

## INVESTIGATION OF BLOOD PARAMETERS OF THE BLACK SEA DOLPHINS (*Tursiops truncatus ponticus*) KEPT AT THE LITHUANIAN SEA MUSEUM

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**Abstract.** The purpose of this investigation was to determine blood morphological and biochemical values of clinically healthy Black Sea dolphins (*Tursiops truncatus ponticus*) kept at the Lithuanian Sea Museum depending on their sex and age. Male dolphins were divided into groups according to their age: dolphins 2–5 years old and over 5 years old. Female dolphins were divided into three groups: 2–5 years old, over 5 years old and under two years old. In 2003–2010, the analysis of blood samples of the eight clinically healthy Black Sea dolphins showed that the average amount of leukocytes amounted to  $6.9 \times 10^9/L$  and erythrocytes to  $4.1 \times 10^{12}/L$ , respectively. Reticulocytes accounted for 2.60%. The concentration of haemoglobin was 163.70 g/L. Packed cell volume was 43.50%. Erythrocyte sedimentation rate was 1.85 mm/h. Segmented neutrophils accounted for 45.3–57.3%, lymphocytes 13.7–33.60%, monocytes 3.20–4.80%, and eosinophiles for 15.40–21.70% of leukocytes. The urea content was 16.82 mmol/L, creatinine level 125.35  $\mu\text{mol}/L$ , and glucose level 5.72 mmol/L. Activities of enzymes studied consisted of aspartate aminotransferase – 220.65 U/L, alanine aminotransferase – 38.65 U/L, gamma-glutamyl transferase – 43.80 U/L, and alkaline phosphatase – 1200.85 U/L, respectively. The level of sodium amounted to 156.48 mmol/L, potassium 3.44 mmol/L, chlorine 118.28 mmol/L, and iron 29.94  $\mu\text{mol}/L$ . The total level of proteins in blood serum was 71.39 g/L. Albumins accounted for 61.62%,  $\alpha_1$  globulins 5.32%,  $\alpha_2$  globulins 8.94%,  $\beta_1$  globulins 4.80%,  $\beta_2$  globulins 5.34%,  $\gamma$  globulins 15.37%, and fibrinogen 1.82 g/L. During growth periods, the hematopoietic dynamics of male and female animals were similar and their sex and age did not demonstrate clear or significant influence on alterations of blood parameters.

**Keywords:** Black Sea dolphins, blood, sex, age.

**Introduction.** The Black Sea dolphins (*Tursiops truncatus ponticus*) are marine mammals belonging to suborder of toothed whales. They are commonly referred to as bottlenose dolphins. The Black Sea is the habitat of three species of dolphins: porpoise, common dolphin and bottlenose dolphin. Some researchers suggest distinguishing Black Sea bottlenose dolphins as a separate subspecies *Tursiops truncatus ponticus* (Sokolov, Romanenko, 1997). Previous researches were based only on few minimal differences of crania of Atlantic, Mediterranean and Black Sea dolphins. Later, genetic analysis also revealed minimal genetic differences between these animals (Viaud-Martinez et al., 2008). Dolphins are easily trained, communicate with people and show no aggression. Atlantic bottlenose dolphins (*Tursiops truncatus*) are most often kept in dolphinariums. In 1994, the only dolphinarium keeping the Black Sea bottlenose dolphins in the eastern coast of the Baltic Sea was opened in the Lithuanian Sea Museum (LSM). These dolphins are rarer than the Atlantic bottlenose dolphins and are only kept in a few dolphinariums in Russia, the Ukraine, Turkey, Israel, and Canada.

Dolphins (living in natural habitats and in dolphinariums) are very sensitive to various environmental factors and, therefore, predisposed to various diseases. Health monitoring of dolphins living in natural environment is rather difficult and reduces the chances of timely help (Newman, Smith, 2006; Dubey et

al., 2008; Waltzek et al., 2012). It has been established that dolphins may get sick with laryngitis, pneumonia and other respiratory diseases which are diagnosed only when there occur behavioural, respiratory and appetite changes in these animals (Venn-Watson et al., 2012).

Blood analysis in the early stages of disease is the most informative and objective way to evaluate the health status of dolphins. Much literary data about the alterations of blood indices of adult healthy dolphins living in natural environment is available in the scientific literature. Yet data about the standard values of haematological indices in dolphins kept in dolphinariums during growth, development and maturation periods are lacking (Hyne et al., 1982; Hall et al., 2007). Taking of blood samples during forced capture may cause stress in aquatic animals, therefore no standard range for the values of haematological indices have been established. Even in adult dolphins, the blood parameters may differ depending on the method of blood sampling (Varela et al., 2006).

The available literary data show that haematological indices of dolphins from different regions of Atlantic Ocean also differ. The variations of blood indices of Atlantic bottlenose dolphins living in natural environment and other species of dolphins have been compared and analysed by few researchers (Ortiz, Worthy, 2000; Aubin et al., 2011). Schwacke et al. (2009) reported that some haematological indices alternate depending on the age and sex of dolphins and fertility periods of female dolphins. It

has been determined that blood parameters may be affected by adaptation time to captivity conditions (Sokolov, Romanenko, 1997). Yet the authors of the present article have not found any literary data about the influence of age and sex on physiological blood parameters of the Black Sea bottlenose dolphins kept in dolphinariums.

Recently, different methods have been developed for drawing blood avoiding stress in animals. Blood samples of dolphins are taken using the so called “medical training of dolphins”. Requested to present fluke by trainer, the trained dolphins stay at the pool edge and allow the specialist to draw blood from the underside of the tail fluke. This is a convenient and safe method both for the dolphins and personnel (Brando, 2010). This method allows avoiding possible changes of blood parameters which may occur during capture and forced fixation.

Ovulation in female dolphins (bottlenose dolphins) may start at the age of 4 years. The males reach their sexual maturity at the age of 5 years (O'Brien, Robeck, 2010). These aquatic animals are distinguished by the fact that female dolphins nurse their calves up to 2 years of age. This is the main reason why researchers commonly analyse and report the standard blood values of adult dolphins. Knowledge of morphological and biochemical standards of blood parameters following the growth, development and maturation curves may be regarded as one of the most important steps in implementing animal health monitoring programs.

**The present research was aimed at** determining the physiological morphological and biochemical blood parameters in the clinically healthy Black Sea dolphins (*Tursiops truncatus ponticus*) kept at the Lithuanian Sea Museum with regard to their age and sex.

**Material and methods.** The research at the dolphinarium of the Lithuanian Sea Museum lasted for 8 years (2003–2010). Adaptation, growth curve and development of dolphins were observed. Within the health monitoring program, blood samples were taken periodically for evaluation of morphological and biochemical indices. Only the blood samples of 8 clinically healthy dolphins were used for analysis. The animals did not receive food for 12–14 hours before drawing blood. The blood samples were taken in the morning between 8.30 a.m. and 10.30 a.m. according to the age bracket and this was done in all seasons. With regard to age, the blood taken from males was attributed to two age groups whereas the females' blood was attributed to three age groups. According to sex and age, the dolphins were put into five groups. Males aged 2–5 years were put into group 1 (n=45 blood samples); males over 5 years old were put into group 2 (n=34 blood samples); group 3 included females aged under two years (n=13 blood samples); group 4 included dolphin females aged 2–5 years (n=29 blood samples); group 5 was comprised of females over 5 years of age (n=83 blood samples). The dolphins were kept in pools with water volume 18000 m<sup>3</sup>. The water salinity was 1.7–1.9‰. The water temperature ranged between 17 to 23 °C. The

concentration of free chlorine was 0.2–0.4 mg/l. The blood was drawn from the underside vein of the tail fluke using a 0.8 x 19 mm needle. The dolphins were trained so as to keep still with the lifted tail fluke during the process of drawing blood. For morphological analysis, the dolphin blood was drawn using sterile 3 ml vacuum test tubes with anticoagulant EDTA K3. For biochemical analysis, the blood was drawn using 4 ml vacuum test tubes with lithium heparin and for fibrinogen analysis the blood was drawn using 3.6 ml test tubes with sodium citrate inside. The analysis of morphological and biochemical blood parameters was conducted at the laboratory of Klaipėda Mariners Hospital. The blood samples were delivered to the laboratory in four hours after blood drawing.

Morphological indices of dolphins' blood were analyzed by use of Cell-Dyn 1700 (Abbott, USA, 2005) and Cell-Dyn Ruby (Abbott, USA, 2007) analyzers. The taken blood smears were dyed with Gimza dye (Merck, Germany) and assessed using a microscope (Nikon instruments, Japan) with magnifying capacity of 50 times. Biochemical indices of dolphins' blood were obtained by use of analyzers RX Daytona TM (Randox, UK, 2006) and Vitros 250 (Ortho Clinical Diagnostic, Janson & Janson, USA, 2002). The values of potassium, sodium and chlorine were assessed by use of Ilyte analyzer (Instrumentation laboratory SpA, USA, Italy, 1994), the value (amount) of fibrinogen was obtained by use of STA compact analyzer Stago (France, 2004). The erythrocytes sedimentation rate was measured at the laboratory of the dolphinarium of Lithuanian Sea museum. The dolphin blood was drawn using “Venoject” 2.4 ml vacuum test tubes (manufactured by “Terum”) with sodium citrate inside. Analysis of the obtained blood samples was performed by Westergren's methodology.

The statistical analysis of dolphins' blood indices was performed by the method of descriptive statistics. The measures used were arithmetic mean of one random variable (M) and standard deviation (SD). The statistical analysis of the blood indices of dolphins of the same age and different sex was performed by the method of single factor dispersion analysis using Anova: Single Factor function of Microsoft Office Excel software and applying Student's criterion (MS Office function t-test Two Sample Assuming Equal Variances) for determining the data changes. The single factor dispersion analysis was performed in case of normal distribution of data. The deviations from the normal distribution were considered significant when excess was  $|Ex| > 1$  and/or asymmetry  $|As| > 0.5$ . The differences were regarded as statistically significant when  $p < 0.05$ .

**Results.** Morphological indices of dolphins' (males and females of *Tursiops truncatus ponticus* species) blood are given in Table 1. The concentration of leucocytes in the blood of *Tursiops truncatus ponticus* kept at the Lithuanian Sea Museum amounted to  $(6.77 \pm 1.54) \times 10^9/L$  on the average. The concentrations of leucocytes in the blood of male and female dolphins aged from 2 to 5 years were comparable yet by 10.9 ( $p < 0.05$ ) to 20.1 ( $p < 0.001$ ) % lower than in the blood of younger or older dolphins.

Table 1. Haematological blood parameters of healthy dolphins' *Tursiops truncatus ponticus*

| Parameters                              | Males                 |                       | Females                   |                       |                       |
|---|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|
|   | 2–5 years old<br>M±SD | > 5 years old<br>M±SD | under 2 years<br>old M±SD | 2–5 years old<br>M±SD | > 5 years old<br>M±SD |
| Leucocytes, 10 <sup>9</sup> /L          | 6.10±1.42*            | 6.85±1.38**           | 7.73±1.00                 | 6.18±1.58*            | 7.17±1.530**          |
| Erythrocytes, 10 <sup>12</sup> /L       | 3.79±0.49**           | 4.06±0.50**           | 4.39±0.41                 | 3.91±0.45**           | 3.83±0.47**           |
| Reticulocytes, %                        | 3.0±1.3               | 2.3±0.8               | 3.2±0.7                   | 2.3±0.7               | 2.0±1.1               |
| Haemoglobin, g/L                        | 161.2±12.4            | 173.7±8.7**           | 153.7±6.2                 | 158.3±7.2             | 159.0±12.9**          |
| Hematocrit, %                           | 42.5±5.0*             | 44.9±4.6**            | 43.9±3.3                  | 42.0±4.18**           | 42.3±4.2**            |
| Erythrocytes' sedimentation rate, mm/h. | 2.0±0.1               | 2.0±0.1               | 1.7±0.0                   | 2.0±0.0               | 2.0±0.0               |
| Eosinophils, %                          | 21.1±5.0              | 20.8±4.4**            | 15.4±2.6                  | 16.7±4.6              | 21.7±6.3**            |
| Band neutrophils, %                     | 2.0±1.6               | 3.4±2.3**             | 1.8±1.2                   | 2.3±1.3**             | 2.8±2.3               |
| Segmented neutrophils, %                | 48.9±6.2**            | 52.9±6.6**            | 46.2±6.8                  | 45.3±9.0**            | 57.3±7.0**            |
| Lymphocytes, %                          | 23.7±6.0**            | 18.3±7.0**            | 33.6±6.7                  | 32.1±9.4**            | 13.7±6.0**            |
| Monocytes, %                            | 4.2±2.2               | 4.8±2.1               | 3.2±1.7                   | 3.2±1.8               | 3.9±2.2               |

\*P<0.05, \*\*P<0.01 – Comparison of blood parameters of males and females with regard to age

The average amount of erythrocytes in dolphins' blood was (3.91±0.49) x10<sup>12</sup>/L. The amount of erythrocytes in the blood of both sexes aged 2–5 years was the same yet it was lower by 5.6% (p<0.05) in the blood of males over 5 years old than in the blood of females of the same age. The blood of young females (below 2 years of age) contained by 11.0–12.6% (p<0.05) more erythrocytes than the blood of older females. Reticulocytes in the blood of all dolphins accounted for 2.40±1.14% on the average. The average haemoglobin concentration was 161.2±12.3 g/L. The haemoglobin concentration in female dolphins' blood did not depend on the age. In males over 5 years old, the concentration of haemoglobin was by 12.4 g/L (7.2%, p<0.01) higher on the average than in younger males and by 14.6 g/L (8.4%, p<0.01) higher than in females of the same age. The average packed cell volume in dolphins' blood was 42.8±4.4% almost bearing no differences between the groups except that in the group of males over 5 years old the packed cell value exceeded the packed cell volume in females of the same age by 2.5% (p<0.01) and in animals aged 2–5 years by 2.4% (p<0.05).

The average rate of erythrocyte sedimentation in the blood of dolphins kept in the Lithuanian Sea Museum was 2.0±0.5 mm/h and was not predetermined by animals' sex or age.

Since the number of leucocytes, the haemoglobin concentration and packed cell values were distributed in a normal pattern, it can be assumed that their average values and differences between sexes and age groups are characteristic of the entire *Tursiops truncatus ponticus* dolphin population. The other average blood values that were studied revealed only the individual peculiarities of the dolphin group that is kept in the LSM dolphinarium.

The analysis of leukocyte formula showed that the segmented neutrophils in the blood of *Tursiops truncatus ponticus* accounted for 52.1±8.5% of the total on the average. In the blood of females from the age group younger than 5 years, the value of segmented neutrophils

was by 11.1–12.0% (p<0.01) lower than in the blood from the older age group. The difference between different age groups of males amounted to 4.0% (p<0.05). In the blood from male group over 5 years of age, the amount of band neutrophils was by 1.3% (p<0.01) and in the blood of females of the same age group the amount of eosinophils was by 5.0–6.3% (p<0.01) higher than in the blood of age group below 2 years. Yet the values of band neutrophils and eosinophils were dispersed with considerable excess and asymmetry (Ex=0.84–3.88, As=0.75–1.62) implying that the mentioned differences cannot be characteristic of the whole *Tursiops truncatus ponticus* dolphin population.

The blood of *Tursiops truncatus ponticus* dolphins contained 20.9±10.0% of lymphocytes (of the total number of leukocytes). The number of lymphocytes in the blood of females below 5 years of age was by 18.4–19.9% (p<0.01) higher than in the blood of older animals. This difference between the groups of males of different age, this difference amounted to 5.5% (p<0.01).

Biochemical blood indices of dolphins of different age and sex are given in Table 2. The average amount of urea in the blood of *Tursiops truncatus ponticus* dolphins was 16.74±2.62 mmol/L. Its amount in the blood of females and males aged 2 to 5 years was by 10.8 (p<0.01) to 16.1% (p<0.01) higher than in the blood of younger and older animals. The average concentration of creatinine in the blood of dolphins kept in the LSM was 131.18±28.80 mmol/L. No differences were determined in creatinine concentrations in the blood of female dolphins of different age. In the blood of dolphins from age group over 5 years, the concentration of creatinine was by 12.3% (p<0.05) higher than in the blood of younger animals. The level of aspartate aminotransferase was comparable in female and male blood from various age groups. Only in the blood of females younger than 2 years its levels were by 45.95 U/L (19.9%, p<0.05) lower than in the blood of older females. The levels of gamma-glutamyl transferase in male blood showed no dependence on the age of animals. However,

in the blood of females its level increased with animal age and in the group of female dolphins aged over 5 years the level of gamma-glutamyl transferase was by 27.5% ( $p<0.01$ ) higher than in the group of females below 2 years. The levels of alanine aminotransferase in the blood of dolphins of both sexes from various age groups was the same except in the blood of females over 5 years of age where the level of alanine aminotransferase was by 10.5 U/L (37.8%,  $p<0.01$ ) lower. The levels of lactate dehydrogenase in the blood of animals from the group aged over 5 years were by 7.79 (in females;  $p<0.05$ ) to 8.16 (in males;  $p<0.01$ ) % lower than in younger animals whereas the levels of alkaline phosphatase were lower even by 1.91–2.57 times ( $p<0.01$ ). However, of all enzymes only lactate dehydrogenase was normally distributed without display of marked excess or asymmetry ( $A_s=0.18$ ,  $E_x=0.74$ ).

No statistically significant differences in the levels of sodium, potassium, chlorine and glucose in different age

groups of both sexes of animals were determined. Only the blood of young females (below 2 years of age) contained higher concentrations of iron (by 17.2 ( $p<0.05$ ) to 22.0 ( $p<0.01$ ) %) than the blood of older females.

The distribution of the levels and fractions of proteins in the animal blood is given in Table 3. The average total level of protein in dolphins' blood was  $70.49\pm 6.93$  g/L. In the blood of males over 5 years of age, the level of proteins was by 14.48%, and in the blood of females by 8.13% higher than in the blood of younger dolphins ( $p<0.05$ ). Albumins accounted for 59.75% of the total of proteins on the average. The average level of fibrinogen was  $1.80\pm 0.25$  g/L. The average levels of different albumin fractions in the blood of examined dolphins were the following:  $\alpha_1$ -globulins –  $5.12\pm 1.98\%$ ,  $\alpha_2$ -globulins –  $8.71\pm 2.19\%$ ,  $\beta_1$ -globulins –  $4.44\pm 1.97\%$ ,  $\beta_2$ -globulins –  $5.34\pm 1.36\%$  and  $\gamma$ -globulins –  $16.61\pm 5.03\%$ . Statistically significant differences in the levels of individual fractions of globulins in female and male blood from different age groups of dolphins were not determined.

Table 2. Haematological blood parameters of healthy dolphins'

| Parametres                      | Males                 |                       | Females                   |                       |                       |
|---------------------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|
|                                 | 2–5 years old<br>M±SD | > 5 years old<br>M±SD | under 2 years<br>old M±SD | 2–5 years old<br>M±SD | > 5 years old<br>M±SD |
| Urea, mmol/L                    | 18.28±3.36*           | 15.35±1.95            | 15.92±2.31                | 17.84±1.90*           | 16.06±2.01            |
| Creatinine, $\mu$ mol/L         | 138.4±15.9            | 157.7±49.4**          | 112.3±9.2                 | 119.2±13.4**          | 125.0±22.9            |
| Aspartate aminotransferase, U/L | 256.1±75.2**          | 250.3±36.1            | 185.2±70.1                | 231.2±48.0**          | 212.0±54.1            |
| Alanine aminotransferase, U/L   | 49.6±39.0             | 48.3±13.3*            | 32.7±12.9                 | 38.1±8.7              | 27.7±14.1*            |
| Gamma-glutamyl transferase, U/L | 42.42±13.0            | 47.34±9.3*            | 36.81±10.6                | 42.86±6.7             | 50.79±10.1*           |
| Alkaline phosphatase, U/L       | 1550.9±347.6*         | 813.5±53.1**          | 1471.1±620.8              | 1736.3±364.7*         | 675.4±318.6**         |
| Laktate dehydrogenase, U/L      | 829.7±84.3*           | 767.1±65.5**          | 806.5±85.8                | 808.8±73.2*           | 750.3±135.6**         |
| Sodium, mmol/L                  | 156.01±1.47           | 156.75±1.19           | 156.06±1.39               | 155.64±3.31           | 157.32±1.78           |
| Potassium, mmol/L               | 3.57±0.30             | 3.34±0.26             | 3.54±0.20                 | 3.52±0.24             | 3.30±0.26             |
| Chlorine, mmol/L                | 119.49±1.82           | 117.72±2.06           | 117.00±1.76               | 119.41±2.31           | 119.56±2.42           |
| Iron, $\mu$ mol/L               | 26.66±7.24**          | 24.71±3.60            | 35.16±10.86               | 29.11±6.30**          | 27.41±5.89            |
| Glucose, mmol/L                 | 5.10±0.78             | 4.87±0.58             | 5.84±0.71                 | 5.43±0.65             | 4.69±0.65             |

\* $P<0.05$ , \*\* $P<0.01$  – Comparison of blood parameters of males and females with regard to age

Table 3. Healthy dolphins' blood proteins parameters

| Parameters               | Males                 |                       | Females                   |                       |                       |
|--------------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|
|                          | 2–5 years old<br>M±SD | > 5 years old<br>M±SD | under 2 years<br>old M±SD | 2–5 years old<br>M±SD | > 5 years old<br>M±SD |
| Total proteins, g/L      | 66,56±5,98*           | 77,83±8,39*           | 68,97±4,79                | 66,06±3,43*           | 71,91±5,18*           |
| Albumins, %              | 61,67±5,30            | 56,86±3,84            | 64,50±4,95                | 62,60±4,03            | 58,74±4,69            |
| $\alpha_1$ -globulins, % | 5,19±1,77             | 6,00±4,02             | 5,30±4,38                 | 6,07±0,35             | 4,65±0,99             |
| $\alpha_2$ -globulins, % | 9,51±1,78             | 8,94±1,24             | 7,60±0,57                 | 10,27±0,76            | 8,16±2,65             |
| $\beta_1$ -globulins, %  | 4,99±1,73             | 5,70±3,98             | 5,35±0,50                 | 3,90±1,20             | 3,75±1,08             |
| $\beta_2$ -globulins, %  | 4,80±1,46             | 5,88±1,40             | 5,45±0,50                 | 5,20±0,72             | 5,44±1,50             |
| $\gamma$ -globulins, %   | 13,81±3,17            | 17,80±7,35            | 11,80±0,99                | 11,97±2,14            | 18,94±4,20            |
| Albumins, g/L            | 41,40±4,31            | 45,16±3,91            | -                         | 42,27±3,27            | 41,69±3,88            |
| Globulins, g/L           | 25,32±3,79            | 35,56±6,29            | -                         | 25,17±2,37            | 29,61±4,09            |
| Fibrinogen, g/L          | 1,77±0,25             | 1,77±0,25             | 1,89±0,21                 | 1,74±0,28             | 1,83±0,24             |

\* $P<0.05$  – Comparison of males and females blood parameters with regard to age

**Discussion.** In the blood of dolphins of both sexes and all age groups kept at the dolphinarium of the Lithuanian Sea Museum, the average value of erythrocytes was  $3.9 \times 10^{12}$  /L and reticulocytes accounted for 2.4%. It was determined that the number of erythrocytes and haemoglobin concentration were highest in the age group of male dolphins over 5 years old. T. S. Venn-Watson et al. (2007), who analysed the blood parameters of adult dolphins, reported similar results. S. R. Noren et al. (2002) determined that erythropoiesis is considerably more intensive in the organism of dolphins over three years of age. The evaluation of reticulocytes using Student's criterion showed that their level in the blood of female dolphins below two years of age was by 26.41–36.38% higher than in the blood of older female dolphins (in the group of females over 5 years old, the data did not match the normal distribution pattern) whereas in the blood of males aged 2–5 years (the distribution displayed a considerable asymmetry  $As=0.60$ ) it was by 23.12% higher than in the group of males aged over 5 years.

In the group of male dolphins aged 2–5 years, the level of leucocytes in the blood was by 10.95% lower than in the group of older male dolphins. In the blood of females aged 2–5 years, the level of leucocytes was by 20.05% lower than in the blood of younger females and by 13.80% lower than in the blood of older females. In the blood of male dolphins over 5 years of age, the level of segmented neutrophils was by 7.56% higher than in the blood of younger males. The level of segmented neutrophils in the blood of females over 5 years old was by 19.37 to 20.94% higher than in younger females. The obtained data are in good correlation with the reported literary data proving that in the blood of older dolphins the level of segmented neutrophils tends to increase (Venn-Watson et al., 2011).

The content of eosinophils in the blood of females aged over 5 years was by 29.03% higher than in the group of females aged below 2 years. The difference of this index in the groups of older and younger dolphins made up only 1.42%. The determined level of eosinophils in the blood of dolphins kept in the Lithuanian Sea Museum amounted to 15.40–21.70%. The researchers who analysed blood parameters of Atlantic bottlenose dolphins reported that the level of eosinophils in their blood only amounted to 10.00–11.00% (Shirai, Sakai, 1997; Bossart et al., 2002). Comparable results also were obtained by other researchers of Black Sea dolphins kept in dolphinariums (Andrejeva et al., 2007). A. G. Miciura, L.H. Bogdanova (2011) and I. V. Masberg (2011) suggested that the levels of eosinophils in the blood of healthy Black Sea dolphins might be elevated: by up to 16.00% in males and by up to 14.00% in females. Therefore, it can be stated that the levels of eosinophils in the blood of healthy Atlantic and Black Sea bottlenose dolphins are different.

The enzyme activity investigations contribute to diagnosing the early stages of liver, heart, blood and kidney diseases and to determining the character and dynamics of pathological process. Aminotransferases catalyse the interconversion of amino acids by transfer of

amino groups. Aminotransferases are of great clinical significance. Determining their levels is especially important for diagnosing the early symptoms of diseases and evaluating metabolism (Venn-Watson et al., 2008). Besides, bearing in mind that aminotransferases are present in all cells of the organism, the levels of aspartate aminotransferase and alanine aminotransferase in the blood serum serve as an indicator of the degree of damage of various organs. It was determined that investigations of blood enzymes and urea provide much valuable information about the sufficiency or deficiency of proteins in foods.

The increase or decrease of alkaline phosphatase may serve as an important prognostic index for evaluation of the health status of dolphins (Romano et al., 2004). Based on the obtained results, we can state that the activity of this enzyme in the blood of different age groups of animals varies considerably. The highest activity of this enzyme was determined in the blood of the youngest dolphins. In the blood of older dolphins, the activity of alkaline phosphatase was twice as low as in the blood of younger animals. Similar results have been reported by researchers from other countries that analysed blood parameters of Atlantic bottlenose dolphins (Goldstein et al., 2006; Venn-Watson et al., 2007). M. B. Fothergill et al., (1991) suggested that the activity of alkaline phosphatase increases in the period of growth and skeleton formation of animals.

As the values of potassium are normally distributed ( $Ex=0.46$ ,  $As=0.03$ ), it can be assumed that they are characteristic of the whole *Tursiops truncatus ponticus* population. The values of potassium in the blood of Black Sea bottlenose dolphins kept in the Lithuanian Sea Museum were lower than the ones reported in some literary sources. P. A. Fair et al., (2006) reported that the average concentration of potassium in the blood of Atlantic bottlenose dolphins was  $3.8 \pm 0.4$  mmol/l. According to our data, the concentration of potassium in the blood of dolphins aged below 5 years was by up to 7.6% lower than in the blood of older animals. Since the chlorine values are normally distributed ( $Ex=0.47$ ,  $As=0.17$ ), it can be assumed that the determined value of chlorine is characteristic of the whole *Tursiops truncatus ponticus* population.

The alternations of protein values in the blood may be a result of different diseases in dolphins. Therefore it is important to know not only the total level proteins but also the level of their different fractions. In the cases of inflammation, the level of albumins decreases whereas the levels of  $a_1$  and  $a_2$  globulins increase and in later stages the fraction of  $\gamma$  globulins, which are antibodies and very important for immune system, also changes. It was determined that in the cases of malignant tumours, the decrease of albumin levels and increase of  $\beta$  globulins are considerable. Researchers, who investigated the parainfluenza cases in dolphins, also observed hyperglobulinemia (Venn-Watson et al., 2008). In the future, the obtained results about haematological indices in dolphins could be used in the dolphin health status monitoring programs. The alternations of blood

parameters could contribute to diagnosing early, latent, signs of illness and some diseases and to adjustment of the process of treatment.

### Conclusions

1. Hemopoiesis dynamics in male and female Black Sea bottlenose *Tursiops truncatus ponticus* dolphins kept in the dolphinarium of the LSM was comparable. The age and sex of animals displayed no considerable and significant influence on alternations of blood parameters.

2. In the blood of healthy dolphins, the average value for leucocytes was  $6.9 \times 10^9/L$ , erythrocytes  $4.09 \times 10^{12}/L$  and reticulocytes 2.6%. The concentration of haemoglobin was 163.7g/L, volume of packed cells 43.5%, sedimentation rate of erythrocytes 1.85 mm/h. Segmented neutrophils accounted for 45.3–57.3%, lymphocytes 13.7–33.6%, monocytes 3.2–4.8%, eosinophils 15.4–21.7% of the total of leucocytes.

3. The average content of urea in the blood of dolphins was 16.82 mmol/L, creatinine 125.35  $\mu\text{mol}/L$ , glucose concentration 5.27 mmol/L, aspartate aminotransferase activity 220.65 U/L, alanine aminotransferase activity 38.65 U/L, gamma-glutamyl transferase activity 43.80 U/L, and alkaline phosphatase activity 1200.85 U/L.

4. The concentration of potassium in *Tursiops truncatus ponticus* dolphins amounted to 156.48 mmol/L, potassium 3.44 mmol/L, chlorine 118.28 mmol/L, and iron 29.94  $\mu\text{mmol}/L$ .

5. The total content of proteins in the blood serum of dolphins was 71.395 g/L. Albumins accounted for 61.62%,  $\alpha_1$  globulins 5.32%,  $\alpha_2$  globulins – 8.94%,  $\beta_1$  globulins 4.80%,  $\beta_2$  globulins – 5.34%,  $\gamma$  globulins – 15.37% and fibrinogen for 1.82 g/L.

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