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Red Blood Cell Indices for Rainbow Trout (*Oncorhynchus mykiss* Walbaum) Reared in Cage and Raceway Culture

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Abstract

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In this study, 161 immature female rainbow trout of weight 331 ± 30 g, raised in cage culture 291 m above sea level at water temperature 17 ± 3 ºC, were used to calculate reference (physiological) haematology values for red blood cell indices. Red blood cell counts (RBCc), haematocrit values (Hct), haemoglobin concentrations (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured and analyzed. The calculated intervals of the reference ranges between the lower and upper quantiles were: RBCc 0.87 – 1.34 T . l-¹, Hct 0.36 – 0.554, Hb 64 – 107 g . l-¹, MCV 347 – 501 fl, MCH 60 – 92 pg and MCHC 0.15 – 0.21. Multiple correlation indices obtained from cage culture fish to determine effects of time (Day), water temperature (WT), dissolved oxygen (O_2), oxygen saturation level of the water (OSW), chemical oxygen demand (COD_{Mn}), biological oxygen demand (BOD_5) and NH4+ were the following: RBCc was explained by Day and QOSW with coefficient of correlation r = 0.611, Hb was explained by $QBOD_5$ and QNH_4^+ with correlation r = 0.783 and MCHC was explained by COD_{Mn}, QDay and QO_2 with correlation r = 0.743. Fish from cage culture had significantly greater red blood cell indices (P = 0.01 and P = 0.0000, respectively) than fish from flow-through tanks 651 m above sea level, water temperature 9 ± 2.5 ºC and equal nutrition. The results have shown that fish farming technology, varying physical and chemical properties of water and availability of natural food may influence erythropoiesis in caged fish.

Red blood cell counts, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reference range

Rainbow trout farming in cage culture is a traditional means of aquaculture in the Czech Republic. It is primarily focused on productive utilization of multi-purpose fresh water retention lakes. In comparison with the classical ways of trout farming using raceway culture it allows reduced energy losses in the fish and offers opportunities to exploit resources of natural food. Irregular upward temperature fluctuations accompanied by oxygen dynamics and the presence of a wide spectrum of wild fish species living naturally outside cages present a risk to fish health. Haematological tests to provide information about the state of erythropoiesis. Previous haematological studies of nutritional effects (Řehulka 1984ab, 1989, 2000), infectious diseases (Řehulka 2002a) and pollutants (Řehulka 2002b) brought knowledge that erythrocytes are a major and reliable indicator of various sources of stress. Erythrocytes reflect the state of the organism over a prolonged period of time. Also, the outcome of a test is only slightly influenced by the timing of sample collection. From a clinical haematology standpoint, knowledge of the reference ranges of red blood cell indices is vital for impartial assessment of the samples. These values were previously described in work by Haider (1970, 1971, 1973), McCarthy et al. (1973, 1975), Sniezsko (1961), Wedemeyer and Nelson (1975), Miller et al. (1983) and Haley and Weiser (1985).

The objective of the current study of fish from cage culture was: (1) to define reference

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ranges for red blood cell counts, haematocrit values, haemoglobin concentrations, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration, (2) to determine whether any relationships could be found between the foregoing red blood cells indices and various physical and chemical indices of water, and (3) to underline differential erythropoiesis in fish from raceway culture as influenced by unequal environmental factors. The reference (physiological) ranges are defined by an upper and lower limit for each parameters that covers the majority of the values obtained for healthy fish.

Materials and Methods

Fish

The trial work was performed in two consecutive years using ten floating net cages of dimmensions $4 \times 4 \times 3$ m in multi-purpose dam lake of 0.365 mil. m^2 submerged area, 291 m above sea level (location A). Additionally, 20 flow-through tanks of dimmensions 5×0.8 0.8 m, 651 m above sea level (location B) were used. Stocking density in cages was 2000 fish, while in tanks it reached 300 fish. Both trial locations were supplied with water from the Oder river. Trials began on June 15 and were completed September 20 each calendar year.

The experimental fish were immature females of the Kamloops strain, equal origin, excellent body condition and health status. The fish were imported to the Czech Republic in 1966 (Kálal et al. 1970; Kálal 1989). The starting weight of the fish was from 88 to 100 g and standard length from 184 to 194 mm. The stage of fish reproductive development was assessed by microscopy. It was important in this study to obtain reliable health status information on the fish so as to avoid any distortion of the results by disease.

During the trial, parasitological examination (Ergens and Lom 1970), histological tests and post-mortem examinations (Roberts 1978; Lucký 1982) were carried out regularly in periods of 14 days.

The fish were fed twice daily with feeding rates corresponding 2% of body weight . day $^{-1}$ with uniform dry pellets with size 5 mm and with the following dietary formulation and chemical composition of the diets (g per kg): Fish meal 150, meat and bone meal 370, soya bean meal (free of fat) 230, wheat flour 140, hay meal 50, milk powder 30, dry matter 900, crude protein ($N \times 6.25$) 400, crude fat 100, ash 110, crude fibre 300 and nitrogen free extract 230. Vitamin and micromineral premix supplied per kg of diet: Vitamin A, 25,000 IU; vitamin D_3, 3,000 IU; α-tocopherol, 100 mg; vitamin K_3, 10 mg; thiamin, 10 mg; riboflavin, 10 mg; niacin, 10 mg; pyridoxine, 10 mg; vitamin B_{12}, 0.025 mg; Ca-pantothenate, 50 mg; ascorbic acid, 1000 mg; folic acid, 2.5 mg; biotin, 0.2 mg; Fe, 50 mg; Cu, 5.5 mg; Co, 0.5 mg; Se, 0.2 mg; Zn, 42 mg; Mn, 40 mg; I, 0.8 mg; choline chloride, 900 mg; inositol, 500 mg. Prior to the experiment, the quality of pellets, fed to the fish, was assessed as defficiency free. Main attention was paid to evaluation of the pellet quality with respect to the fat component using the peroxide value and 2-thiobarbituric acid number (Hilton and Slinger 1981; Řehulka and Jirásek 1987; Řehulka 1990).

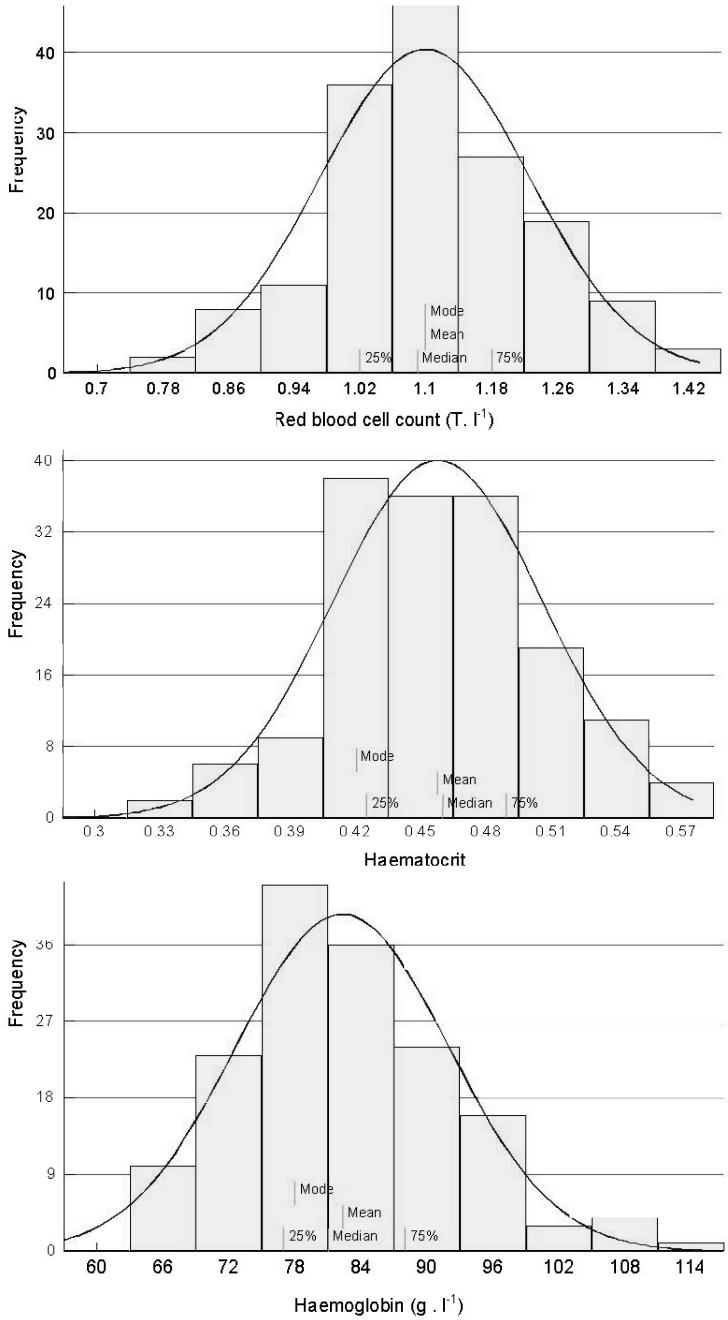
In both locations, a total of 307 fish were processed, of which 161 fish were from location A and 146 fish were from location B.

Water temperature and chemical properties through both trial periods were monitored. Mean values recorded were 17 ± 3 °C (A) and 9 ± 2.5 (B), dissolved oxygen 8.7 ± 0.77 mg l^{-1} (A) and 9.4 ± 0.5 (B) and the O_2 saturation level of the water was 93 ± 12.1 % (A) and 86 ± 4.2 (B). Additional chemical properties of water from location A or location B (in parentheses) were the following: pH 6.8 (7.6), $ANC_{4.5}$ (acid neutralizing capacity) 0.8 mmol . l^{-1} (0.7), $BNC_{8.3}$ (base neutralizing capacity) 0.02 mmol . l^{-1} (0.03), COD_{Mn} (chemical oxygen demand) 1.7 mg . l^{-1} (0.8), BOD_5 (biological oxygen demand) 2.3 mg . l^{-1} (1.6), NH_4^+ 0.12 mg . l^{-1} (0.09), NO_2^- 0.07 mg . l^{-1} (0.03), NO_3^- 14.8 mg . l^{-1} (6.8), PO_4^{3-} 0.01 mg . l^{-1} (0.02), SO_4^{2-} 24.9 mg . l^{-1} (9.7), Cl^- 7.1 mg . l^{-1}(2.8), Mg^{2+} 9.7 mg . l^{-1} (4.2), Ca^{2+} 24 mg . l^{-1} (17), total iron 0.06 mg . l^{-1} (0.10) and total hardness 5.6 ºN (3.4).

Experiments to test relationships among haematological indices and time (Day), water temperature (WT), dissolved oxygen (O_2), oxygen saturation level of the water (OSW), chemical oxygen demand (COD_{Mn}), biological oxygen demand (BOD_5 and NH_4^+ were carried out only in the first year in location A (n = 68). Choice of dependent variables and method of data collection were performed in a similar way to the work preceeding this study (Řehulka 1997).

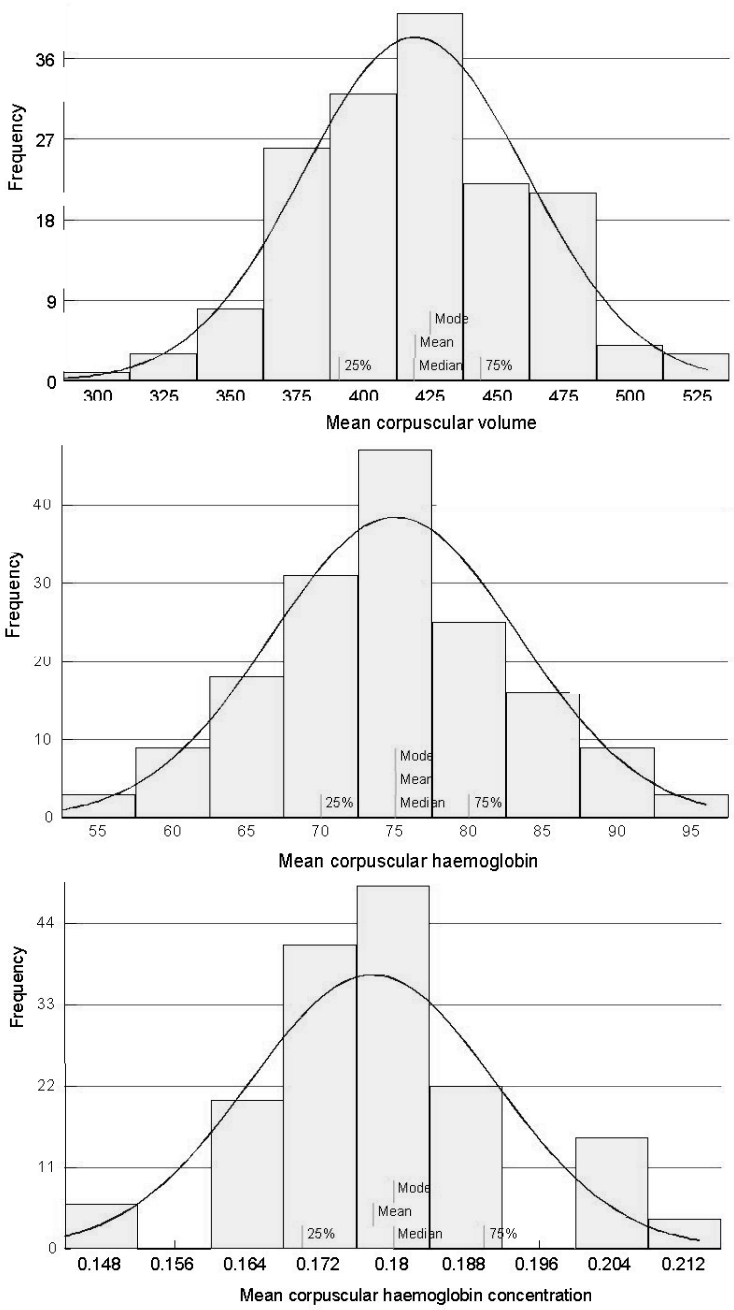
Preparation of blood samples

Blood samples were collected at the end of the experiment when water temperature reached 15 ºC (A) and 7 ºC (B). The fish were anesthetized with Menocain (Spofa) (3-aminobenzoic acid ethylester natrium hydrogen sulphate) (Král 1988) at a concentration of 0.05 g . l^{-1} and the blood samples were taken by puncturing the caudal vessels from 08.00 to 12.00 h. EDTA (ethylenediaminetetraacetate) was used as anticoagulants. The red blood cell counts (RBCc T . l^{-1}) were determined by using a Bürker couting chamber and Hayem solution and the erythrocytes were counted in 2×20 rectangles per sample. Haematocrits (Hct) were determined in duplicate by using microhaematocrit-heparinized capillary tubes and a microhaematocrit centrifuge (15 250 *g* for 3 min.). Hct values were determined within 30 minutes following blood centrifuging which, in turn, had followed immediately after taking the blood samples (Soivio and Nyholm 1973).Haemoglobin concentrations (Hb g . l^{-1}) were determined by the cyanhaemoglobin method, using a wavelength of 540 nm. RBCc and Hb values were determined within 6 hours after blood sampling. The derived blood

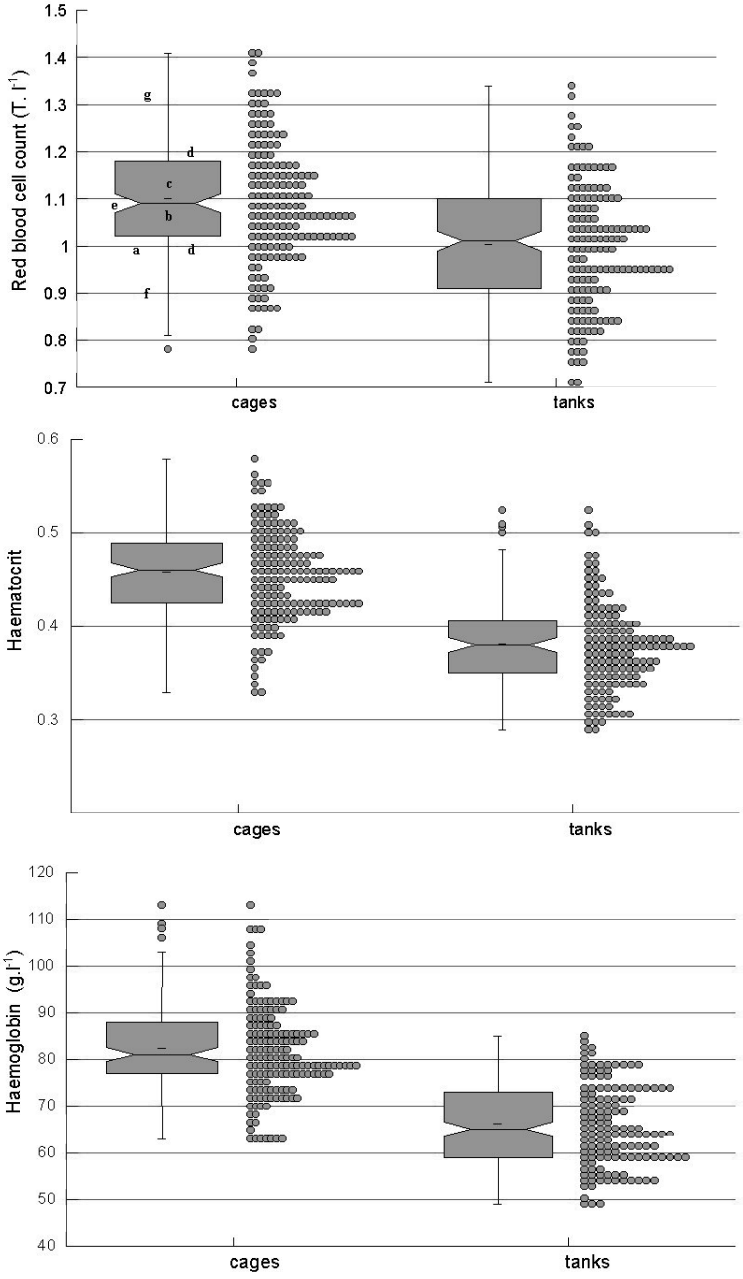


Figs 1-6 (top to bottom). *Oncorhynchus mykiss*. Frequency distribution for haematological parameters of immature female rainbow trout from cage culture

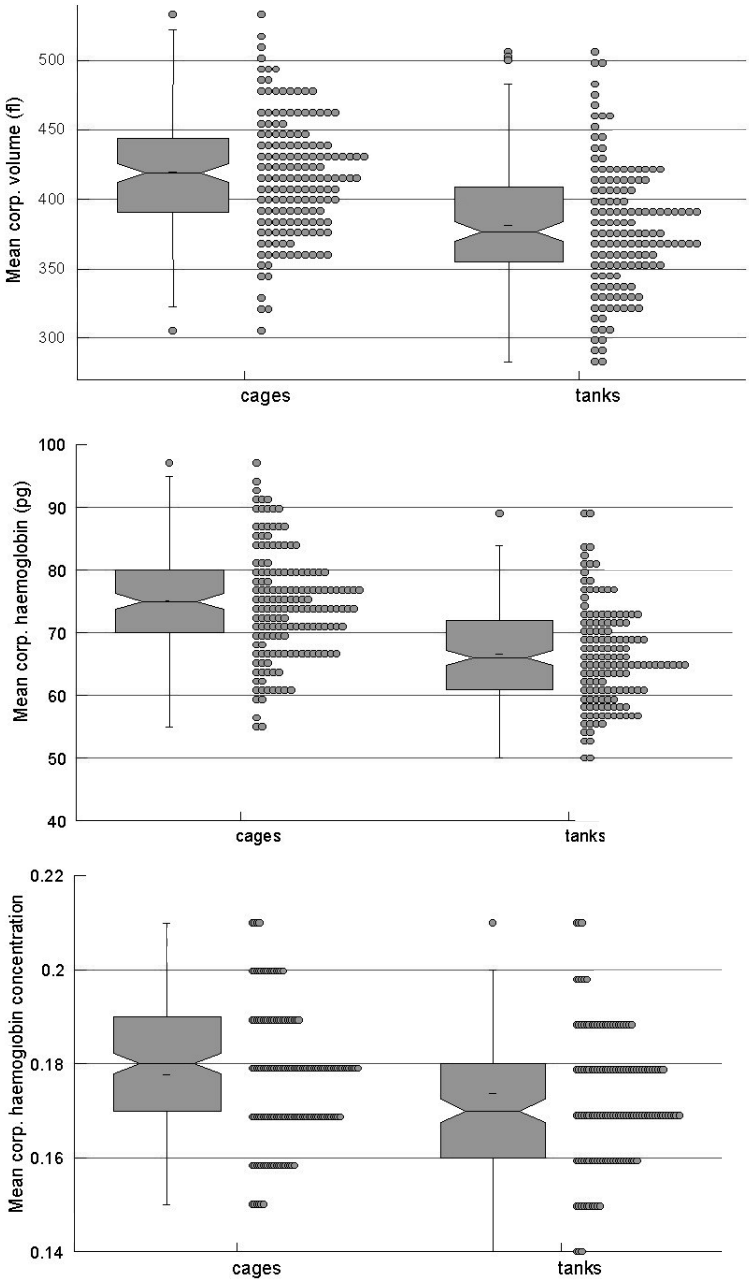
indices of mean corpuscular volume (MCV fl), mean corpuscular haemoglobin (MCH pg) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae. The findings and instructions published



by Blaxhall and Daisley (1973), Soivio and Nikinmaa (1981), Svobodová et al. (1986) and Wells and Weber (1991) were respected when the RBC indices were determined. Blood sampling in location A was performed in the first year from 77 fish at water temperature between 16.8 and 18.1 ºC. In the second year it occurred at water temperature 15 to 16.3 ºC. In location B, 70 fish were examined in the first year, while in the second year 76 fish were processed at water temperature 7 to 9 ºC. At the end of trial, haematological analysis was performed in each location within three days.



Figs 7-12 (top to bottom). *Oncorhynchus mykiss.* Haematological indices of immature female rainbow trout from cage and flow-through tanks: *a* = width of the box, indicating the size of the set; *b* = mid - diagonal of the box, representing the position of the median in relation to the y - axis; **c** = mark inside the box, showing the position of the arithmetic mean; *d* = lower and upper edge of the box, indicating successively the position of the lower and upper quartiles; *e* = width of the notch, corresponding to the confidence interval around the median; *f* = the lower filament, with a length corresponding to the value of the lower quartile reduced by 1.5 times the span of the quartiles.



If this value is lower than the minimum value in the set, the length of the filament corresponds to this minimum value. If values lower than those corresponding to the coordinate of the end point of the lower filament do occur in the set, then these values are signalled as remote; *g* = the upper filament, with a length corresponding to the value of the upper quartile enlarged by 1.5 × the span of the quartiles. If this value is higher than the maximum value in the set the length of the filament corresponds to this maximum value. If values higher than those corresponding to the coordinate of the end point of the upper filament do occur in the set, then these values are signalled as remote.

Blood sampling to determine relationships between haematological indices and physical and chemical characteristics of water were done in 10 trout, seven times in regular 14-day intervals. After blood samples were collected, characteristics of fish weight and external measurements were obtained.

The haematological parameters are expressed in international units (SI).

Statistical analysis

The basic variable characteristics were used during the statistical processing and a direct non-parametric method (Racek 1999) that permits assessmens of data that does not have a normal frequency distribution, was used for determining the reference boundaries for each haematological parameter. The reference boundaries were defined as $100\ \frac{p}{2}$ % (lower reference limit) and $100 - 100\ \frac{p}{2}$ % (upper reference limit) quantiles. 100 – 100p % of individuals of the reference group staying between the reference limits and these were determined by the defined values of the lower and upper quantiles. The remaining 100p % of the individuals with the lowest and highest values were outside the reference limits. Quantile for $P = 0.05$ was chosen, which means 95% probability, that the calculated values of the reference group were within the entire reference population.

To test normality, we used the Kolmogorov-Smirnov test, which compares the absolute frequency sums kn (x_1) for i = 1,2,…, with the theoretical frequencies on basis or the distribution function for normal distribution $\varphi(x_i)$. Having performed the Kolmogorov-Smirnov test, we prepared a graphical representation of the results. The experimental data were represented by in each case a histogram and a normal distribution curve, with delineation of the position of the median and mode. The $n\varphi(x_i)$ values for the individual x_i were plotted on lines perpendicular to the horizontal (x) axis.

To compare the RBC indices among fish kept in cage or flow-through tanks, we used the F-test to establish the homogeneity of distribution and t-test to establish the differences between the levels.

To test the effects of physical or chemical characteristics of water on RBCc, Hct, Hb, MCV, MCH, MCHC stepwise regression method was selected. Quadratic function appeared to be the most optimal. Variables Day, WT, O_2, OSW, COD, BOD_5 and NH_4^+ were treated as independent variables.

Results

The calculated intervals of the reference values between the lower and upper quantiles in fish from cages were calculated for RBCc 0.87 - 1.34, Hct 0.36 - 0.554, Hb 64 - 107), MCV 347 - 501, MCH 60 - 92 and MCHC 0.15 - 0.21. The distribution and density of the tested characteristics in the selected reference groups is made possible by the use of histograms (Figures 1 - 6). The histograms showed normal distributions for the haematological parameters except for MCHC. During evaluations of RBC indices, it was confirmed that fish from cages had significantly greater values of RBCc, Hct, Hb, MCV and MCH ($p = 0.0000$) and in MCHC ($p = 0.01$) relative to the tank technology in both experimental years. RBCc were found in range from 0.78 to 1.41 vs. 0.71 – 1.34, Hct 0.329 – 0.579 vs. 0.289 – 0.524, Hb 63 – 113 vs. 49 – 85, MCV 305 – 533 vs. 283 – 506, MCH 55 – 97 vs. 50 – 89, MCHC 0.15 – 0.21 vs. 0.14 – 0.21 in fish from cages or tanks, respectively. These apparent differences are shown in Figures 7 to 12.

During the experiment, no fish ailments were observed in any location and subsequently preventive veterinary measures were not required. The findings recorded during the regular health checks and during blood collections, included mild infections by *Trichodina* sp. on the skin and fins and a sporadic occurrence (2 to 4 specimens) of *Gyrodactylus bohemicus*.

To assess the dependencies between RBC indices and Day, WT, O_2, OSW, COD_{Mn}, BOD_5, and NH_4^+, the statistical model included only those explanatory variables from the 14 original ones, which displayed a significant coefficient when describing variation of response variable. For individual RBC indices the following regression conclusions were found: RBCc was explained by Day and QOSW with determination coefficient of 37% and coefficient of correlation r = 0.611 and regression equation $RBCc' = 0.7312 + 0.0030QDay + 0.0000OSW$. Hb is explained by $QBOD_5$ and QNH_4^+ with determination coefficient of 61% and coefficient of correlation r = 0.783 and regression equation $Hb' = 67.2552 + 1.7263QBOD_5 + 563.0897QNH_4^+$. MCHC is explained by COD_{Mn}, QDay and QO_2 with determination coefficient of 55%, correlation coefficient of 0.743 and equation $MCHC' = 0.0837 + 0.0317COD + 0.0000QDay + 0.0003QO_2$.

Discussion

This study enhances knowledge of physiological values of RBC indices in rainbow trout farmed in cage culture and follows the findings described in prior work of Řehulka (1997), devoted to evaluations of the dynamics. Methodology chosen for this study provided further elucidation of the factors influencing erythropoiesis in accordance with the applied farming technology. This was emphasized in an early Czech study by Pravda (1984) who monitored Hct, Hb a MCHC in rainbow trout farmed in flow-through tanks, cages and ponds. For cultured fish from cages or flow-through tanks a reduction of Hct by 31%, of Hb by 15% and of MCHC by 8.5% was reported relative to fish raised in ponds. The current values from both types of technology generally agree with the range of values reported by McCarthy et al. (1973) in juvenile rainbow trout of Kamloops variety. The exception was Hct, where the present range of values became wider, especially in cage farmed fish (0.329 – 0.579 vs. 0.5 – 0.49). Especially interesting were results reported by the following authors (McCarthy et al. 1975) in juvenile rainbow trout of Shasta variety. Especially extreme values of RBCc (0.46), Hct (0.24) and Hb (42) in comparison with fish from cages and flow-through tanks. Rather unusual were wide ranges of values in RBCc (0.28 – 1.72) of Shasta mature female together with MCV (238 – 916) and MCH (65.6 – 336) and also low value of Hb (15) in lower limit percentile u two-year-old rainbow trout of Whytheville strain (Miller et al. 1983). Such extremely small values of RBCc were not encountered in the present diagnostic and experimental research except for fish, where poor health status was caused by food contaminated with oxidized lipids and (or) defficient with tocoferol (Řehulka 1989), or when infections of bacterial (*Aeromonas*) (Řehulka 2002) or viral (VHS) etiology (Řehulka 2003) were present. Extremely low water temperatures caused the values of Hb (29), Hct (0.1) and RBCc (0.2), which was explained by Dobšinská et al. (1980) with temperatures of 7.9 °C lead us to the conclusion that water temperature above 15 °C observed in cage technology could have influenced increase of RBC indices more profoundly than farming technology or chemical characteristics of water. It can be worthwhile to verify conclusions of Denton and Yousef (1975) with additional trials, who found seasonal fluctuations in Hb, Hct, MCV, MCH and MCHC. Also, they reported, that water temperature does not seem to be responsible for variations in haematology of rainbow trout and that diet, metabolic adaptations and activity were probable causes of seasonal changes. These authors underscored activity of fish, which was in our experiments higher in flow-through tanks and caused increased appetite in comparison to the cage technology. In cages, frequent increases of water temperature up to 18 or 20 ºC caused probably the smaller appetite. Evidence to this conclusion were noticeable differences in weight at the closure of the experiment with greater weight gains in fish from flow-through tanks (331 ± 30 vs. 197 ± 53 g). To elucidate the topical research questions, trials to test several factors simultaneously (Martinez et al. 1994) should be performed. The authors investigated the effect of the simultaneous influence of weight, temperature, stocking density and O_2 concentration in the water on RBCc, Hct, Hb, MCV, MCH and MCHC. Temperature ranged from 15 to 20 °C and O_2 concentration in the water 5.8 ± 2 to 8.6 ± 2 ppm. Multiple correlation and regression analyses showed strong dependence of RBCc, Hct and Hb on the factors considered, of which the most influential was temperature. Multiple correlation (r_m) and determination (r^2) coefficients were 0.62 and 38.44% for RBCc, 0.66 and 43.36% for Hct and 0.71 and 50.41% for Hb. Values of multiple correlations to test effects of chemical properties of water and temperature on RBC indices were only slightly informative.

Results from this study contribute to the knowledge of RBC indices in cage farmed rainbow trout in the Czech Republic. At the same time, it was shown, that the most thoroughly constructed haematological profile of rainbow trout requires repeated

measurements is some cases. We were able to enrich the applied and basic research, needs of practical farming and maintaining the optimum physiology and prevention of fish losses in large-scale trout farming. From the veterinary point of view it is important to know that both systems of fish breeding technology, in which erythropoiesis is influenced by a number of environmental factors, must be taken into account when RBC indices are evaluated. This fact encourages the effort to assess in an experimental manner the causes underlying erythropoiesis and to evaluate the RBC indices in the traditional raceway culture where rainbow trout are for the most part reared in the Czech Republic.

Ukazatelé červeného krevního obrazu u pstruha duhového *(Oncorhynchus mykiss* Walbaum*)* v plovoucích klecových odchovnách a průtočných bazénech

U 161 juvenilních pstruhů duhových samičího pohlaví o hmotnosti 331 ± 30 g odchovávaných v plovoucích klecích na víceúčelové nádrži (291 m.n.m.) při teplotě vody 17 ± 3 °C bylo vypočteno referenční (fyziologické) rozmezí počtu erytrocytů (RBCc), hematokritu (Ht), hemoglobinu (Hb), středního objemu erytrocytu (MCV), hemoglobinu erytrocytu (MCH) a střední barevné koncentrace (MCHC). Vypočtené intervaly referenčních hodnot vymezené dolním 2,5 % a horním 97,5 % kvantilem byly následující: RBCc 0,87 – 1,34 $T.l^{-1}$, Hct 0,36 – 0,554, Hb 64 – 107 g . l^{-1}, MCV 347 – 501 fl, MCH 60 – 92 pg, MCHC 0,15 – 0,21. Mnohonásobné korelace prováděné ve stejných podmínkách za účelem ověření vlivu času (Day), teploty vody (WT), rozpuštěného kyslíku (O_2), nasycenosti vody kyslíkem (OSW), chemické spotřeby kyslíku (COD_{Mn}), biologické spotřeby kyslíku (BOD_5) a NH_4^+ na hematologické ukazatele, přinesly tyto výsledky: RBCc vysvětluje Day a QOSW s korelačním koeficientem r = 0,611, Hb vysvětluje $QBOD_5$ a QNH_4^+ s koeficientem korelace r = 0,783 a MCHC vysvětluje COD_{Mn}, QDay a QO_2 s koeficientem korelace 0,743. Bylo zjištěno, že ryby z klecí měly signifikantně (P = 0,01 a P = 0,0000) vyšší hodnoty všech hematologických ukazatelů než pstruzi z průtočných bazénů (651 m.n.m., teplota vody 9 ± 2,5 °C, stejná výživa). Výsledky ukázaly, že odlišné fyzikální a chemické vlastnosti vody, způsob chovu a možnost příjmu přirozené potravy u ryb z klecí mohly ovlivnit krvetvorbu.

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