MORPHOMETRIC AND HAEMATOLOGIC PARAMETERS IN *SALMO LETNICA* IN NATURAL CONDITIONS

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Salmo letnica (Karaman 1924) is one of the most important endemic species of Ohrid Lake in scientific and economic aspects. The aim of this study was to evaluate season effects on morphometric and hematological parameters of *Salmo letnica* in natural conditions. 100 individuals of fish, respectively 52 in winter season and 48 in summer season, have been analyzed in the Albanian side of Ohrid Lake. The analyzed haematological parameters were erythrocites, haematocrit and haemoglobin; while morphometric measured parameters were body weight, length and height. Morphometric and haematological parameters of *Salmo letnica* showed slight differences between sampling seasons, but these differences often have not a statistical significance ($P < 0.95$).

Key words: *Salmo letnica*; seasonal effects; haematological parameters; morphometric parameters

СЕЗОНСКИ ЕФЕКТИ ВРЗ МОРФОМЕТРИЧКИТЕ И ХЕМАТОЛОШКИТЕ ПАРАМЕТРИ НА *SALMO LETNICA* ВО ПРИРОДНИ УСЛОВИ

Salmo letnica (Караман, 1924) е еден од најважните ендемски видови на Охридското Езеро, од научен и од економски аспект. Целта на оваа студија беше да се проценат сезонските ефекти врз морфометричките и хематолошките параметри на *Salmo letnica* во природни услови. 100 индивидуи на риба, 52 во зимската и 48 во летната сезона, беа анализирани на албанската страна на Охридското Езеро. Од хематолошките параметри беа анализирани еритроцитите, хематокриот и хемоглобинот, додека од морфометричките параметри беа измерени тежината на телото, должината и висината. Морфометричките и хематолошките параметри на *Salmo letnica* покажуваат незначителни разлики кај испитуваните примероци во двете сезони, но тие разлики често немаат статистичка значајност ($P<0,95$).

Клучни зборови: *Salmo letnica*; сезонски ефекти; хематолошки параметри; морфометрички параметри

INTRODUCTION

Haematological parameters are increasingly used as the indicator of the physiological stress response to endogenous or exogenous changes in fish (Lermen et al., 2004). One of the most interesting of exogenous factors is the seasonal difference which is accompanied with water temperature changes and different levels of oxygen dissolved in it.

Since an increase in temperature decreases oxygen availability in water and increases oxygen consumption, mechanisms ensuring adequate adaptation of fish organism to elevated temperatures occur. One of the factors involved in this adaptation is the temperature dependence of haemoglobin-oxygen binding. Haemoglobin-oxygen affinity decreases with increasing temperature (Nikinmaa et al., 1980; Tetens et al., 1984). One well-documented mechanism to compensate elevated oxygen consumption is to increase blood oxygen-carrying capacity by raising a red blood cell number (Yamamoto et al., 1980). The studies on many fish species have demonstrated that water temperature changes might affect changes in haematological parameters and morphometric parameters, as results in the publications of Denton & Yousef (1975), Andersen et al. (1985), Härdig & Höglund (1984), Jonassen et al. (1999), Lermen et al. (2004).

Salmo letnica (Karaman, 1924) is considered as the most important autochthonous fish of Ohrid Lake, from scientific and commercial point of view. There are some studies on morphometric and haematological parameters of *Salmo letnica* (Beqiraj, 2007; Beqiraj, et al., 2006; Latifi et al., 2008; etc), but there is no data on the seasonal effect on these parameters.

The present study is focused on the effect of seasonality on morphometric and haematological parameters in *Salmo letnica* in natural conditions.

MATERIALS AND METHODS

The analyzed haematological parameters have been erythrocytes count (Er), haematocrit value (Htc) and haemoglobin level (Hb).

Fish specimens have been collected from different stations in the Albanian side of Ohrid Lake between December 2005 and September 2006 (Fig. 1). These samples included specimens of the fish caught by fishermen. In the study specimens aged over 3 years were used, which, according to Rakaj (1995) belong to the weight over 120 g and to the length over 24 cm.

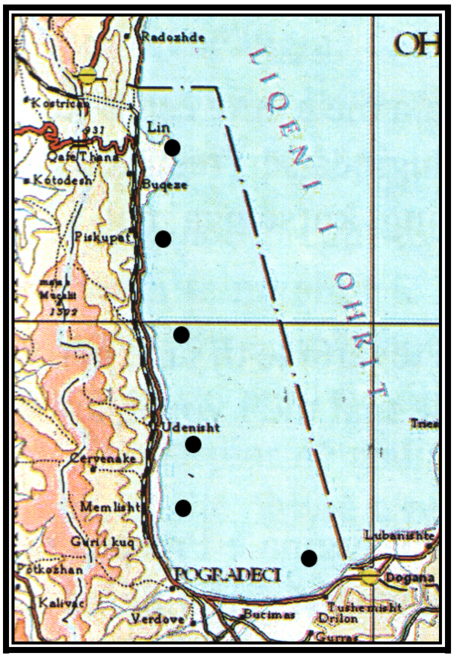

Figure 1. Sampling stations in the Albanian side of Ohrid Lake

Blood samples have been taken after anesthesia with MS 222; 0.1 g l^{-1} water (according to Handy & Depledge, 2000).

Blood was collected from the caudal vein of a 100 fish specimens with 5 ml sterile syringes and kept at ice-cold tubes to which heparin (70 IU ml^{-1}) had been added (according to Ballarin et al., 2004).

Blood was immediately analyzed (for Hb and Hct level) and blood smears were prepared in the field condition. Blood smears were prepared and stained according to the May-Grünwald-Griemsa technique and examined with microscope (1250 × magnification).

Red blood cells were counted using a Bürker haemocytometer after 1 : 1000 dilution of the whole blood with phosphate buffered saline (PBS: 0.8% NaCl, 0.02% KCl, 0.02% KH_2PO_4, 0.115% Na_2HPO_4) according to Blaxhall & Daisley (1973). Haematocrit was determined using capillaries filled blood and centrifuged at 8700 *g for 5 min and expressed as the percentage of the total blood volume (Alexander & Griffiths, 1993). The total blood haemoglobin content has been determined using the cyanmethaemoglobin method (Alexander & Griffiths, 1993) based on the oxidation of haemoglobin to cyanmethaemoglobin in the presence of potassium ferricianide and the subsequent absorbance reading at 540 nm.

Each specimen has been weighed and measured (Fig. 2).

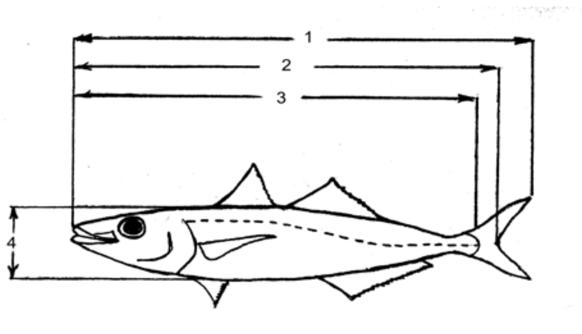

Figure 2. Schematic presentation of biometric measurements: 1 – maximal length (L), 2 – length till the tail bifurcation (l), 3 – length till the end of scales (C), 4 – maximal body height (H)

Samples have been grouped according to the season: winter period (January – February) and summer period (May, July and September).

Statistical parameters which were used are mean values (M), standard deviations (St. dev.), maximal and minimal values (max; min), variance (Var), standard error of the mean (m), mean difference (t_{mes}) and comparison of two sample means (t_D), according to Merkurjeva (1964), Elliot (1997) and Kaneko et al. (1997). The analysis of variance (ANOVA) has also been carried out.

RESULTS AND DISCUSSION

Tables 1 and 2 below show data on morphometric parameters (total body weight, length till the tail bifurcation, length till the end of scales and maximal body height) according to sampling season (52 winter samples and 48 summer samples) and their statistical elaboration.

Mean values of summer and winter sampling present a high significance: P > 0.999. Student criteria value (t_{mes}) goes from 10.9 – 57.64, while theoretical t_{mes} for the significance level P > 0.999 is 3.5. This report relatively slightly changes on individual values of samples.

Comparing morphometric parameters values of *Salmo letnica* presented in Tab. 1 and Tab. 2 have showed small differences of these parameters between sampling seasons. The difference seems to be more evident in the body weight. Statistic comparison of two sample means (showed from the t_D index) for each morphometric parameter between two sampling seasons is presented in Table 3.

The slight effect of season in the body weight is related to the impact of changes in water temperature and oxygen content in metabolic pathways. The increasing water temperature causes the intensification of some enzyme activity (like lactatdehydrogenase, hydroksi-butiril-CoA-dehydrogenase, creatininecinase, glutamatdehydrogenase, glutamate-pyruvat-transaminase) (Sauer & Haider, 1977). The increased activity of these enzymes, like "adaptation" enzymes of the fish in a new condition, proves the intensification of catabolic pathways, which might induce decreasing of the body mass and restraining of the development of other morphometric parameters

Table 1

Morphometric parameters of Salmo letnica, (n = 52) of the winter season

	Weight	L	l	C	H
M	425.4054	34.35135	32.83514	29.98649	7.135135
St. Dev.	281.1642	7.288561	7.303166	6.570649	2.046843
Min.	150	25	23	22	0.5
Max.	1150	51	50	45	11
Var.	79053.3	53.12312	53.33623	43.17342	4.189565
m	38.99	1.01	1.012	0.911	0.283
t_{mes}	10.9***	34***	32.44***	32.9***	25.21***

P > 0.95*, P > 0.99**, P > 0.999***

Table 2

Morphometric parameters of Salmo letnica, (n = 48) of the summer season

	Weight	L	l	C	H
M	326.6071	32.80357	31.53571	29.41071	7.732143
St. dev.	154.3688	3.878572	3.853638	3.564365	1.364104
Min.	150	24	23	22	6
Max.	1000	47	45.5	42.5	12
Var.	23829.73	15.04332	14.85053	12.7047	1.86078
m	22.7	0.569	0.56	0.52	0.2
t_{mes}	14.38***	57.64***	56.3***	56.55***	38.65***

P > 0.95*, P > 0.99**, P > 0.999***

Table 3

t_D of mean values of morphometric parameters of Salmo letnica between seasons

t_D	Weight	L	l	C	H
winter/summer	2.2*	1.237	1.124	0.54	–1.72

P > 0.95*

.Data were also analyzed by the analysis of variance (ANOVA). Elaborated data of ANOVA for the effect of season in morphometric parameters (weight and length) are showed in Table 4.

Table 4

The effect of season in weight and maximal length (L) of Salmo letnica
ANOVA

Parameters	*SS*	*df*	*MS*	***F***	***P-value***	*F crit*
Weight	789978	1	789978	0.53105	0.46887	3.99336
Length (L)	38.1825	1	38.1825	1.03748	0.3123	3.99336

Table 4 of ANOVA shows no significant difference on the average value of weight of *Salmo letnica* in different seasons, so season has no statistical effect on the body weight of *Salmo letnica.* From the same table it is also shown that season has no statistical effect on the mean maximal length (L) of *Salmo letnica.* Blood smears from *Salmo letnica* are showed in Figure 3.

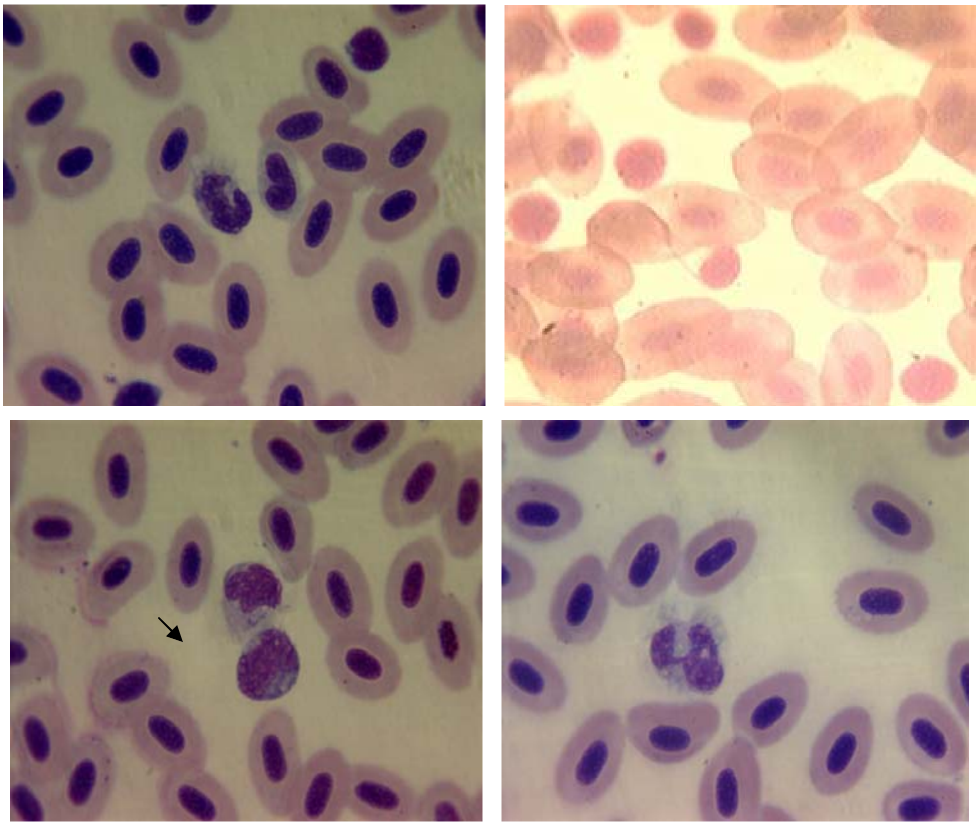

Figure 3. View of blood smears from *Salmo letnica*

Values of haematological parameters of *Salmo letnica* are also grouped based on the period of sampling. The data were statistically elaborated and presented in the Table 5 below. This table shows mean values of Er, Hct and Hb of 52 specimens sampled in winter season and 48 specimens sampled in summer. Statistically elaborated data present a high significance: P > 0.999. t_{mes} goes from 18.74 for the haematocrit level in winter to 47.37 for the haemoglobin level in winter. Theoretical t_{mes} for the significance level P > 0.999 is 3.5. This reports relatively slight changes on the individual values of samples.

Table 5

Haematological parameters of Salmo letnica in winter and summer

	Winter (*n* = 52)			Summer (*n* = 48)		
	Er ($*10^6/mm^3$)	Hct (%)	Hb (g/dl)	Er ($*10^6/mm^3$)	Hct (%)	Hb (g/dl)
M	1.1148	35.62	9.36	1.042	38.75	8.86
St. dev	0.221	13.9	2.53	0.174	5.66	1.87
Min.	0.921	3	3.55	0.81	29.73	5.33
Max.	1.59	60	15.25	1.21	53.33	13.70
Var.	0.049	193.32	6.39	0.03042	30.60	3.51
m	0.0306	1.93	0.35	0.025	0.82	0.27
t_{mes}	36.27***	18.48***	26.74***	41.68***	47.37***	32.81***

P > 0.95*, P > 0.99**, P > 0.999***

Values in the Table 5 show that season has not the same effect in all haematological parameters analyzed. The table shows that differences in the mean values of erythrocytes, haematocrit and haemoglobin level of *Salmo letnica* attributed to season are small. These values are also analyzed based on the criteria of comparison of two samples means and analysis of variance ANOVA. We observed differences on haematological parameters of *Salmo letnica* in different seasons, but these differences have not a statistical significance (P < 0.95). These data are showed in Tables 6 and 7.

Table 6

t_D of haematological parameters of Salmo letnica according to the sampling season

t D	Er	Hct	Hb
Winter/Summer	1.823	1.138	0.635

P > 0.95*, P > 0.99**, P > 0.999***

Table 7

The effect of season in erythrocytes number (Er), haematocrit (Hct) and haemoglobin (Hb) level of Salmo letnica ANOVA

Parameters	*SS*	*df*	*MS*	*F*	*P-vlera*	*F crit*
Er	0.00178	1	0.00178	0.04344	0.83556	3.99336
Hct	235.861	1	235.861	3.9793	0.05039	3.99336
Hb	0.24702	1	0.24702	0.03388	0.85455	3.99336

Elaborated data from ANOVA emphasize that season has not a significant effect on the mean value of the erythrocytes number, has quite a slight significant effect on the mean value of haematocrit, has not a significant effect on the mean value of haemoglobin level of *Salmo letnica*.

One of the factors influencing the lack of statistically significant differences between seasons in the analyzed blood parameters may be related to the fact that analyzed specimens live in natural conditions. We considered that *Salmo letnica* lives in deep and well oxygenated waters, at a depth of 60 – 80 m, or at the so-called hypolimnion stratum, the cold stratum of water, generally with stable temperature, according to Filipi (1954).

There is no detailed information how *Salmo letnica* tolerates temperature changes, but like other salmonids (ex. rainbow trout *Oncorhynchus mykkis*) it might be an eurythermal species that tolerates relatively wide range of temperatures.

This consideration is also due to the fact that *Salmo letnica* prefers deep and cold water, but it also experiences the natural fluctuation of temperature (remember that *Salmo letnica* comes near the coast during spawning period or searching food, after Filipi (1954) (especially the population of *S. letnica aestivalis* which spawns in July–August, after *Spirkovski & Ilić-Boeva*, 2002).

CONCLUSIONS

Morphometric and haematological parameters of *Salmo letnica* showed slight differences between sampling seasons.

Based on the criteria of comparison of two samples means, the morphometric parameter with more expressed season differences is the total body weight.

The analysis of variance (ANOVA) expresses the lacking of seasonal effect on the weight and maximal length of *Salmo letnica*.

The analysis of variance (ANOVA) shows that season has not a significant effect on the mean value of erythrocytes number, has a slightly significant effect on the mean value of haematocrit and has not a significant effect on the mean value of the haemoglobin level of *Salmo letnica.*

REFERENCES

[1] Alexander R. R., Griffiths J. M. (1993): *Basic Biochemical Methods*. Wiley, Liss. 187–189.

[2] Andersen N. A., Laursen J. S., Lykkeboe G. (1985): Seasonal variations in hematocrit, red cell hemoglobin and nucleoside triphosphate concentrations, in the European eel *Anguilla anguilla. Comparative Biochemistry and Physiology.* **81A**: 87–92.

[3] Ballarin L., Oro M., Bertotto D., Libertini A., Franceson A., Barnaro A. (2004): Hematological parameters in Umbrina cirrosa (Teleostei, Scienidae): A comparison between diploid and triploid specimens. *Comparative Biochemistry and Physiology*. Part A, 138: 45–51.

[4] Beqiraj Kalamishi D. (2007): *Studim i treguesve metabolikë të koranit (Salmo letnica) dhe formave të tij në kushte natyrore*. PhD Thesis. Agricultural University of Tirana.

[5] Beqiraj Kalamishi D., Mane B., Dhaskali L., Dimço E., Latifi F. (2006): Metabolic and hematological indices in *Salmo letnica* of Ohrid Lake. *II International Symposium of Ecologists of Montenegro*. p. 92.

[6] Blaxhall P., Daisley K. W. (1973): Routine haemotological methods for use with fish blood. *Journal of Fish Biology*. 5: 771–781.

[7] Denton J. E., Yousef, M. K. (1975): Seasonal changes in hematology of rainbow trout, *Salmo gairdneri. Comparative Biochemistry and Physiology.* 5lA: 151–153.

[8] Elliot J. M. (1997): *Some methods for the statistical analysis of samples of benthic invertebrates*. 160 p.

[9] Filipi N. (1954): Ihtiofauna e Liqenit të Pogradecit dhe e afluentëve të tij, *Buletini për Shkencat Natyrore.* III. 140–173.

[10] Handy R. D., Depledge M. H. (2000): Physiological Responses: Their measurement and use as environmental biomarkers in ecotoxicology. *Ecotoxicology.* 8: 329–349.

[11] Härdig J., Höglund L. B. (1984): Seasonal variation in blood components of reared Baltic salmon, *Salmo salar* L. *Journal of Fish Biology.* **24**: 565–579.

[12] Jonassen, T. M., Imslaand, A. K., Stefansson, S. O. (1999): The interaction of temperature and fish size on growth of juvenile halibut. *Journal of Fish Biology*. **54** (3): 556–572.

[13] Kaneko, J. J., Harvey, J. W., Bruss, M. L. (1997): *Clinical Biochemistry of Domestic Animal*s. Academic press. 931 p.

[14] Latifi F., Dhaskali L., Passantino L., Dimco E., Beqiraj, D., Mane B. (2008): Vlerësimi citomorfometrik i eritrociteve të koranit (*Salmo letnica*) në kushte natyrore. Kerkime, Nr. 16. Akademia e Shkencave dhe e Arteve të Kosovës. Seksioni i Shkencave të Natyrës. KDU5/6 : 061. 12 (496.51) 05: 105–112.

[15] Lermen C. L., Rosiele L., Crestani V. P. V., Gioda C. R., Schetinger M. R. C., Baldisserotto B., Moraes G., Morsch V. M. (2004): Effects of different temperature regimes on metabolic and blood parameters of silver catfish, *Rhamdia quelen*. *Aquaqulture.* 239: 497–509.

[16] Merkurjeva E. K. (1964): *Biometria v Zhivotnovodstvje*. 310 p.

[17] Nikinmaa M., Tuurala H., Sovio A. (1980): Thermoacclimatory changes in blood oxygen binding properties and gill secondary lamellar structure of *Salmo gardnieri. Journal of Comparative Physiology.* **B**. 140: 255–260.

[18] Rakaj N. (1995): *Iktiofauna e Shqipërisë.* ShBLU, Tiranë. 700 fq.

[19] Sauer D. M., Haider G. (1977): Enzyme activities in the serum of rainbow trout, *Salmo gardnieri* Richardson; the effects of water temperature. *Journal of Fish Biology.* **11** (6): 605–612.

[20] Spirkovski Z., Ilić-Boeva D. (2002): *Lake Ohrid Fish*. 79–82.

[21] Tetens V., Wells R. G. M., Devries A. L. (1984): Antarctic fish blood: respiratory properties and the effect of thermal acclimation. *J. Exp. Biol*. 109: 265–279.

[22] Yamamoto K., Itazawa Y., Kobayashi H. (1980): Supply of erithrocites into the circulating blood from the spleen of exercised fish. *Comparative Biochemistry and Physiology.* **65A**: 5–11.