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Hematological values of wild tucuxi (*Sotalia fluviatilis*) from the Central Amazon[1]

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Blood analysis is a valuable tool that is routinely applied in assessing the health and physiological status of free-ranging animals (Borjesson *et al*., 2000). However, very few hematological studies have been performed on wild cetaceans, at least partially because of the major logistical difficulties in obtaining samples from such large, mobile, and wholly aquatic animals (St. Aubin *et al*., 2000). Accidental or intentional entrapments by the commercial fishing industry has granted some opportunities to collect and examine the blood of harbor porpoises (*Phocoena phocoena*) (Koopman *et al*., 1999), and beluga whales (*Delphinapterus leucas*) (St. Aubin *et al*., 2000), while directed capture and release efforts has resulted in a robust data series for bottlenose dolphins (*Tursiops truncatus*) (Asper *et al*., 1990; Goldstein, *et al*., 2006).

The tucuxi (*Sotalia fluviatilis*) is endemic and widely distributed throughout the Amazon River Basin. A long-term project involving the capture and release of freshwater dolphins in the Amazon region of Brazil has created a unique opportunity to collect blood samples from wild tucuxis (da Silva and Martin, 2000). The present study reports the first hematological values determined from blood samples taken from two wild adult tucuxis. Comparisons to closely related species were conducted to determine relative blood values. Given the importance of properly storing blood samples taken from wild animals in remote locations, we also present an evaluation of the viability of samples stored at 5°C for up to two weeks at a field camp in central Amazonia.

Blood samples were collected at Mamirauá Sustainable Development Reserve (3°3'S, 64°51'W), located in the central Amazon Brazil. The entire seining, handling and release procedure lasted approximately nine minutes and each tucuxi was kept out of the water for a maximum duration of five minutes (da Silva and Martin, 2000). Blood was drawn from the superficial caudal peduncle vessels using a 19-gauge, 1.9cm long, butterfly catheter (Becton Dickinson Indústrias Cirúrgicas Ltda., São Paulo, Brazil, 04717-004) and stored at 5°C in 5ml ethylenediaminetetracetic acid (EDTA) Vacutainer tubes (Becton Dickinson Indústrias Cirúrgicas Ltda., São Paulo, Brazil, 04717-004) within six hours from the time of collection. Manual hematological techniques (Delaney and Garratty, 1969) were employed for red blood cells (RBC) and white blood cells (WBC) counts using Hayem and Turck reagents, respectively (Newprov Produtos para Laboratório Ltda., Pinhais, Paraná, Brazil, 83.323-020). Platelet counts were determined using a saline-formalin 40% solution (Hughes-Jones, 1979), binocular microscope (Nikon E-200, Nikon Instruments Inc., N.Y. 11747-3064, U.S.A.), and a Neubauer-Improved counting chamber (LO - Laboroptik GmbH, Friedrichsdorf, Germany). Microhematocrit capillary tubes were centrifuged at 12,850 X G for 10min and inspected against a standard calibration to determine packed cells volume (PCV). Samples for hemoglobin (Hb) analysis were prepared by pipetting 20µl whole blood into 5ml Drabkim's reagent (Newprov Produtos para Laboratório Ltda., Pinhais, Paraná, Brazil, 83.323-020) for subsequent photometric determination (Lima *et al*., 1992). Erythrocyte cell indices were calculated by mean cell volume (MCV)(fl) = PCV(%) x 10/RBC(10^6/mm^3) and by mean cell hemoglobin concentration (MCHC)(g/dl) = Hb concentration(g/dl) /PCV(%). Blood smears were treated with Wright's staining solution (Laborclin Produtos para Laboratório Ltda., Pinhais, Paraná, Brazil, 83.321-210) to conduct differential counts of 100 WBC per slide. Analyses of blood stored for 7 and 14 days were all performed using the same samples and methodology in order to evaluate possible changes in hematological values over time.

Comparisons to 59 wild *T. truncatus* from Indian River Lagoon, Florida, and nine healthy Guiana dolphins (*Sotalia guianensis*) maintained for at least four months in captivity at European dolphinaria, were also performed as an attempt to identify possible diversity of common blood values among several species (Goldstein *et al*., 2006; van Foreest, 1980).

Despite the low number of individuals in the present study (n = 2), a few differences occurred between wild freshwater and marine *Sotalia* species, although hematological values were very similar overall (Table 1). While PCV and Hb appear to be slightly higher in wild specimens, the RBC and MCV values fall outside the range estimated for samples from captive individuals. These observations may reflect changes due to certain conditions of their very different environments or be a result of stress in captivity influencing blood values. Previous studies have shown that captive common seals (*Phoca vitulina*) have consistently lower levels of the same blood component values (PCV, RBC, and Hb) compared to wild seals and attributed these observations to decreased activity caused by limited space and water depth in captive conditions (McConnell and Vaughan, 1983).

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Comparison of the values for *Sotalia spp*. to wild *T. truncatus* reveals that RBC values are still higher in *S. fluviatilis*, suggesting an interspecific difference rather than an effect of environmental conditions. All values estimated for *S. fluviatilis* fall within the ranges of values estimated for *S. guianensis*, although some differences were observed with respect to the WBC differential count. Again, it is difficult to conclude whether or not these differences are the result of conditions in captivity or simply a reflection of interspecific variation, especially considering that the antigenic stimulation of these three species is probably very different and promotes the production of distinctly different types of WBC.

Comparisons of seven hematological values between fresh and stored refrigerated blood (7 and 14 days) revealed important changes in all parameters (Table 2). While RBC, WBC and platelets values decreased during storage, values of PCV, Hb, MCV and MCHC all showed small increases. The observed decrease in values of RBC, WBC and platelets are all due to the effects of cell lysis that occurs naturally in stored blood. The most significant decrease occurred between the first and seventh days for RBC, 36.6% in tucuxi A and 17% in tucuxi B. WBC showed a decrease of 14.4% between the first and seventh day and 20% between the seventh and 14th day in tucuxi A, and 23.7% between the first and seventh day and 26.5% between the seventh and 14th day in tucuxi B. Platelets showed a more pronounced decrease (58.3%) between the seventh and 14th day in tucuxi A and between the first and seventh day (36.1%) in tucuxi B. While hematological values noticeably decreased over time, no patterns allowing for more precise inference regarding cell decrease with respect to time of storage prior to analysis emerged.

We observed that stored blood samples became browner and darker over time, promoting interferences in light measurements by spectrophotometry, but falsely increasing measurements of hemoglobin concentration and consequently increasing estimates of MCHC values. Slight increases in PCV, due to erroneous measurements caused by hemolysis and combined with decreasing RBC estimates, gave the false impression of increases in MCV. Hemoglobin concentration showed the largest increase (18%) between the seventh and 14th day in tucuxi B. MCV increased 62% and CHCM increased 5% between the seventh and 14th day in tucuxi A.

Table 1. Hematological values from two wild adult male *Sotalia fluviatilis*, mean and range values from nine captive *S. guianensis* (van Foreest, 1980**)** and mean, SD and range from 59 wild *T. truncatus* (Goldstein *et al*., 2006).

HEMATOLOGICAL VALUES	*Sotalia fluviatilis* (A)	*Sotalia fluviatilis* (B)	*Sotalia* ***guianensis***	*Tursiops* truncatus
PCV (%)	42	43	40.5 (38–43)	40 ± 2.41 (35–46)
RBC (10^6/mm^3)	5.98	5.16	4.27 (3.74–4.98)	3.61 ± 0.28 (2.8–4.8)
Hb (g/dl)	15.85	14.36	13.4 (12.9–14.5)	14.47 ± 1.11 (11.3–18.2)
MCV (fl)	70	83	94 (86–101)	112.2 ± 6.46 (96–126)
MCHC (g/dl)	37	33	33 (34–40)	35.93 ± 1.01 (32–38)
ESR[a] (mm/hour)	60	42	ND[b]	ND[b]
Platelets (10^3/mm^3)	240	144	ND[b]	167.12 ± 41.68 (73–281)
WBC (10^3/mm^3)	10.8	15.6	9.16 (5.7–14.1)	10.31 ± 2.58 (5.8–19.5)
Neutrophil (%)	51	48	56.5 (40–80)	44.55 ± 9.89 (25.3–68.4)
Band neutrophil(%)	0	2	0.5 (0–2)	0.04 ± 0.31 (0–2.41)
Lymphocyte (%)	40	38	27 (13–47)	19.29 ± 7.8 (2.04–47.33)
Monocyte (%)	2	4	2.5 (1–5)	3.29 ± 2.24 (0–10.69)
Eosinophil (%)	7	8	13.5 (7–22)	32.4 ± 9.28 (13.7–52.8)
Basophil (%)	0	0	0 (0–2)	0.04 ± 0.08 (0–0.3)

(ESR[a]) Erythrocyte sedimentation rate; (ND[b]) Not determined.

Table 2. Effect of time on hematological values in blood samples from two wild adult male *Sotalia fluviatilis*, fresh and stored for 7 and 14 days at 5°C.

	TIME	PCV (%)	RBC (10^6/mm^3)	Hb (g/dl)	MCV (fl)	MCHC (g/dl)	WBC (10^3/mm^3)	Platelets (10^3/mm^3)
Sotalia fluviatilis (A)	Day 0	42	5.98	15.81	70.23	37.64	10.8	240
	Day 7	43	3.77	16.99	114.05	39.51	9.25	192
	Day 14	44	3.64	18.50	120.88	42.04	7.40	80
Sotalia fluviatilis (B)	Day 0	43	5.16	14.36	83.36	33.39	15.6	144
	Day 7	44	4.28	16.18	102.80	36.77	11.9	92
	Day 14	ND[a]	4.27	19.12	ND[a]	ND[a]	8.75	64

(ND[a)] Not determined.

Changes in MCV between the seventh and 14th day were not so pronounced in tucuxi B, showing an increase of 23%, and 10% in CHCM. PCV of tucuxi B could not be estimated from the seventh day samples due to a high degree of hemolysis and, consequently, MCV and MCHC could not be calculated. Blood samples from tucuxi showed great sensitivity to storage time, even when maintained in a continuously refrigerated state, and should be analyzed within 24 hours from the time of collection to achieve the most accurate estimates of hematological values possible.

There is a clear and urgent need for reliable, representative baseline hematological value data from wild *Sotalia* spp. for long-term population monitoring. The value estimates reported here are from only two individuals and do not represent statistically valid normal ranges, and thus should be used with caution. However, these data do provide important preliminary estimates of blood values for wild *S. fluviatilis* and will be useful in future conservation efforts.

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