Erythrocyte Profile of Diploid and Triploid Silver Crucian Carp (*Carassius auratus*)

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Abstract

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Haematological analysis was performed on 27 adult specimens of *Carassius auratus* irrespective of sex in 2003 and on 32 juveniles of distinguished sex in 2004. In this study we found that the ploidy level affected significantly ($p < 0.01$) the values of the erythrocyte count, mean corpuscular volume and mean corpuscular haemoglobin. Although we did not prove any significant effect of sex in juvenile diploids of *C. auratus* on the values of erythrocyte profile, the erythrocyte count, haematocrit value and haemoglobin content value were higher for males than for females. The erythrocyte count decreased significantly ($p < 0.01$) with increasing ploidy level. The index of haemoglobin content followed the same trend of a decreasing mean value with increasing ploidy level. Mean corpuscular volume and mean corpuscular haemoglobin increased with the increasing ploidy level ($p < 0.01$). Haematocrit value and mean corpuscular haemoglobin concentration did not significantly differ from the point of view of the ploidy level.

Carassius auratus, ploidy level, complete red blood count, erythrocyte nuclear dimensions

The alien species of silver crucian carp (*Carassius auratus*) has become an integral part of ichthyofauna of the Czech Republic in the last third of the 20th century (Lusková et al. 2004). Original populations of *C. auratus* that have begun to occupy this region were considered to consist exclusively of triploid females with gynogenic reproduction (Peňáz et al. 1979). During the last decade, there have been increasingly frequent findings of males and specimens with various ploidy levels (diploid, triploid, tetraploid) in both sexes (Halačka et al. 2003; Lusková et al. 2004). Populations of this "species" gain the character of a diploid-polyploid complex with the majority of triploid individuals. In association with these changes, we studied a possible effect of ploidy level on the erythrocyte profile, as it has never been studied yet in native populations of *Carassius auratus* "*gibelio*". Most published studies which compared haematological indices to the ploidy level were performed in association with artificially induced changes of original natural ploidy level of given specimens (Beck and Biggers 1983; Svobodová et al. 1998; Flajšhans and Vajcová 2002, and others). Comparison of haematological indices of di- and triploid fish was most frequently performed in salmonids (Benfey and Sutterlin 1984ab; Benfey et al. 1984; Small and Benfey 1987; Cogswell et al. 2001 and others), sturgeons (Palíková et al. 1999; Flajšhans and Vajcová 2000), exceptionally in other fishes, as e.g. *Ictalurus punctatus* (Wolters et al. 1982), diploid and triploid hybrids of *Ctenopharyngodon idella* and *Hypophthalmichthys nobilis* (Beck and Biggers 1983), *Tinca tinca* (Flajšhans 1997; Svobodová et al. 1998), or *Umbrina cirrosa* (Ballarin et al. 2004). Different sizes of erythrocytes of diploids and triploids (Sezaki et al. 1977; Benfey et al. 1984; Flajšhans 1997; Svobodová et al. 1998) are frequently used for determination of ploidy level in some fish species, as e.g. *Cobitis biwa* (Sezaki et al. 1988), *Cobitis taenia* (Boron 1994) and *Carassius auratus* (Halačka and Lusková 2000).

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Materials and Methods

In the course of research on *C. auratus* from the area of confluence of Dyje and Morava rivers, we analyzed a total of 59 specimens. The first sampling was performed in August 2003 when we caught 27 adult specimens of *C. auratus* (5 diploids, 21 triploids and 1 tetraploid). Standard length (SL) and weight (W) were registered. Fish were released after blood sampling, and therefore sex was not determined. During the second sampling in October 2004, we caught 32 juvenile *C. auratus* (11 diploid males and 8 diploid females, and 13 triploid females). These fish were sacrificed after blood sampling, and their sex could be detected. Blood was sampled by a punction of the caudal vessel into a heparinized syringe. Analysis of the ploidy level was performed by the method of computer-assisted analysis of microscopic image (image cytometry, ICM, Plate VI, Fig. 1). Blood smears for ICM were stained 10 min in Harris haematoxylin (Sigma - Aldrich). Ploidy-diagnostic dimensions (area, perimeter, maximum and minimum diameter of erythrocyte nucleus) were determined by means of bright field microscopy, image capture by CCD camera and processing the image information by Olympus MicroImage 4.0 software (Flajšhans 1996; Flajšhans 1997; Halačka and Lusková 2000). The following indices of erythrocyte profile were registered for statistic evaluation: erythrocyte count (Er), haematocrit value (PCV), haemoglobin content (Hb) and derived indices: mean corpuscular volume (MCV = PCV/Er), mean corpuscular haemoglobin (MCH = Hb/Er) and mean corpuscular haemoglobin concentration (MCHC = Hb/PCV). Methodology followed Svobodová et al. (1986) and Lusková (1996). The effect of the ploidy level on the haematological indices was tested using Mann - Whitney test in the Statistica 6.0 software.

Results

Values of standard length, weight and of haematological indices of 27 adult specimens of *C. auratus* captured in 2003 without sex differentiation are given in Table 1. The erythrocyte count decreased significantly with the increasing ploidy level: 1.69 ± 0.19 T.l^{-1} in diploid specimens, 1.06 ± 0.12 T.l^{-1} in triploids and 0.59 T.l^{-1} in a tetraploid specimen. The index of haemoglobin content followed the same trend of a decreasing mean value with the increasing ploidy level: 79.00 ± 5.79 g.l^{-1} in diploid specimens, 74.38 ± 11.58 g.l^{-1} in triploid specimens, and 59.00 g.l^{-1} in the tetraploid specimen (Table 1). Mean values of the mean corpuscular volume (223.50 ± 21.75 fl in diploids, 319.47 ± 72.34 fl in triploids and 508.47 fl in a tetraploid specimen) and mean corpuscular haemoglobin (46.99 ± 3.89 pg in diploids, 71.18 ± 12.53 pg in triploids and 100.00 pg in a tetraploid specimen) indices increased with the increasing ploidy level in accordance with their mathematic interpretation. Haematocrit value and mean corpuscular haemoglobin concentration did not significantly differ from the point of view of the ploidy level (Table 1).

Table 1. Comparison of values of erythrocyte profile in *C. auratus* specimens differing with ploidy level (2n, 3n, 4n) in 2003 (**$p < 0.01$).

Indices	Unit	2n		3n		4n
		mean	SD	mean	SD	mean
Number of individuals (n)		5		21		1
Standard length (SL)	mm	233	39.17	261	22.53	230
Body weight (W)	g	406	212.56	601	158.35	418
Erythrocyte count (Er)	T·l^{-1}	1.69**	0.19	1.06**	0.12	0.59
Haematocrit value (PCV)	l·l^{-1}	0.38	0.05	0.33	0.06	0.30
Haemoglobin content (Hb)	g·l^{-1}	79.00	5.79	74.38	11.58	59.00
Mean corpuscular volume (MCV)	fl	223.50**	21.75	319.47**	72.34	508.47
Mean corpuscular haemoglobin (MCH)	pg	46.99**	3.89	71.18**	12.53	100.00
Mean corp. hem. concentration (MCHC)	l·l^{-1}	0.21	0.03	0.23	0.03	0.20
Nuclear area	µm^2	10.89**	0.45	14.83**	0.41	17.76
Nuclear perimeter	µm	12.39**	0.34	15.12**	0.28	16.93
Nuclear major axis	µm	4.69**	0.19	5.97**	0.15	6.73
Nuclear minor axis	µm	2.66**	0.03	2.85**	0.04	3.00

Values of standard length, weight and of haematological indices of 32 juveniles of *C. auratus* captured in 2004 with distinguished sex are given in Table 2. As no effect of sex

was found on the values of haematological parameters, the category of juvenile diploids was pooled and assessed regardless of sex. The category of triploids was female monosex. The erythrocyte count decreased significantly with the increasing ploidy level (see Table 2). The index of the haemoglobin content followed the same trend of a decreasing mean value with the increasing ploidy level. Mean values of the mean corpuscular volume and mean corpuscular haemoglobin indices increased with the increasing ploidy level. Haematocrit value and mean corpuscular haemoglobin concentration did not significantly differ from the point of view of the ploidy level (Table 2).

The ploidy level affected significantly ($p < 0.01$) the values of Er, MCV and MCH (Tables 1, 2). A highly significant increment in values of erythrocyte nuclear area, nuclear perimeter, nuclear major axis and nuclear minor axis in triploid specimens compared to diploid ones (Tables 1, 2) were also reflected in the MCH and MCV indices.

Table 2. Comparison of values of erythrocyte profile in *C. auratus* specimens differing with ploidy level (2n, 3n) in 2004 (*$p < 0.05$; **$p < 0.01$).

Indices	Unit	2n males + females		3n females	
		mean	SD	mean	SD
Number of individuals (n)		19		13	
Standard length (SL)	mm	89	4.45	91	3.95
Body weight (W)	g	22	3.16	24	2.90
Erythrocyte count (Er)	$T \cdot l^{-1}$	1.70**	0.12	1.05**	0.10
Haematocrit value (PCV)	$l \cdot l^{-1}$	0.31	0.04	0.31	0.03
Haemoglobin content (Hb)	$g \cdot l^{-1}$	68.95*	5.64	65.43*	9.04
Mean corpuscular volume (MCV)	fl	185.09**	20.85	297.84**	40.52
Mean corpuscular haemoglobin (MCH)	pg	40.51**	2.69	62.27**	6.94
Mean cor. hem. concentration (MCHC)	$l \cdot l^{-1}$	0.21	0.03	0.21	0.03
Nuclear area	μm^2	11.01**	0.48	15.52**	0.35
Nuclear perimeter	µm	12.65**	0.44	15.67**	0.22
Nuclear major axis	µm	4.89**	0.23	6.29**	0.12
Nuclear minor axis	µm	2.64**	0.05	2.83**	0.04

Discussion

There are no current erythrocyte profile values in *C. auratus* from Central European wildlife. Only Sezaki et al. (1991) compared the effect of ploidy level on erythrocyte profile of diploid and triploid specimens of *C. auratus langsdorfii*. Considering the range of weight (7 - 25 g) stated by these authors, their specimens were probably juvenile. Value of Er significantly decreased with the increasing ploidy level (1.97 ± 0.18 $T.l^{-1}$ in diploids and 1.44 ± 0.09 $T.l^{-1}$ in triploids). Haemoglobin content followed the same trend of decreasing mean value with increasing ploidy level (96.00 ± 7.00 $g.l^{-1}$ in diploids, 91.00 ± 9.00 $g.l^{-1}$ in triploids). Haematocrit value (0.42 ± 0.03 $l.l^{-1}$) and the derived MCHC value (0.23 ± 0.01 $l.l^{-1}$) did not differ significantly with ploidy levels. Mean values of MCV and MCH did not increase significantly with the increasing ploidy level (Sezaki et al. 1991). All values registered in this subspecies showed a similar trend, but absolute values were higher than ours.

Although we did not prove any significant effect of sex in juvenile diploids of *C. auratus* on the values of erythrocyte profile, Er, PCV and Hb values were higher for males than for females. Most studies give either significantly, or non-significantly higher values of Er, PCV and Hb for males than for females. Hlavová (1993) came to this conclusion in *Thymallus thymallus*, Lusková et al. (1995) in *Chondrostoma nasus*, Lusková and Halačka (1996) in *Barbus barbus* and Svobodová et al. (1998) in *Tinca tinca*.

According to Ihssen et al. (1990) and Benfey (1999), in polyploid specimens the volume of the entire cell increases with the increasing volume of cell nucleus, which is associated with reduction of the number of cells in the particular tissue.

Although we did not measure the oxygen content in the blood of diploids and triploids, our values of erythrocyte profile indices support hypotheses of the authors mentioned below, on higher oxygenation capacity of blood complex of diploid fish compared to triploids, in association with decreased Er and Hb with increased ploidy level of *C. auratus*. Svobodová et al. (1998) mentioned a possibility of lower oxygenation capacity of blood in triploid *Tinca tinca*. Enhanced respiration frequency in triploid *C. auratus langsdorfii,* compared to diploids, was reported by Sezaki et al. (1991). According to Graham et al. (1985) in *Salmo salar* the oxygen content in the blood of triploids gained only 68% of those in diploids. According to Benfey and Sutterlin (1984b), there was no difference between diploid and triploid *Salmo salar* in the oxygen consumption as the fish probably compensated it by enhanced respiration frequency (Sezaki et al. 1991).

According to conclusions of Benfey (1999), the majority of haematological studies of polyploid fish mention similar haematocrit value of specimens with different ploidy levels, in accordance with our results.

Červený krevní obraz u diploidního a triploidního karasa stříbřitého (*Carassius auratus*)

Hematologické vyšetření jsme provedli v roce 2003 u 27 adultních jedinců *Carassius auratus* bez diferenciace pohlaví a v roce 2004 u 32 juvenilních jedinců pohlavně diferencovaných. V této studii ploidie významně ovlivnila ($p < 0,01$) hodnoty ukazatelů počtu erytrocytů, středního objemu erytrocytů a hemoglobin erytrocytu. I když jsme u juvenilních diploidů *C. auratus* statisticky významně neprokázali vliv pohlaví na hodnoty červeného krevního obrazu, počet erytrocytů, hematokritovou hodnotu a koncentraci hemoglobinu měli samci vyšší než samice. Počet erytrocytů se statisticky významně snižoval ($p < 0,01$) se zvyšováním ploidie. Shodný klesající trend průměrných hodnot s růstem úrovně ploidie měl ukazatel koncentrace hemoglobinu. Střední objem erytrocytů a hemoglobin erytrocytu se s růstem ploidie zvyšovaly ($p < 0,01$). Hematokritová hodnota i hodnota střední barevné koncentrace se z hlediska ploidie statisticky významně nelišily.

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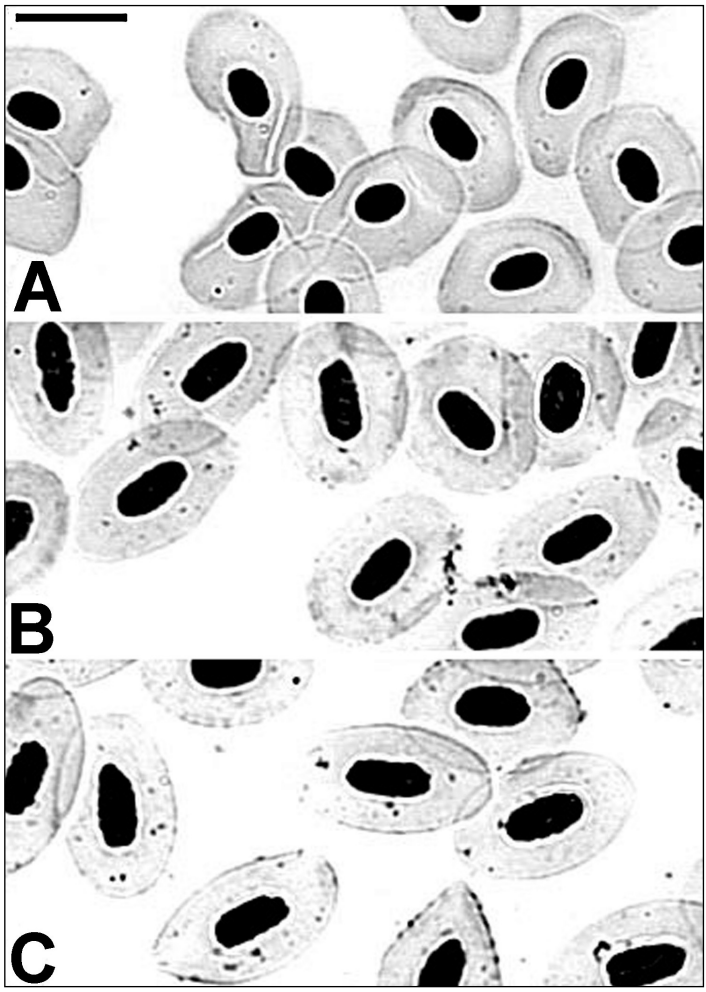

Size comparison of erythrocyte nuclei of *C. auratus* according to the ploidy level (A - diploid, B - triploid, C - tetraploid). Bar indicates 10 µm