Effects of Eugenol and MS-222 Anaesthesia on Siberian Sturgeon *Acipenser baerii* Brandt

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Abstract

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The aim of the present study was to assess changes induced in the organism of Siberian sturgeon (*Acipenser baerii*) by eugenol and MS-222 anaesthesia on the basis of haematological indices, biochemical blood profile values and histological examinations. The haematological and biochemical indices were assessed in fish immediately and 24 h after anaesthesia. The results showed that despite no mortality occurred after anaesthesia in both 125 $mg \cdot l^{-1}$ of MS-222 and 0.075 $ml \cdot l^{-1}$ of eugenol, these chemical substances severely influenced the constituents of Siberian sturgeon blood and resulted in some histological changes in the gills and liver. Both eugenol and MS-222 anaesthesia caused erythrocyte swelling and haemolysis. The severe depletion of leukocyte number occurred 24 h after both eugenol and MS-222 anaesthesia (mainly due to depletion of lymphocyte, neutrophil segments and eosinophil fractions). Total protein, total globulin, triacylglycerol concentration and alanine aminotransferase activity in blood plasma was significantly elevated ($p < 0.01$) after both eugenol and MS-222 anaesthesia. The concentration of Ca^{2+}, inorganic phosphate, NH_3 and alkaline phosphatase activity were significantly decreased ($p < 0.01$) compared to control. No significant changes were noticed in the albumin and glucose concentrations and the activity of lactate dehydrogenase, aspartate aminotrasferase and creatinkinase. Results of the examinations suggest that the use of MS-222 (125 $mg \cdot l^{-1}$) and eugenol (0.075 $ml \cdot l^{-1}$) does not cause irreversible damage in Siberian sturgeon.

Anaesthetics, blood, stress, Acipenseridae

Sturgeons are considered to be ‘‘living fossils’’ (Bemis et al. 1997). Primitive characteristics, such as a heterocercal tail and cartilaginous skeleton, have been maintained over approximately 100 - 200 million years despite major environmental changes (Baker et al. 2005; Asadi et al. 2006). Sturgeons have undergone multiple genome duplications during their evolution, which may account for their resistance to deleterious mutations, since there are probably several functional copies of every gene (Blacklidge and Bidwell 1993). Their primitive characteristics make sturgeons intriguing animals for study, since their biochemical haematological profile may differ substantially from the teleost profile.

All sturgeon species worldwide are covered under the provisions of CITES. Several species are considered threatened with extinction as a result of over-fishing, poaching, water pollution, damming and destruction of natural watercourses and habitats (Anonymous 2002). The culture of sturgeons is one of the on-growing aquaculture branch in Europe due to the need of active protection of natural populations and the high demand for caviar.

Usually the big size and sharp bony shields on the body surface make handling of sturgeon spawners difficult and dangerous for the operating personnel. The use of general anaesthetics is a common practise in sturgeon culture, especially during artificial

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propagation. Anaesthetics are also used during sorting, tagging, surgery and other stress-inducing procedures.

Propiscin (Polish Inland Fisheries Institute; active substance is etomidate), administered on gills in the form of spray, is commonly and successfully used for immobilisation of sturgeons in Poland. However, preparations based on etomidate are not suitable for general anaesthesia because they probably do not bring analgesic effects and the recovery time is quite long (Brown 1988, own experience).

MS-222 (tricaine methanesulphonate) is probably the most frequently used anaesthetic world-wide in sturgeons (Wilga and Lauder 1999; Kirschbaum et al. 2000; Jodun et al. 2001; Jackson et al. 2002; Mohler 2003). It induces general anaesthesia in a short time and produces analgesia and good myorelaxation. Recovery time is usually short. However, the use of MS-222 is not recommended in some sturgeon species (pallid sturgeon *Scaphirhynchus albus*) due to adverse effects, including mortality (Wanner 2006).

In the last decade, many authors have published data addressing the anaesthetic properties of clove oil in fish. Clove oil is distilled from *Eugenia aromatica* or *Eugenia caryophyllata.* Its active ingredient, eugenol (4-allyl-2-methoxyphenol), makes up 70 to 90% by weight of clove oil. Clove oil also contains eugenol acetate (> 17%) (Sato and Burhanuddin 1995; Isaacs 1983; Briozzo et al. 1989; Keene et al. 1998). Despite the common use of anaesthetics in fish, there is little information about their influence on the sturgeon organism.

The aim of the present study was to assess the changes induced in the organism of Siberian sturgeon (*Acipenser baerii*) by eugenol and MS-222 on the basis of haematological indices, biochemical blood profile values and histological examinations.

Materials and Methods

Fish

Young sturgeons with the mean length of 253 ± 75 mm (mean ± SD) and mean weight of 94.90 ± 55.23 g were used for the experiments. The fish were obtained from the Acipol Ltd. fish farm and were classified as Siberian sturgeon based on the morphological characteristics and farmer declaration. The fish were acclimated for 14 days to laboratory conditions. They were kept in 500l fibreglass flow-through tanks equipped with automatic temperature regulation and artificial aeration and fed with commercial sturgeon pellets (AllerAqua 4515). The experiment was carried out at 17 °C ± 1 °C of water temperature. Water oxygen saturation was maintained above 80%. Water pH was 7.8 ± 0.2 and hardness was 245 ppm.

Anaesthetics

MS-222 (tricaine methanesulphonate) and eugenol were delivered by Sigma-Aldrich Chemicals Ltd. The most adequate concentration for general anaesthesia in Siberian sturgeon was determined to be 125 $mg \cdot l^{-1}$ for MS-222 and 0.075 $ml \cdot l^{-1}$ for eugenol in a pre-experiment. The above concentrations meet the criteria proposed by Marking and Meyer (1985) most closely: induction of general anaesthesia in a time shorter than 3 min; recovery time shorter than 10 min and no mortality after 48 h. The mean recovery time in case of eugenol was actually longer then 10 min (13'26" ± SD 2'52").

Test procedure

The fish were divided into 5 groups (N = 10). They were netted from the tank individually and immediately placed in 30l glass tanks filled with MS-222 or eugenol solution for a 10 min exposure. Blood samples for further analysis were collected immediately after exposure (MS-0 and Eug-0 groups, respectively) and 24 h after exposure (MS-24 and Eug-24 groups, respectively) (still bleeding fish were kept in 300l flow-through glass tanks). Fish not exposed to anaesthetic solution constituted the control group. Control group sampling was done at the beginning of the test. All blood samples were taken by a syringe from caudal vessels within 2 min after netting fish out of the water. To stabilize blood samples, aqueous solution of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodová et al. 1986). Fish feeding was stopped 24 h before experimental anaesthesia and fish were not fed during the entire test course.

Haematological indices

Procedures based on the unified methods for haematological examination of fish (Svobodová et al. 1986) were used to evaluate the haematological profile of sturgeon blood. Determined indices included the erythrocyte count (Er), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (MCH), leukocyte count (Leuko) and the differential leukocyte count (Leukogram).

Histological examination

Samples of tissues for histological examination were taken from five control sturgeons at the start of the experiment, and from six fish from both MS-222 and eugenol anaesthetized groups 24 h after anaesthesia. In all cases, tissue samples were taken after blood sampling. Tissues were immediately fixed in Bouin's solution, drained and embedded in paraffin and stained with haematoxylin-eosin.

Biochemical blood plasma profile

Blood plasma was obtained by centrifuging blood samples in a cooled centrifuge (4 °C, 837 × *g*). Biochemical indices determined in blood plasma included glucose (GLU), total proteins (TP), albumins (ALB), total globulins (GLOB), triacylglycerols (TAG), ammonia (NH_3), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), calcium (Ca^{2+}) and inorganic phosphate (PHOS).

For plasma biochemical analysis, VETTEST 8008 analyser (IDEXX Laboratories Inc., USA; Medisoft Co.) was used. The apparatus is based upon dry chemical technology and colorimetric reaction. Sample analysis was carried out on selective testing discs (Multi-layer film slides, Kodak) by means of laser reading of the bar codes. Detection limits of the methods were as follows: GLU (0.01 mmol·l^{-1}), TP (1.0 g·l^{-1}), ALB (1.0 g·l^{-1}), GLOB (1.0 g·l^{-1}), TAG (0.01 mmol·l^{-1}), NH_3 (1.0 µmol), LDH (0.0167 µkat·l^{-1}), AST (0.0835 µkat·l^{-1}), ALT (0.0835 µkat·l^{-1}), ALP (0.0167 µkat·l^{-1}), CK (0.0167·µkat·l^{-1}), Ca^{2+} (0.01 mmol·l^{-1}) and PHOS (0.01 mmol·l^{-1}).

Statistical analysis

Results of the experiment were tested by non-parametric variance analysis (Kruskall-Wallis) and Mann-Whitney test using the Toxicologist 1.0 software (The Euro-Mediterranean Centre on Marine Contamination Hazards 1989).

Results

Haematological indices

Results of haematological examinations are shown in Table 1. No statistically important differences were noticed in mean blood haemoglobin concentration. Both eugenol and MS-222 caused a severe decrease of leukocyte count 24 h after exposure. The erythrocyte count was significantly lower ($p < 0.01$) in the eugenol group immediately after anaesthesia compared to all the other groups. The mean PCV value was higher ($p < 0.05$) in the MS-0 group compared to the control; however, this value was not significantly different compared to other groups. In the Eug-0 group, PCV was slightly higher compared to the Eug-24 group ($p < 0.05$). MCV and MCH were significantly higher ($p < 0.01$) in both Eug-0 and MS-0 groups compared to other groups. The mean MCHC value in MS-0 group was significantly much higher than in all other groups ($p < 0.01$).

Table 1. Effects of eugenol and MS-222 anaesthesia on haematological indices in Siberian sturgeon

Indices	Control	Eugenol		MS-222	
		Immediately after anaesthesia	24 h after anaesthesia	Immediately after anaesthesia	24 h after anaesthesia
Er (T/L)	0.32 ± 0.08	0.19 ± 0.03*^{a}	0.32 ± 0.09 ^{b}	0.26 ± 0.04 ^{c}	0.39 ± 0.10^{b}
Leuko (G/L)	6.08 ± 2.51	4.99 ± 1.46^{a}	1.68 ± 0.59*^{b}	5.87 ± 3.57^{a}	2.68 ± 1.47*^{b}
Hb (g/L)	28.16 ± 6.74	34.76 ± 10.98^{a}	29.56 ± 7.87^{a}	32.92 ± 13.88^{a}	33.28 ± 6.54^{a}
PCV	0.19 ± 0.03	0.22 ± 0.03^{a}	0.18 ± 0.03^{b}	0.24 ± 0.04*^{a}	0.21 ± 0.04^{ab}
MCV (fl)	641 ± 207	1374 ± 269*^{a}	605 ± 156^{b}	1194 ± 544*^{a}	550 ± 133^{b}
MCH (pg)	92.8 ± 37.8	184.3 ± 69.6*^{a}	96.9 ± 32.3^{b}	149.4 ± 48.1*^{a}	87.1 ± 17.7^{b}
MCHC (g/L)	146.0 ± 2.59	153.0 ± 6.00^{a}	158.0 ± 2.86^{a}	665.0 ± 17.68*^{b}	162.0 ± 1.93^{a}

values marked with * are statistically different compared to control
values marked with different letter index are statistically different

Table 2 shows the results of differential leukocyte count in experimental and control sturgeons. The number of lymphocytes was significantly decreased ($p < 0.01$) in both eugenol and MS-222 group after 24 h compared to control and respective groups immediately after anaesthesia. The number of neutrophil segments was significantly lower

Table 2. Effects of eugenol and MS-222 anaesthesia on the differential leukocyte count in Siberian sturgeon

Cell type		Control group	Eugenol		MS-222	
			Immediately after anaesthesia	24 h after anaesthesia after	Immediately anaesthesia	24 h after anaesthesia
		mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Lymphocytes	G/L	4.05 ± 1.72	3.50 ± 1.14[a]	1.09 ± 0.39*[b]	4.10 ± 2.22[a]	1.55 ± 0.90*[b]
	%	66.1 ± 12.4	69.8 ± 6.9[a]	68.9 ± 7.6[a]	73.8 ± 6.2*[a]	59.0 ± 9.9[b]
Monocytes	G/L	0.01 ± 0.02	0.0	0.01 ± 0.01 [a]	0.0	0.01 ± 001[a]
	%	0.2 ± 0.3	0.0	0.4 ± 0.4 [a]	0.0	0.3 ± 0.3 [a]
Neutrophils - segments	G/L	1.04 ± 0.46	0.99 ± 0.35[a]	0.24 ± 0.12*[b]	0.91 ± 0.71[a]	0.61 ± 0.39[a]
	%	19.2 ± 9.6	20.4 ± 5.8[a]	14.9 ± 6.1[b]	16.3 ± 6.7[ab]	22.1 ± 8.1[a]
Neutrophils - rods	G/L	0.09 ± 0.08	0.11 ± 0.06[a]	0.09 ± 0.05[a]	0.15 ± 0.13[a]	0.16 ± 0.09[a]
	%	1.6 ± 1.1	2.4 ± 1.2[a]	5.6 ± 2.8*[b]	2.4 ± 1.6[a]	6.0 ± 1.9*[b]
Neutrophiles - myeloid sequence	G/L	0.04 ± 0.06	0.03 ± 0.04[a]	0.03 ± 0.02[a]	0.03 ± 0.05[a]	0.04 ± 0.03[a]
	%	0.7 ± 0.9	1.2 ± 2.6[a]	1.6 ± 1.1[a]	0.5 ± 0.5*[b]	1.6 ± 0.5*[a]
Eosinophils - segments	G/L	0.20 ± 0.26	0.23 ± 0.13[a]	0.02 ±0.02*[b]	0.24 ± 0.20[a]	0.04 ± 0.02*[b]
	%	3.1 ± 2.6	4.4 ± 2.0[a]	1.3 ± 1.1[b]	3.9 ± 2.9[a]	1.4 ± 0.8[b]
Eosinophils - rods	G/L	0.38 ± 0.25	0.10 ± 0.08*[a]	0.11 ± 0.08*[a]	0.12 ± 0.14*[a]	0.26 ± 0.24[a]
	%	6.3 ± 3.9	2.1 ± 1.6*[a]	6.9 ± 3.4[b]	2.1 ± 2.1*[a]	8.9 ± 6.2[b]
Eosinophils - myeloid sequence	G/L	0.25 ± 0.60	0.02 ± 0.03*[a]	0.01 ± 0.01*[a]	0.06 ± 0.14[a]	0.02 ± 0.01[a]
	%	2.7 ± 5.5	0.3 ± 0.5*[a]	0.6 ± 0.4[a]	0.8 ± 1.8[a]	0.7 ± 0.7[a]

values marked with * are statistically different compared to control
values marked with different letter index are statistically different

($p < 0.01$) in the Eug-24 group compared to both control and Eug-0 group. There was no significant difference between the groups in the number of both the neutrophil rods and the neutrophil myeloid sequence. Similarly to lymphocyte numbers, the number of eosinophil segments was lower ($p < 0.01$) in both "24" groups compared to control and "0" groups. The number of eosinophil rods was significantly lower ($p < 0.01$) in Eug-0 and Eug-24 groups compared to control. The number of eosinophil myeloid sequence was significantly decreased ($p < 0.05$) in both Eug-0 and Eug-24 groups compared to control.

Biochemical blood plasma profile

The results are shown in Table 3. Significant increase in TP and GLOB occurred in both the eugenol groups and MS-0 group compared to control ($p < 0.01$). All the experimental groups revealed a significant decrease in Ca, PHOS and NH_3 concentrations in blood plasma ($p < 0.05$) and a significant increase in TAG concentration ($p < 0.05$) compared to control. The activity of ALT was significantly increased in all the experimental groups ($p < 0.05$) and the activity of ALP was significantly decreased in all groups compared to control. No significant differences were noticed between control and experimental groups in ALB, GLU, LDH, AST and CK indices. No significant differences were noticed between respective "0" and "24" groups for both eugenol and MS-222 anesthetized sturgeons.

Histopathological findings

Evident congestion of sinusoid capillaries was observed in the liver of Siberian sturgeons 24 h after MS-222 anaesthesia (Plate XIII, Fig. 1 A). Sections taken from gills of fish treated with MS-222 showed a swelling of primary and secondary lamellae (Plate XIII, Figs 2B, 2E). The spaces between secondary lamellae were decreased due to hypertrophy of the epithelial cells. Lamellar hypertrophy was also observed in sturgeons treated with eugenol, however, these lesions were less intensive (Plate XIII, Figs 2C, 2D). Slight congestion of capillaries and cell necrosis occurred rarely in both experimental groups.

Table 3. Effects of eugenol and MS-222 anaesthesia on the biochemical blood indices in Siberian sturgeon

Indices	Control	Eugenol		MS-222	
		Immediately after anaesthesia	24 h after anaesthesia	Immediately after anaesthesia	24 h after anaesthesia
TP ($g \cdot l^{-1}$)	9.67 ± 1.97	12.12 ± 4.39*	13.33 ± 2.55*	12.64 ± 2.84 *	10.57 ± 1.72
GLOB ($g \cdot l^{-1}$)	8.83 ± 2.14	11.37 ± 4.53*	12.56 ± 2.92*	12.00 ± 3.03*	9.86 ± 1.95
ALB ($g \cdot l^{-1}$)	0.83 ± 0.41	0.75 ± 0.46	0.78 ± 0.441	0.82 ± 0.40	0.71 ± 0.49
GLU ($mmol \cdot l^{-1}$)	1.73 ± 0.27	1.73 ± 0.38	1.85 ± 0.134	1.83 ± 0.38	1.58 ± 0.26
TAG ($mmol \cdot l^{-1}$)	1.02 ± 0.10	1.76 ± 0.99*	1.43 ± 0.05*	1.67 ± 0.28*	1.36 ± 0.19*
Ca^{2+} ($mmol \cdot l^{-1}$)	1.89 ± 0.22	1.47 ± 0.11*	1.44 ± 0.11*	1.47 ± 0.17*	1.35 ± 0.18*
PHOS ($mmol \cdot l^{-1}$)	2.90 ± 0.10	2.16 ± 0.15*	1.95 ± 0.56*	2.27 ± 0.22 *	2.24 ± 0.13*
NH_3 ($\mu mol \cdot l^{-1}$)	584.0 ± 51.3	525.1 ± 32.7*	520.7 ± 70.1*	534.9 ± 28.0*	504.6 ± 88.5*
LDH ($\mu kat \cdot l^{-1}$)	18.02 ± 0.26	17.98 ± 0.45	18.21 ± 0.73	17.91 ± 0.60	17.69 ± 0.74
AST ($\mu kat \cdot l^{-1}$)	2.42 ± 0.33	2.73 ± 0.45	2.56 ± 0.18	2.77 ± 0.26	2.61 ± 1.32
ALT($\mu kat \cdot l^{-1}$)	0.10 ± 0.01	0.19 ± 0.04*	0.18 ± 0.04*	0.18 ±0.04*	0.18 ± 0.04*
ALP ($\mu kat \cdot l^{-1}$)	1.01 ± 0.12	0.67 ± 0.08*	0.66 ± 0.10*	0.66 ± 0.24*	0.66 ± 0.11*
CK ($\mu kat \cdot l^{-1}$)	15.21 ± 0.48	15.17 ± 0.48	15.33 ± 0.59	15.07 ± 0.18	15.23 ±0.61

Values marked with * are statistically different compared to control; no statistical difference was found between experimental groups

Discussion

Careful and controlled MS-222 anaesthesia is thought to be useful in haematological examinations due to lack of a significant effect on the haematological indices in fish (Bourne 1984). No changes in haematological indices were noticed in teleost fish following eugenol anaesthesia (Velíšek et al. 2005, 2006). However, our results seem to indicate a severe impact of both eugenol and MS-222 on haematological indices in Siberian sturgeon. Anaesthesia probably resulted in erythrocyte swelling (high increase of MCV immediately after anaesthesia), followed by an increase of the PCV value in the MS-0 group. The PCV value was not increased in eugenol-anaesthetised sturgeons. However, the number of erythrocytes was severely depleted, probably due to haemolysis. The high increase of MCH in both groups immediately after anaesthesia is probably an apparent phenomenon, as the method used for measuring haemoglobin concentration measures total blood volume haemoglobin, not specifically erythrocyte haemoglobin. The MCH value is virtually shifted during calculations in case of haemolysis. We concluded that both eugenol and MS-222 caused the swelling and destruction of erythrocytes. Changes were less severe in case of MS-222 and could be compensated by an erythrocyte release from blood storage organs, which resulted in lack of significant changes in the erythrocyte number and the significant increase of PCV, MCH and MCHC value in this group (Table 1). The TP and GLOB shift probably resulted from the erythrocyte destruction. All the above changes were reversible and returned to normal after 24 h, except for TP and Glob in the eugenol anaesthetized group. It seems that repeated eugenol or MS-222 anaesthesia could result in anaemia in Siberian sturgeon.

Hatting (1977) has showed that MS-222 produces an increase of haematocrit value and haemolysis in some cyprinid fish in the *in vitro* experiment. Smit et al. (1979) stated that MS-222 affects erythrocyte osmotic fragility. Ferreira et al. (1981) attributed high erythrocyte osmotic fragility following benzocaine and MS-222 anaesthesia to low blood plasma pH induced during anaesthesia.

The leukocyte number was severely depleted 24 h after both MS-222 and eugenol anaesthesia, mainly due to the depletion of lymphocytes, neutrophil segments and eosinophil fractions (Tables 1 and 2). According to Angelidis et al. (1987) lymphopaenia

is one of the results of stress reaction in fish. However, another stress indicator, GLU, was not affected in our experiment. Decreased PHOS and NH_3 concentration (Table 3) seems to be the result of a decreased metabolic rate. Baker et al. (2005) found no changes in haemoglobin, haematocrit and MCHC following severe experimental hypoxia in both Atlantic and shortnose sturgeons. The plasma cortisol and plasma glucose and lactate were elevated; however, the extent of this elevation was lower than in teleost fish. Beyea et al. (2006) concluded that shortnose sturgeon respond to external stress to a negligible extent. They speculated that sturgeon may be able to mobilize energy stores using alternative to corticosteroid stress response pathway. They suggested that hyperlipidaemia initiated through the adrenaline release from chromaffin cells (Matty 1985) may be such alternative pathway. This is in agreement with our results; both MS-222 and eugenol anaesthetized Siberian sturgeons showed elevated level of TAG immediately and 24 h after anaesthesia. Our experiment showed that anaesthesia with both 125 $mg \cdot l^{-1}$ of MS-222 and 0.075 $ml \cdot l^{-1}$ of eugenol severely impact the constituents of Siberian sturgeon blood and result in some histological changes in the gills and liver.

Vliv anestetik eugenolu a MS-222 na jesetera sibiřského (*Acipenser baerii* Brandt)

Cílem studie bylo posoudit změny v organismu jesetera sibiřského (*Acipenser baerii*) po anestézii eugenolem a MS-222 na základě hematologického, biochemického a histologického vyšetření. Hematologické a biochemické vyšetření bylo provedeno u ryb ihned a 24 h po anestézii. Výsledky experimentu ukázaly, že navzdory nulové mortalitě po anestézii oběma látkami MS-222 (125 $mg \cdot l^{-1}$) a eugenolu (0,075 $ml \cdot l^{-1}$), měla anestézie vliv na hematologické a biochemické ukazatele jesetera sibiřského a způsobila histopatologické změny na žábrech a játrech. Výsledky ukázaly, že anestézie eugenolem a MS-222 vyvolala zvětšení objemu erytrocytů a hemolýzu. 24 h po anestézii oběma anestetiky došlo ke snížení počtu leukocytů (a to hlavně v důsledku snížení počtu lymfocytů, neutrofilních segmentů a eosinofilů). Koncentrace celkových bílkovin, celkových globulinů, triacylglycerolů, a aktivita alanin aminotransferázy v krevní plazmě byla signifikantně zvýšena ($p < 0,01$) po anestézii eugenolem a MS-222. Koncentrace Ca^{2+}, anorganického fosfátu, amoniaku, a aktivita alkalické fosfatázy byla signifikantně snížena ($p < 0,01$) oproti kontrole. Nebyly zaznamenané významné změny u koncentrace albuminu, glukózy a aktivity laktát dehydrogenázy, aspartát aminotrasferázy a kreatinkinázy. Výsledky ukázaly, že MS-222 (125 $mg \cdot l^{-1}$) a eugenol (0,075 $ml \cdot l^{-1}$) jsou bezpečné pro jesetera sibiřského.

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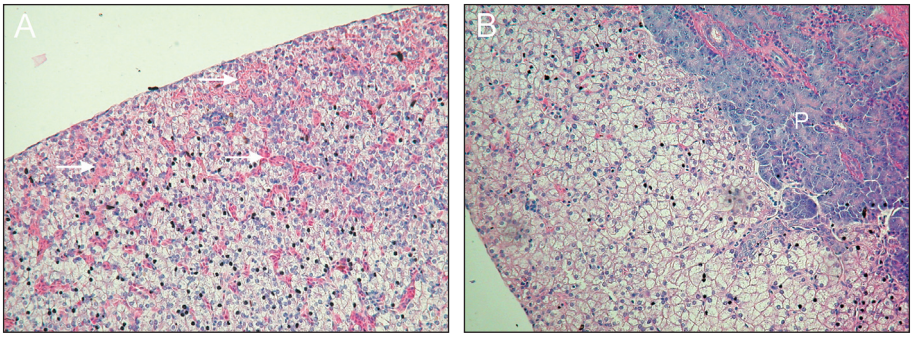

Fig. 1. Section through the liver of Siberian sturgeon: A - 24 h after anaesthesia with MS 222; the congestion of sinusoid capillaries (arrows), B - control fish; p – pancreas [× 100; H & E].

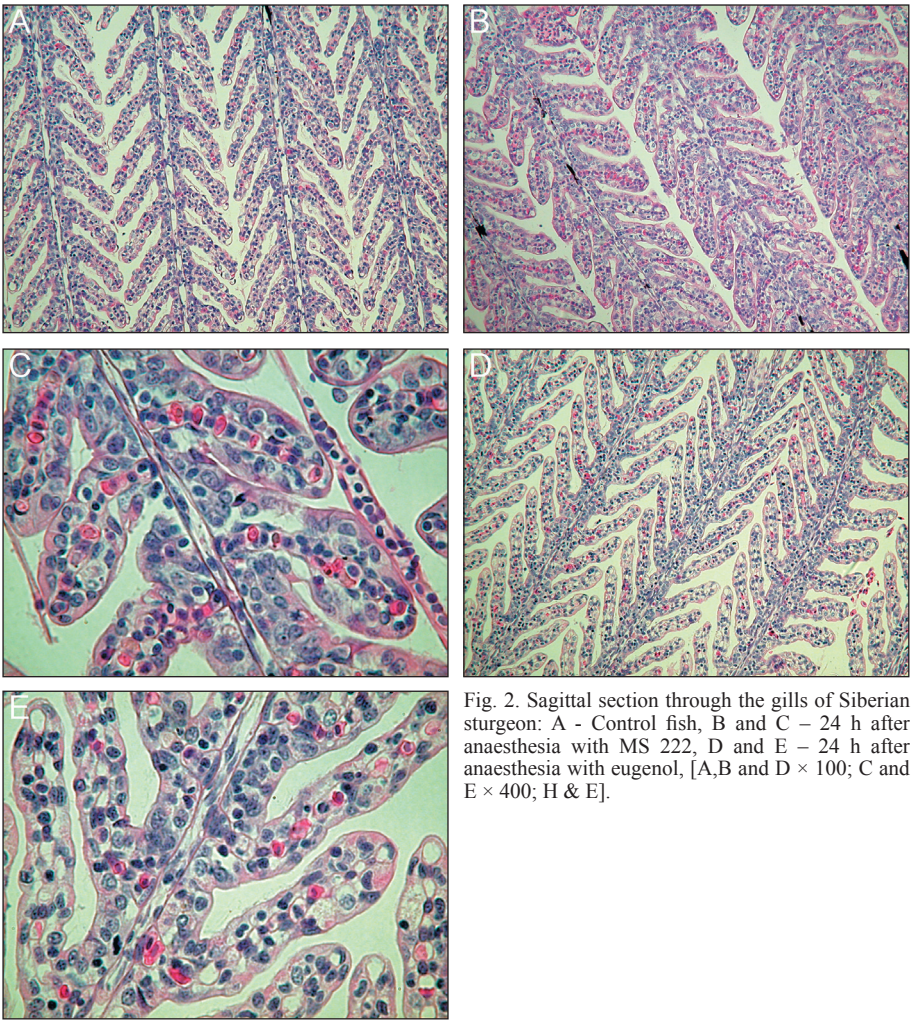

Fig. 2. Sagittal section through the gills of Siberian sturgeon: A - Control fish, B and C – 24 h after anaesthesia with MS 222, D and E – 24 h after anaesthesia with eugenol, [A,B and D × 100; C and E × 400; H & E].