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Amazonian Manatee Urinalysis: Conservation Applications

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1. Introduction

The Amazonian manatee (*Trichechus inunguis* Natterer, 1883) is an aquatic mammal (Family Trichechidae) that inhabits freshwater environments. It is endemic to the Amazon Basin, and occurs from Marajó Island (at the mouth of the Amazon River in Brazil) to the headwaters of the floodplain in Colombia, Peru and Ecuador.

Historically, the Amazonian manatee has been subjected to strong hunting pressure, and was a source of food not only for indigenous and fishery communities of the Amazonian region, but also a target of large-scale commercial fisheries throughout the 19th and early 20th centuries (Best, 1984; Rosas & Pimentel, 2001). In the year 1650 tons of meat and fat of these animals were sent to Europe (Best, 1982, 1984, Da Silva & Best, 1979; Junk and Da Silva, 1997). Later, between 1935 and 1954, its skin was used industrially. Due to durability, this material was used for the manufacture of pulleys, belts and hoses (Best, 1982, 1984), but with the advent of synthetic materials, its use in industry became less common (Rosas, 1991; Vianna et al., 2006

Today, despite being illegal, Amazonian manatees are still hunted (Rosas, 1994) since their flesh is still commonly consumed regionally. Manatee bones, skin and fat are also used for commercial purposes such as drug manufacturing. (M. Marmontel pers. comm., 2009). Due to the persistence of hunting, the animal has been listed as endangered species by the Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA Portaria n° 1.522/89 - and included in the International List of endangered animals of the International Union for Conservation of Nature - IUCN, as a vulnerable species (Ayres & Best, 1979, Costa et al. 2005; MMA, 2001, Rosas, 1991, Trujillo et al., 2006, 2008, Vianna et al., 2006). In Colombia, the species is also included in the Red Book of endangered species (Trujillo et al., 2006). Although protected by law in Brazil since the 1967 (Rosas, 1994), subsistence hunting, and to a lesser degree, commercial hunting – both of which still persist – have kept the species among those with "vulnerable" status (Hilton-Taylor, 2000).

In addition to hunting, other factors threaten the populations of the Amazonian manatee through occasional degradation and even loss of habitat for the species, leading to a decreased availability of habitat for performing key events in its cycle life (feeding, reproduction, etc.). Deforestation of riparian environments, water pollution (a serious threat to an herbivorous aquatic species, by damaging its habitat and a decrease of dietary plants), construction of hydroelectric plants, and accidental capture by fishing nets are also potential risks to this species (Rosas, 1991; Trujillo et al., 2006, 2008).

Urinalysis can be useful in determining the health status of captive animals. In this context, chemical, physical and sedimentological urinalysis could be a useful tool to monitor the health status of wild Amazonian manatees , increase our knowledge of its physiology, and provide a scientific foundation for future physiological studies of this species.

Due to the difficulty of obtaining biological samples, few data are available on the characterization, collection and production of urine in aquatic mammals in general, and very little is known about the composition of Amazonian manatee urine. The only available information on the urine of this species derives from analyses conducted in the Laboratory of Aquatic Mammals (LMA) of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazon, Brazil. Some of these results were recently published (Pantoja et al., 2010), using urinalysis in order to establish normal ranges of urinary parameters to help monitor the health of this species in captivity. To accomplish this, the authors performed chemical urinalysis to obtain quantitative values of glucose, urea, creatinine, uric acid and amylase levels obtained using colorimetric spectrophotometry.

Other important information derived from analyses conducted over thirty years ago with seven captive specimens kept in the Laboratory of Aquatic Mammals (LMA) of the same institute. This information came from urine collection carried out by Robin Best with captive Amazonian manatees in 1978. These data were not published, but could be accessed from LMA's files, and were used as a comparison for the data obtained in this investigation, which presents the results from chemical, physical and sedimentological analysis of Amazonian manatees' urine in order to continue research useful for monitoring health in captivity.

We believe that any decisions about an animal's release or return to the wild should be made with caution and security. The safety of the individual animal as well as the continued well-being of the wild host population should be carefully considered. The information provided by this study can help support scientifically based reintroduction programs to re-establish or reinforce endangered native wild populations of Amazonian manatee. Urinalysis can be a useful tool since it can help assess the health status of animals proposed to be released or returned to the wild.

By establishing baseline data on physiological parameters using healthy captive animals, this study may also contribute to Amazonian manatee conservation since urine collection from wild manatees can help evaluate the condition of wild populations. Likewise, urinalysis could be used to monitor the health of other captive animals, such as those that may be reintroduced to former areas of habitat from which the species was removed by excessive hunting. Finally, this study extends our knowledge of the manatee's basic physiology, hopefully contributing to its effective management and conservation.

2. Material and methods

All animals studied were judged to be clinically healthy, based on their general appearance. Within a twelve-month period, 21 animals were sampled - nine females (F) and twelve males (M), classified into the following age classes: calves (0-2 years old/2 F and 2 M), juveniles (3-5 years old/4 F and 3 M), sub adults (6-9 years old/1 F and 4 M) and adults (over 10 years old/2 F and 3 M).

All juveniles, sub adults and adults were kept in three big pools (197 m^3 each) and the calves were distributed in four smaller pools (6.4 m^3 each). The animals were fed with grass (*Brachiaria mutica*), lettuce, cabbage and other vegetables at approximately 10% of their body weight per day. In addition, the calves were nursed by their mothers or fed with an artificial milk formula (Rodriguez et al., 1999).

Urine was collected once a month when the tanks were drained, by placing stainless steel containers under the genital slit of females until their urination (Fig. 1a). Males were turned on their side and abdominal massages were applied to stimulate micturition (Bossart et al., 2001) (Fig. 1b). When this latter procedure did not work, the same method employed to females was applied to males.

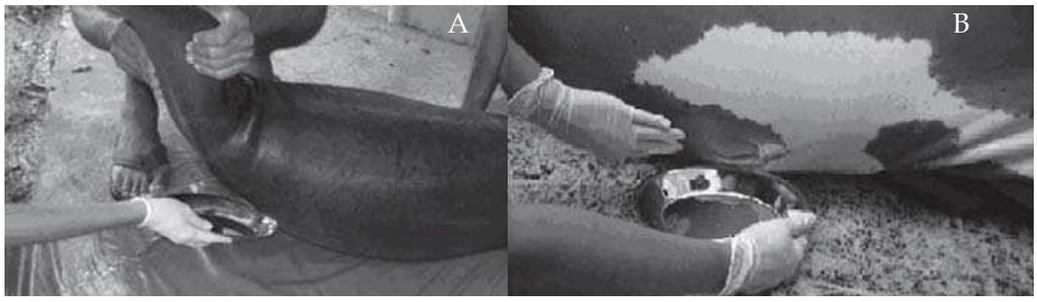

Fig. 1. A) Stainless steel containers being placed under the genital slit of female Amazonian manatee until their urination (Photo: Mattos, G. E.); B) Urine collection in male Amazonian manatee, turned on its side, with belly being massaged to stimulate urination. (Photo: D'Affonseca Neto, J. A.).

Chemical urinalysis was conducted by using dip strips (URISCAN™) just after urine collection of each sample by soaking the strips in fresh urine and making a comparison of the resulting color of the strip with the standardized color chart provided with the kit. Urinary parameters measured by dip strips were: protein, ketones, urobilinogen, bilirubin, pH, blood cells (erythrocytes), leukocytes, nitrite, specific gravity (actually a physical parameter), and glucose, which was also analyzed quantitatively by colorimetric spectrophotometry chemistry analyzer "Dimension AR" (Dade Behring) after centrifugation of samples to 300 RPM. All these compounds are physiologically related to homeostasis equilibrium, whose monitoring can be a useful tool to management actions (e.g. environment protection programs that aim to reintroduce these mammals to their natural environment). Urinary compound levels are indicative of the health status of manatees and other aquatic mammals (Gürtler et al., 1987; M. S. Matos & P. F. Matos, 1995; Kantek Garcia-Navarro, 1996).

The physical examination consisted in the observation of color, appearance and density of urine samples. The first two were made with the naked eye, in conjunction with sediment analysis, always using good light for easy viewing and characterization (Strasinger, 1996). The terminology used for recording urine color was: colorless, yellow, lemon yellow and reddish. Appearance was recorded as: clear, semi-cloudy and cloudy. Although the specific gravity can be considered a physical parameter, this was considered along with other chemical parameters following the method described for the reactive strips.

For sediment urinalysis, samples were centrifuged and the remaining solid was placed between slide and coverslip for examination under a light microscope (10x and 40x magnification). Elements present in the urinary sediment were counted per visual field (10X magnification). Leukocytes and erythrocytes were counted in five visual fields and then averaged between them for each urine sample. Epithelial cells and crystals were recorded as absent, rare (only a few elements in the visual field), frequent (half the visual field containing these elements) or numerous (elements filling the entire visual field). Bacterial abundance was recorded as "absent", "low" (few bacteria per visual field), moderate (half the visual field containing bacteria) and "high" (bacteria fill the entire visual field). For hyphae, these were recorded as present or absent. Little light was used to facilitate the search of urinary sediment elements (Bossart et al. 2001; Kantek Garcia-Navarro, 1996).

Qualitative results obtained by reactive strips (protein, ketones, urobilinogen, bilirubin, pH, blood cells (RBCs), leukocytes, nitrite, specific gravity and glucose) and data obtained from analysis of color, appearance and sediment were grouped in tables, according to sex and age. Some elements of the sediment were photographed through a camera attached to the microscope and these results (boards with such elements) will be presented throughout the chapter.

3. Results and discussion

A total of 188 tests were performed, 108 of them with urine samples of males and 80 with urine samples from females[1].

3.1 Chemical urinalysis

3.1.1 Protein

Protein levels indicated by reactive strips were minimal (<10 mg/dL) and constant throughout the experiment, for both males and females, and for different age groups.

Normally, proteins do not cross the glomerular membrane. When this occurs, it is in a small quantity which is then reabsorbed in the tubules. If some protein is not reabsorbed, a little can be present in urine but in such small quantities that can remain undetectable, or at most, appears as "traces". This can occur in more concentrated urine, like the first morning urination or that produced after muscular effort. Proteinuria (protein in urine) may have a renal or a post-renal origin, and the former may be physiological or caused by a glomerular

[1]In the first month of collection (June) it was not possible to collect urine from a juvenile female (Adana).

lesion (Gürtler et al., 1987) characterized by the presence of cylinders in the sediment. Proteinuria without casts (cylinders in the urine), suggests that its origin is post-renal, or passing, without further associated injury (Kantek Garcia-Navarro, 1996).

Low levels of protein (1 g/L (100mg/dL)) were detected in *Halichoerus grypus* (gray seal) urine by Schweigert (1993). Although low, these values were higher than those detected in this study in Amazonian manatees, possibly due to the difference in protein consumption, which is lower in *T. inunguis,* because it is an herbivorous animal.

Despite the small sample size, the constant and negative results observed over the nine months of the experiment (<10 mg/dL) ruled out the possibility of the occurrence of pathological or physiological proteinuria in manatees sampled and these results were considered as normal for the qualitative detection of proteins by reactive strips for the species.

3.1.2 Ketones

The measured levels of ketones in urine samples were also constant for 100% of the samples, both for males and females in different age groups, and always corresponded to the minimum detectable by reactive strips (<5 mg/dL).

When the diet is low in carbohydrates, the body uses its fat reserves for energy. The metabolism of fatty acids results in the formation of so-called ketone bodies (ketones), which when excessive can be observed in urine (ketonuria). Such an occurrence may be indicative of: 1) diabetes mellitus (usually accompanied by hyperglycemia and glucosuria), 2) starvation (as ketone bodies are just from the metabolism of body fat stores in case of famine), 3) intake grazing of poor quality, i.e., containing a few digestible carbohydrates, in the case of cattle, 4) prolonged vomiting or diarrhea (which cause ketonuria similar to that caused by starvation), or 5) other causes such as acute febrile diseases and toxic states (especially when accompanied by vomiting or diarrhea), endocrine disorders such as hyperactivity of the anterior pituitary and adrenal cortex, acute or chronic liver disease (Kantek Garcia-Navarro, 1996), and ketosis in ruminants (Gürtler et al ., 1987).

A non-detection of ketone bodies by qualitative analysis using reactive strips in urine from the gray seal was reported by Schweigert (1993). Similar results were obtained in this study with Amazonian manatees, and the constant qualitative results of ketone bodies (<5 mg/dL, negative value) suggests, in addition to discarding the hypothesis of occurrence of any of the pathologies listed above, that this should be the normal value expected for *T. inunguis* urine.

3.1.3 Urobilinogen

Urobilinogen values detected in urine samples from males and females did not vary throughout the experiment and showed minimum value measured by reactive strips (0.1 mg/dL).

Urobilinogen is the reduced form of conjugated bilirubin. The elimination of a part of this component in urine is normal, since it is the main urinary pigment that gives yellow coloration to the urine. The main causes of urobilinogenuria (increased urine urobilinogen)

are: 1) liver disease (in which urobilinogen gets to be detected in dilutions up to 1:40) or 2) hemolytic jaundice. The decrease in urinary urobilinogen, in turn, may be due to: 1) total or partial obstruction of the bile ducts, 2) diarrhea (which may decrease the intestinal absorption of urobilinogen, causing a slowdown in its elimination), 3) action of certain antibiotics that cause inhibition of normal intestinal flora that are responsible for its production, or 4) chronic nephritis (that is accompanied by polyuria, which causes dilution of urobilinogen in urine) (Kantek Garcia-Navarro, 1996).

The constant results (0.1 mg/dL, negative values) ruled out urobilinogenuria and suggest that these values can be considered as normal for qualitative research by reactive strips of urobilinogen in the urine of Amazonian manatee. The possibility of a reduced elimination of urobilinogen in urine can be ruled out, since, in addition to the uniformity of the results, samples showed minimal yellowing.

3.1.4 Bilirubin

Except for an examination of a juvenile male (Mapixari) in July and two other examinations performed on two subadult males (Erê and Guarany) in November, which resulted in 0.5 mg/dL, bilirubin values obtained for all other males and all females were the least detectable by reactive strips (<0.5 mg/dL).

Bilirubin is a pigment derived from hemoglobin resulting from the degradation of erythrocytes by the macrophage-monocytic phagocytic system. When released by macrophages, bilirubin binds to albumin and thus does not appear in urine as the latter does not cross the glomerular barrier. Bilirubin in the liver turns off the albumin and conjugates with glucuronic acid to form conjugated bilirubin, which in turn is excreted in small amounts but not in all animals (only 20% of dogs, 5% of cats and 25% of cattle). An increase in bilirubin in urine (bilirubinuria) may be due to: 1) obstruction of the bile ducts, causing bile reflux (conjugated bilirubin) into the circulation, causing bilirubinuria, 2) liver disease (such as infectious canine hepatitis, leptospirosis, liver cirrhosis, various cancers of the liver, liver toxicosis and other) 3) hemolytic jaundice, or 4) intestinal obstruction (since there is decreased elimination of bilirubin through the gut, thereby increasing the rate of plasma conjugated bilirubin , which happens to be excreted in the urine) (M. S. Matos & P. F. Matos, 1995; Kantek Garcia-Navarro, 1996).

Negative results (<0.5 mg/dL) observed in most quality tests by reactive strips in both males and females of *T. inunguis,* and the results of sub-adult males (Erê and Guarany) and of a juvenile male (Mapixari) (which reached 0.5 mg/dL, still considered "traces") did not point to any of the conditions listed above. Low values of bilirubin, according to M. S. Matos & P. F. Matos (1995), can usually be found in 25% of healthy cattle, corroborating that the presence of bilirubin as "traces" does not confirm the occurrence of diseases. The absence of bilirubin was also reported in earlier studies performed in *T. inunguis* (Best, unpublished data[2]); in only one male (Xingu, 1/19/1978) were "light strokes" of bile pigments were

[2]This information came from urine collection carried out by Robin Best (in memorian) in captive Amazonian manatees in 1978. These data can be found in LMA's files, and it was possible to access them with the permission of the LMA directorships: Dr. Fernando César Weber Rosas and Dr. Vera Maria Ferreira da Silva.

detected. Therefore, negative values of bilirubin were regarded as the normal condition expected for *T. inunguis* urine. The "traces" of this pigment detected by reactive strips does not consist in a diagnosis of any of the physiological dysfunctions mentioned above.

3.1.5 pH

The minimum pH value measured by reactive strips in the urine of the Amazonian manatee was 5.0 and the maximum was 9.0. The pH 8.0 was the most observed in the samples (n=132), differing only in female infants, whose pH in most samples (n=4) was 6.0.

Urine pH is related to the maintenance of acid/base equilibrium maintained by the renal elimination of nonvolatile acids and alkalis. It is determined by metabolism and diet, being more acid in animals with high protein diets (meat eaters), and more basic in animals with diets high in carbohydrates (herbivores) (Gürtler et al. 1987; Kantek Garcia-Navarro, 1996).

With respect to organic metabolism, the pH of the urine normally accompanies the body pH, except in cases of aciduria (urine with an acid pH) found in the alkalosis existing in severe hypochloremia accompanied by vomiting (paradoxical aciduria) (Kantek Garcia-Navarro, 1996). Table 1 below shows the reference values of urinary pH of some domestic and laboratory animals, compared with Amazonian manatee detected values.

Animal group	pH	Reference
Cattle[H]	7.4 to 8.4	
Canidae[C]	5.5 to 7.5	
Felidae[C]	6.0 to 7.0	
Equine[H]	7.0 to 8.0	Benjamin (1976)
Sheep and goat[H]	7.0 to 8.0	
Pig[O]	5.5 to 8.5	
Human[O]	4.8 to 7.5	
Amazonian manatee[H]	6.0 to 9.0	Best (unpublished data)
Amazonian manatee[H]	5.0 to 9.0	This study

C=carnivorous; H=herbivorous, O=omnivorous

Table 1. Urinary pH values of some domestic and laboratory animals, humans, and the Amazonian manatee.

Urine acidification may be due to: 1) starvation (when the body uses its own plasma proteins resulting in an acidic urine), 2) respiratory or metabolic acidosis (the former occurs by the accumulation of CO_2 in the lungs in patients with emphysema, pulmonary fibrosis and cardiopulmonary failure, and the latter occurs when the body loses bicarbonate or accumulate acids, a condition observed in diabetes mellitus and chronic renal failure with uremia, i.e., a large quantity of urea in the blood), 3) rapid action drug therapy, such as methionine or sodium chloride, calcium, or ammonia, 4) increased protein catabolism, as with fever or great muscle effort, 5) rapid absorption of cavity fluids (which have high protein value); 6) paradoxical aciduria (mentioned above), or 7) diseases that cause tissue disintegrations, such as diabetes, Fanconi syndrome and proximal tubular acidosis (Kantek Garcia-Navarro, 1996).

On the other hand, urine alkalinization may be due to: 1) time taken to perform the exam (since there may be bacterial growth transforming urea to ammonia; however, in the present study this possibility was excluded by carrying out the pH determination immediately after urine collection) (Bossart et al., 2001), 2) cystitis, especially when accompanied by urinary retention, and subsequent bacterial action on urea, as above, 3) treatment with salts of alkaline reaction, such as baking soda, or sodium lactate, citrate or sodium or potassium acetate, potassium nitrate , acetazolamide and amphotericin B or 4) metabolic alkalosis (due to accumulation of bicarbonate), or respiratory failure (when there is increased ventilation resulting from CO_2 elimination at a greater rate than its production, a situation that can occur in acute cardiorespiratory disease accompanied by hypoxia) (Kantek Garcia-Navarro, 1996).

The range of pH values recorded in this study (5.0 to 9.0) was similar to that observed originally for *T. inunguis*, which was 6.0 to 9.0 (Best, unpublished data). In most of our qualitative analysis by reactive strips, detected pH was alkaline (pH=8.0) for both males (n=85) and for females (n=47). This finding corresponds to that expected for herbivore urine (Kantek Garcia Navarro, 1996; M. S. Matos & P. F. Matos, 1995), agreeing with the results found by Best (unpublished data). Manire et al. (2003) observed in two males (11 and 14 years) of *Trichechus manatus latirostris* an average pH=8.0 ±0.31 (n = 15) and 7.99 ±0.30 (n=13) – these values are very similar to those observed in most samples analyzed in this study. However, Bossart et al. (2001) had reported more acidic values in *T. manatus latirostris* (6.0 to 7.5).

More acidic pH values (between 5.0 and 6.0) were recorded in gray seals by Schweigert (1993), as well as for the southern sea lion (*Otaria flavescens*), whose pH values of 31 samples ranged from 5.69 to 7.0 (mean 6.25 ±0.26) (Le Bas, 2003). pH values around 6.0 were also observed in the urine of three freshwater dolphins *Inia geoffrensis, Platanista indi* and *P. gangetica (*De Monte & Pilleri, 1972), which have a high protein diet due to feeding on fish. In this study, the registration of more acidic pH (5.0 and 6.0) was not attributed to: 1) starvation, since none of the animals stopped eating at this point, 2) the action of acid-acting drugs, which were not administered to the manatees in the period of the experiment, or 3) any of the diseases listed above as possible causes of urinary acidification, since the animals were apparently healthy throughout the experiment. However, the following factors: 1) increased protein catabolism, caused by intense muscular effort (possibly caused when the animal was restrained for urine collection) and 2) rapid absorption of cavity fluids could be considered as well as normal individual variations. However, these factors need further studies to confirm occurrence in manatees as the cause of urinary acidification.

The small sample size did not allow a more robust statistical check on the changes in pH observed, and although the value 8.0 was the most frequent, there is a need for more conclusive studies that would allow the establishment of a normal value expected by qualitative assessment of this parameter by reactive strips.

3.1.6 Blood cells (erythrocytes – red blood cells RBC's)

Blood cells (RBCs) detection by reactive strips pointed to following values: <10, 10 and 50 RBC/mL. The value of 10 RBC/mL was observed in 86.7% of the tests (n=163) of both males and females of different age classes.

When there is blood in urine, it has reddish-brown color. This occurrence can sometimes manifest as haematuria, sometimes as hemoglobinuria. The difference will be made by the presence of erythrocytes (RBCs) in the sediment and by the appearance of urine. Haematuria is confirmed when red blood cells are present in whole urine, appearing cloudy. It follows from hemorrhage or renal urogenital tract, glomerulonephritis, vasculitis, or renal infarction (when red blood cells pass into the tubules). Hemoglobinuria, in turn, is the presence in solution of hemoglobin in the urine, cloudy and semi-looking brown or red (due to the fact that hemoglobin is free in plasma) (Kantek Garcia-Navarro, 1996).

According to its origin, hemoglobinuria can be true or false. In the true form, it appears in the urine half blurred due to the presence of free hemoglobin in plasma (hemoglobinemia). This hemoglobin passes through the glomerular barrier and, owing to its great quantity, is not fully reabsorbed and may be preset in urine suggesting intravascular hemolysis, which occurs in hemolytic anemia with intravascular hemolysis and hemoglobinemia. The latter include babebioses and hemolytic disease in newborn individuals. The hemoglobinemia with intravascular hemolysis and hemoglobinuria may be due to either 1) *Clostridium haemolyticum* or *C. perfringens* infections, 2) postpartum hemoglobinuria (M. S. Matos & P. F. Matos, 1995), 3) ingestion of certain toxic plants, 4) severe burns with destruction of large amounts of tissue, 5) the action of toxic agents (e.g. copper , mercury and sulfa drugs), or 6) the action of hemolysins produced by *Leptospira pomona* (Kantek Garcia-Navarro, 1996). Even if the latter could be considered in the diagnosis of *T. inunguis* since Marvulo et al. (2003) detected antibodies against *Leptospira sp.* in blood samples from two Amazonian manatees in captivity, suggesting that these animals had had contact with leptospires. There is a need for further studies to elucidate this finding.

Regarding the false presence of hemoglobin, this is a consequence of the breakdown of red blood cells present in very dilute or alkaline urine, a fact that usually occurs *in vitro*, and may also occur within the urinary bladder. In fact, it's a haematuria masquerading as hemoglobinuria. To confirm it is necessary to search erythrocytes in whole sediment, since such hemolysis, not rare, is partial (Kantek Garcia-Navarro, 1996).

The strips are highly sensitive to reactive detection of hemoglobin, but have low sensitivity to intact erythrocytes, requiring microscopic examination for their confirmation ("URISCAN™ Urine Strip" bull). The qualitative results obtained by reactive strips were disregarded, since in the sediment survey we observed different amounts of red blood cells detected by reactive strips. Therefore, in the present study, detection of red blood cells during microscopic analysis of the sediment was considered as the most efficient. Additionally, the "URISCAN™ Urine Strip" bull alert to the fact that reaction to the test strip may vary from one patient to another, and that even in case of detection of intact red blood cells or hemoglobin, its necessary to analyze the sediment for confirmation.

3.1.7 Leukocytes

The amount of leukocytes in the samples did not exceed 25 WBC/mL, and, for 90.4% of them (n=170) less than 25 WBC/mL were detected for both male and females of different age groups.

Leukocytes are white blood cells that may appear in urine as small cells, with round and granular cytoplasm; 7 per field is the amount considered normal for leukocytes in urine (Kantek Garcia-Navarro, 1996). In case of a reaction to an infection, it appears in its degenerate form (pus cells), and when present in large numbers may be indicative of disease (Gürtler et al., 1987) as inflammation of the renal system, prostate, uterus or vagina (M. S. Matos & P. F. Matos, 1995), or simply be due to fever or strenuous exercise (in this case appears only temporarily) (Kantek Garcia-Navarro, 1996). The quality tests for reactive strips for leukocytes detection in samples proved to be ineffective, as the results never coincided with those observed by microscopic analysis of the sediment, which was considered the most accurate research of leukocytes in samples.

3.1.8 Nitrite

The detection of nitrite by reactive strips in males was variable throughout the experiment. Despite the absence of nitrite in most samples of males (n=73), in a few months nitrite was detected in at least half of them. The survey of nitrite in the samples of females, in turn, followed a pattern throughout the experiment, indicating the absence in the urine analyzed, except the samples for the months of July and October, in an adult female (Tukano).

Nitrites can be produced by bacteria present in urine, so the presence of nitrite in urine may indicate urinary tract infection. Apparently, the test has not had the same use in animals and in humans, although a correlation was recently demonstrated between the presence of nitrites in urine and urinary tract infections in pigs (Kantek Garcia-Navarro, 1996).

Because the results for nitrite in urine is directly related to the presence of bacteria in the urine, it will be discussed later, along with discussion of elements of urinary sediment. It is noteworthy that the results of the presence or absence of nitrite in the urine, detected by reactive strips, also did not coincide with the appearance of bacteria in urine during microscopic analysis of the sediment, a method that, as in the case of leukocytes, was considered more accurate for confirming the presence of nitrite in the samples.

3.1.9 Specific gravity

Values observed in urine specific gravity of *T. inunguis* ranged from 1.000 to 1.015. The value 1.005 was observed in most samples (n=153). A single urine sample had density 1.015 (a female juvenile (Adana) in October).

The specific gravity of urine determines the ability of renal reabsorption. It defines the urine specific gravity compared to the density of the same volume of distilled water at the same temperature. Since urine is actually water that contains dissolved chemicals, urine specific gravity is nothing more than a measure of the density of these chemicals dissolved in the sample (Strasinger, 1996). Therefore, since the density measures the degree of solutes present in the sample, it evaluates renal ability to concentrate urine. The higher the density value, the more concentrated (hypertonic) is the urine, i.e., an increase in density indicates a decrease in glomerular filtration and/or increased water reabsorption, facts that lead to a large reduction in urine flow (olyguria) (Kantek Garcia-Navarro, 1996).

Decreased urine specific gravity typically accompanies polyuria (except in diabetes mellitus, in which polyuria is associated with high density), and may be due to: 1) uremia (clinical condition in which there is an increased rate of urea in the blood, by renal (primary) or systemic (secondary) cause, and in aggravated cases, density may be extremely low due to the inability of the kidney to dilute the urine), 2) diabetes insipidus, 3) uterine empyema (pyometra) which produces excessive thirst (polydipsia) and large increase in urine flow (polyuria) in female domestic dogs and 4) corticosteroids therapy, parenteral fluids or diuretics, usually accompanied by polyuria; 5) isosthenuria (urine with the same density of filtered plasma before passing through the glomerular meshwork) resulting from renal failure in dilute or concentrate urine; 6) chronic interstitial nephritis (due to kidney inability to concentrate urine), or 7) simple excessive water consumption (M. S. Matos & P. F. Matos, 1995; Kantek Garcia-Navarro, 1996).

Related to the increase in urine specific gravity, usually associated with olyguria (except diabetes mellitus), this can be seen in cases of: 1) acute interstitial nephritis (with density values between 1.030 and 1.060, due to the initial phase of the disease in which there is inability of renal elimination of water), 2) general acute nephritis (where there is reduced glomerular filtration rate (producing a more concentrated urine), 3) diabetes mellitus and primary renal glucosuria (an increased density accompanied by polyuria, as that glucose "load" a greater amount of water), 4) dehydration, sometimes due to vomiting, diarrhea, or excessive sweating as they reduce the amount of water available in the kidney, thus producing a more concentrated urine (values greater than 1.050 in dogs and cats up to 1.060, suggest severe dehydration), 5) fever (since body attempts to retain water, thus producing a more concentrated urine), 6) edema (due to a circulatory dysfunction, caused by excessive fluid retention in the body, resulting in olyguria with high density), 7) shock, hypotension which causes a sudden fall in renal perfusion, producing olyguria, which can reach the cessation of urine flow (anuria) (Kantek Garcia-Navarro, 1996); 8) cystitis, or 9) debilitating illnesses tissue destruction (M. S. Matos & P. F. Matos, 1995).

Although little is known about the maintenance of water in manatees, Maluf (1989) analyzing the anatomy of nine manatee kidneys (*Trichechus manatus*), suggested that they have an increased ability to concentrate urine. Since *Trichechus manatus* inhabits both freshwater and marine, it can be considered an interesting model for osmoregulation study in Sirenians. Drawing on this, Ortiz et al. (2001) conducted studies with that species, and concluded that manatees are excellent osmoregulators, regardless of the medium in which they live. In *T. inunguis,* the results of our analysis pointed to a little concentrated urine, tending to low density, which in freshwater animals, serve to expel the excess water, while solutes are retained (Schmidt-Nielsen, 2003). Low specific gravity was also reported by Manire et al. (2003) for two males of *T. manatus latirostris* (1.008 ±0.002, n=15 and 1.010 ±0.004, n=13) that did not respond significantly between the manipulations made in the experiment (reduction in the amount of food, change of diet cabbage, apples, carrots and beans to "seagrass" and change from freshwater to saltwater), indicating that manatees probably would not concentrate their urine to conserve fluid, even when in hemoconcentration (dehydration). The amplitude of density urinary density values measured by Best (unpublished data) was between 1.000 and 1.013, very similar to that observed in our study (1.000 to 1.015). Higher levels of density were observed by Schweigert

(1993) for gray seal, whose values ranged between 1.033 and 1.052, given the exceptional ability of marine mammals' reniculate kidneys to concentrate urine.

Although 1.005 has been the value observed in most samples (n=153), both in males and females, variations that occurred in some tests were considered acceptable, since they did not exceed 1.015 (maximum value observed in the samples analyzed in this study), which would be a much more concentrated urine than those previously reported for *Trichechus*. However, further studies are needed to establish a normal value expected from qualitative assessment of reactive strips for urine density in *T. inunguis.*

3.1.10 Glucose

In our previous research (Pantoja et al, 2010), we measured quantitative levels of glucose by a chemical analyzer, and found no statistically significant difference between sexes and age classes, leading to the establishment of a normal range (3.0 to 3.6 mg/dL) for this parameter. Since the present examination of glucose levels by reactive strips in both males and females, regardless of age group, was the minimum value detectable by the strips (<100 mg/dL), we should take this result as a confirmation of the adequacy of reactive strips as a rapid, cheap and efficient method to access this condition in manatee urine samples.

When glucose is present in normal amounts in the blood, it is absent in the urine, since it is completely reabsorbed in the proximal renal tubules, appearing only as traces in urine (Gürtler et al. 1987; M. S. Matos & P. F. Matos, 1995; Kantek Garcia- Navarro, 1996). Its appearance in urine occurs when the amount of glucose in the glomerular filtrate exceeds the capacity of the tubule reabsorption (when glucose is increased in the blood due to diabetes mellitus), or when there is insufficient tubular reabsorption. The occurrence of glucosuria (increased glucose in the urine), should be confirmed by measuring the amount of glucose in the blood of the animal during fasting (M. S. Matos & P. F. Matos, 1995; Kantek Garcia-Navarro, 1996).

Glucosuria may have physiological or pathological origin. The physiological glucosuria is usually transient and can result from ingestion of large amounts of carbohydrates. Emotional glucosuria may occur during animal restraint during urine collection, since a sudden release of adrenaline occurs. According Kantek Garcia-Navarro (1996) there are cases of glucosuria in domestic cats under physical stress, or severe bleeding in the bladder. When pathological, glucosuria is evident both in animals fasting or at rest and can indicate the following conditions: 1) diabetes mellitus, 2) acute pancreatic necrosis (when insulin production drops, thereby determining the subsequent hyperglycemia and glucosuria), 3) hyperthyroidism (and quick absorption of carbohydrates in the gastrointestinal tract), 4) acute renal failure, in which there is deficiency in the tubular reabsorption of glucose, or 5) chronic liver disease, in which there is an inability to regulate the liver glycogen stores (Kantek Garcia-Navarro, 1996). In the case of diabetes mellitus, glucose concentration varies according to the severity of the disease, which occurs mostly in older dogs and cats (Gürtler et al., 1987).

The consistently negative results of glucose (<100 mg/dL) measured qualitatively by reactive strips suggests that the expected levels of glucose in *T. inunguis* should be low. Quantitative analysis by means of chemical analysis "Dimension AR" ("Dade Behring")

resulted in low levels of glucose, with an amplitude in males 0 to 10 mg/dL and in females 0 to 13 mg/dL. These values were considered very low, corroborating the results obtained by reactive strips. These low levels obtained by both methods ruled out the occurrence of glucosuria, and therefore the diseases related to it, and suggest the applicability of reactive strips in routine examinations in Amazonian manatees in captivity because of the ease, convenience and low cost of this method.

The failure to detect glucose was also reported in gray seals by Schweigert (1993). De Monte & Pilleri (1972) found traces of glucose in an individual of *Platanista minor* (Indus river dolphin), claiming that this was the first detection of this substance in cetaceans by these authors. The detection of high levels of glucose (200 mg/dL) was also reported by Ridgway et al. (1968) in one specimen of bottle-nosed dolphin *Tursiops truncatus* in captivity without clinical symptoms of diabetes. The data from urinalysis of manatees in the Amazon conducted by Best (unpublished data) did not find glucose in any examinations (n =13) of individuals of both sexes. Although not quantitative, and considering the low variation in food that these animals were given at that time (*Cabomba sp.* and *Brachiaria mutica*), compared with the more diverse diet currently offered to manatees in captivity, these data are consistent with the low glucose values observed in *T. inunguis* urine in this study.

3.2 Physical and sedimentological urinalysis

3.2.1 Urine color

Urine samples showed the following colors: colorless, yellow, lemon yellow and red. Most, both in males (n=73), and females (n=48) were characterized as "yellow". In female infants, half of the samples were yellow (n=5) and in subadult females this coloration appeared in four samples. The red coloration was seen in a male infant (Tuã) in January and February, in a subadult male (Guarany) in November, and in a subadult female (Cunhataí) in January.

The change in urine color is due to the amount of urinary pigments (urochrome and uroerythrin) (M. S. Matos & P. F. Matos, 1995; Kantek Garcia-Navarro, 1996; Strasinger, 1996), and to the elimination of drugs or metabolic products of organic dyeing properties, which may occur due to ingestion of certain foods, medications, or result from various physiological or pathological states (M. S. Matos & P. F. Matos, 1995; Kantek Garcia-Navarro, 1996).

In domestic animals, urine color varies from light yellow to dark brown. While the urine of omnivores and carnivores is usually pale yellow, the herbivores' ranges from light yellow to dark brown. The exact color of the urine depends on exogenous dyes (via food) and endogenous dyes (from hemoglobin and protein metabolism). The urine after voiding tends to be clear, however, in herbivores, darkening can occur after urine collection due to the presence of oxygen or through bacteria putrefaction (Gürtler et al., 1987).

De Monte & Pilleri (1972) reported a color ranging from light yellow to lemon yellow or olive for the urine of *I. geoffrensis, P. indi* and *P. gangetica.* The amber color of the urine was observed in other cetaceans and pinnipeds which, by feeding on fish, have a diet rich in animal protein, resulting in this color (Medway & Geraci, 1986, as cited in Bossart et al., 2001). Amber color was not observed in any sample of urine from Amazonian manatees.

However, according to Bossart et al. (2001) this is the color normally found in the urine of *T. manatus latirostris.*

The color "yellow", in polyuria occurs when urine is too diluted and the specific gravity is consequently low (except in the occurrence of diabetes mellitus, in which case urine is colorless, but with high specific gravity) (Kantek Garcia-Navarro, 1996). By tracking the urinary staining presented in the course of the experiment, it was observed that the yellow staining was more frequent in *T. inunguis* urine. The absence of color (urine "colorless") observed in some samples also appears to be a normal finding for this species, which presents urine with low specific gravity (more diluted). The color "red" may be due to haematuria or hemoglobinuria, or ingestion of sugar beet (Kantek Garcia-Navarro, 1996, Strasinger, 1996). In this study, urine samples that showed this color were certainly due to the ingestion of sugar beets, because sediment red blood cells were not detected in any of these samples by microscope examination. Moreover, the presence of sugar beet in the diet of manatees whose urine showed that coloration reinforces the assumption that this was the cause of staining observed. The staining results recorded during the experiment may be used in future comparative urinalysis in Amazonian manatees.

3.2.2 Appearance

Urine samples of Amazonian manatees showed the following appearance: clear, semi-cloudy and cloudy. In males, only two samples: in one juvenile male (Tapajós) in December and in a subadult male (Guarany) in September, showed cloudness. Most samples of females (n=63) presented semi cloudy aspect, however, some samples were clear or cloudy.

The aspect corresponds to a general term that refers to the transparency of the urine sample (Strasinger, 1996). Urine of any species may become cloudy if left to stand for some time due to precipitation of salts that may be present. Other causes of cloudy urine is the presence in large amounts of epithelial cells, erythrocytes, leukocytes, bacteria, mucus and crystals, from either urinary organs, sometimes of the genitals (Kantek Garcia-Navarro, 1996), and also the presence of lipids, semen, lymph, yeast, fecal matter or even external contamination (Strasinger, 1996). Appearance determination needs to be confirmed by the analysis of the sediment depending on the occurrence of each of these cellular elements.

Normal urine is usually transparent just after being excreted; however, it may acquire certain opacity generated by the precipitation of amorphous phosphates and carbonates in the form of white mist. The opacity caused by the presence of the above elements, especially epithelial cells in females, does not necessarily mean pathogenesis (Strasinger, 1996). The registration of some samples in this study classified as semi-turbid was due to the presence of such elements. The presence of large quantities of white blood cells, red blood cells or bacteria, on the other hand, may indeed be evidence of pathogenicity and the fact that the sample recently eliminated was blurred, may be a cause for concern (Strasinger, 1996).

None of the samples that presented high turbidity immediately after collection showed high amounts of substances that could indicate a serious illness by the analysis of sediment. The aspect "semi cloudy," recorded in most samples, appeared to be related to the aforementioned elements (semen, mucus, lymph, yeast, etc.) when the sediment was

analyzed. The temporal record of urine appearance in this study may serve as a basis for future observations on *T. inunguis* urine.

3.2.3 Elements of the urinary sediment

Among the possible elements to be found in urine sediment, those detected included: epithelial cells, leukocytes, bacteria, crystals and hyphae. In none of the samples cylinders were observed.

3.2.3.1 Epithelial cells

Epithelial cells were recorded in all urine samples as rare, frequent (Figure 2) or numerous, regardless of gender. In males the most common finding was "rare" epithelial cells (n=84), whereas in females, frequent epithelial cells were recorded in most samples (n=50).

Epithelial cells are the squamous cell type most frequently observed in urine samples (Gürtler et al., 1987, Bossart et al., 2001). They may come from the bladder, urethra, renal pelvis or ureters, and may be present either singly or sometimes in small clumps (Bossart et al., 2001). In the Amazonian manatee they were present in both forms, mostly in urine samples from females. This may be due to physiological condition of increased shedding of the vaginal epithelium, especially if the animal is in heat (Gürtler et al. 1987; Kantek Garcia-Navarro).

Even in males this finding was observed in some samples mostly as "rare" cells. The detection of "numerous" cells was not considered worrisome, because it may be due to the normal shedding of urogenital tracts.

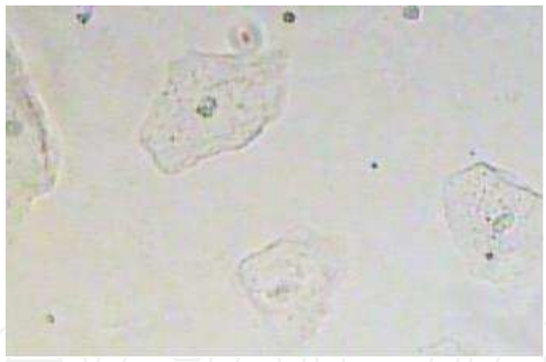

Fig. 2. Squamous epithelial cells found in urine samples from female *Trichechus inunguis* registered as "common".

3.2.3.2 White blood cells WBC's

White blood cells observed microscopically were presented in their degraded form (pus cells). The count of these elements in most examinations of males (n=93) was insignificant (1-3 per field**)** (Fig. 3), with three exceptions: a juvenile male in the month of June (Mapixari) and two subadult males in August (Anamã and Guarany), with 8, 10 and 8 pus cells by visual field, respectively. Most samples of females (n=58) also showed negligible amounts of pus cells (1-3 per field). However, adult females showed varying amounts of pus cells in their samples. High amounts of leukocytes were observed in the following tests: a female infant (Barreirinha) in August (30-35 pus cells by visual field), and an adult female (Cambá)

in June (26 pus cells by visual field), September (18 pus cells by visual field) and October (30-40 pus cells by visual field).

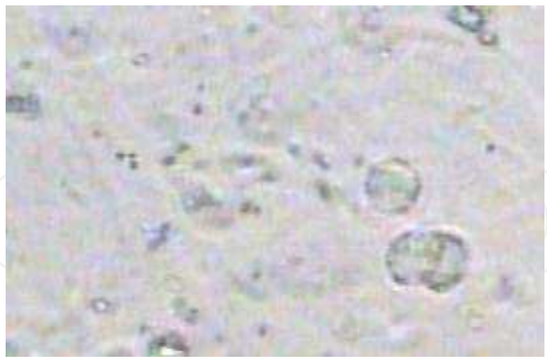

Fig. 3. Pus cells found in urine samples of male *Trichechus inunguis* (3 pus cells per field).

Leukocytes are found in normal urine, in relatively small amounts (Kantek Garcia-Navarro, 1996), as in the case of urine samples analyzed in this study. Leukocyte reaction (increased number of leukocytes in urine), may be due to inflammation somewhere in the urogenital apparatus. The large increase in the number of leukocytes (pus cells) in urine, a phenomenon observed in one infant and one adult female, is called pyuria (pus in the urine) (Kantek Garcia-Navarro, 1996) and in both of them, the increase of pus cells was accompanied by clouding of urine. However, these findings were not alarming, because the pyuria was not accompanied by cylinders, which could be indicative of more severe renal inflammation (Kantek Garcia-Navarro, 1996). In fact, cylinders were not found in any of Amazonian manatee's urine samples.

3.2.3.3 Bacteria

In male samples the occurrence of "bacteria" was recorded as absent, mild, moderate (Fig. 4) and increased. In most male urine samples (n=68) bacteria was absent, except in three cases: a young infant (Tuã), a subadult (Puru) and an adult (Yanomami) in December, when the "bacteria" was recorded as "increased" (bacteriuria). No females presented bacteriuria, and most of the samples (n=61) showed no bacteria.

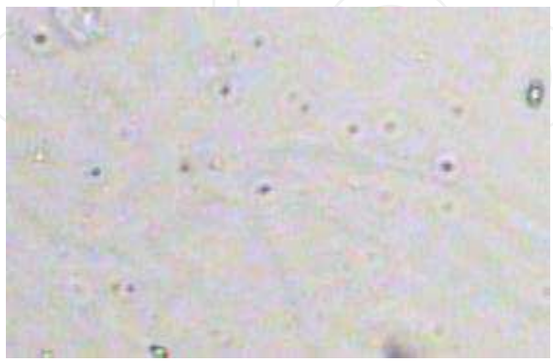

Fig. 4. Bacteria found in a sample of male *Trichechus inunguis* ("Bacteria" "moderate").

Bacteria in the urine may appear as small dots or dashes, both at rest, and in Brownian motion. Their presence in small amounts in urine is considered normal and can sometimes

be due to environmental contamination (Bossart et al., 2001), or can derive from urinary meatus. Increased presence in urine may represent urinary tract infection, coming to form in more severe cases, elongated masses similar to cylinders. For the interpretation of cases in which the bacteria is increased in the urine, one should take into account the amount of leukocytes present. Only when both are found in increased amounts in urine, the existence of a severe infection may be considered (Kantek Garcia-Navarro, 1996).

In the rare cases where bacteriuria was observed in Amazonian manatee, this was not accompanied by increased leukocyte counts, which ruled out the possibility of severe infection (Kantek Garcia-Navarro, 1996). It is necessary to take into account that the presence of bacteria in the urine will be detected with nitrite reactive strips. In this study, the reactive strips showed low efficiency in the detection of nitrite, since in many samples, we obtained a positive value for nitrite, and yet, no bacteria were observed in them. Others, in turn, had bacteria when subjected to examination of sediment, but no nitrite was indicated by previous analysis by reactive strips. One possible explanation for this would be an accidental contamination of the sample prior to the study of urine sediment. This fact did not complicate diagnosis in our study, as we did not consider this result as an indicative of severe infection.

3.2.3.4 Crystals

The following crystals were observed microscopically in the urine samples of Amazonian manatees: amorphous phosphate crystals, triple phosphate crystals, amorphous urate crystals and calcium oxalate crystals (Figs. 5, 6, 7 and 8).

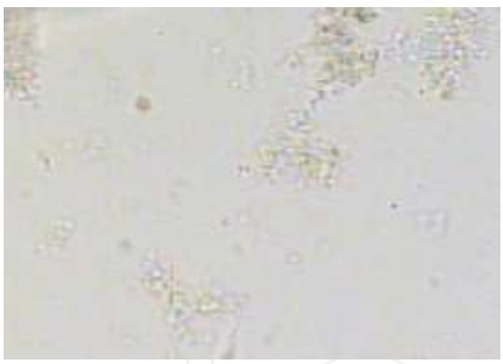

Fig. 5. Amorphous phosphate crystals found in urine samples of female *T. inunguis* recorded as "rare".

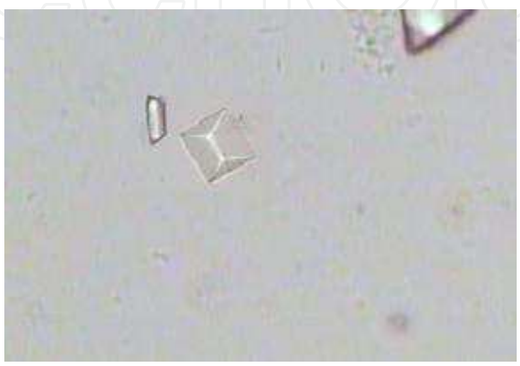

Fig. 6. Triple phosphate crystals found in urine samples of male *T. inunguis* recorded as "rare".

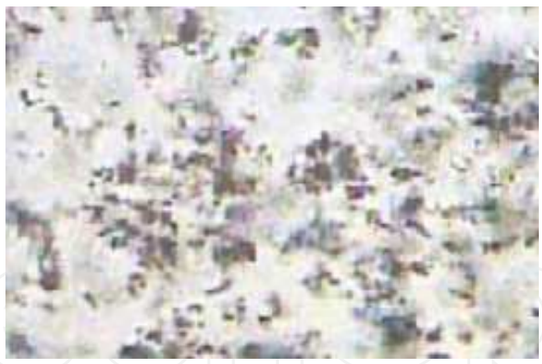

Fig. 7. Amorphous urate crystals found in urine samples of female *T. inunguis* recorded as "common".

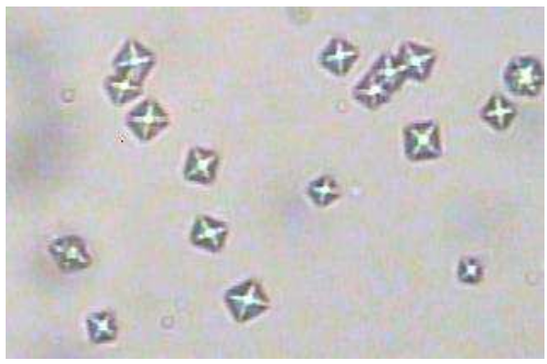

Fig. 8. Calcium oxalate crystals found in urine samples of *T. inunguis* recorded as "common".

In most examinations of males (n=78) amorphous phosphate crystals were not found, although the quantities observed in the samples varied widely and was also reported as rare, frequent and numerous.

In most samples of females (n=48) amorphous phosphate crystals were also not found. In the remaining ones, these crystals were recorded as rare or frequent. Unlike males "numerous" amorphous phosphate crystals were not found in any female samples.

The triple phosphate crystals were not recorded in most samples of males (n=82). When found they appeared as "rare", "frequent" or "numerous". Most samples of females (n=73) presented no triple phosphate crystals. However, in some samples they appeared as "rare," "frequent" or "numerous."

Except for one infant male calf (Piraí), which in the months of June and July had "numerous" and "frequent" amorphous urate crystals, these crystals were not registered in any other sample from males. There was also no evidence of amorphous urate crystals in the samples of most females (n=75).

Finally, calcium oxalate crystals were observed in only four samples of males: as "rare" in a young infant (Piraí) in June and in a juvenile (Tapajós) in September, and as "frequent" in a juvenile male (Tapajós) and in a subadult (Anamã), both in August. The remaining samples of males, as well as all the females' samples, showed no calcium oxalate crystals.

Crystals are formed by minerals present in urine, so it may appear amorphous or crystalline. The crystals can be either in the bladder of the animal (*in vivo*), as *in vitro*. In fact, the presence of crystals is usually nonspecific, with no diagnostic value, and it has to take into account other factors such as diet, patient medication, urine specific gravity, time and storage conditions of the sample, and especially urinary pH to obtain a better interpretation of its occurrence in the urine (Kantek Garcia-Navarro, 1996).

The pH is actually a regulator of crystal formation, as shown in Table 2, which discriminates the crystals observed in Amazonian manatee urine and the respectively urinary pH related to their occurrence. In all cases, the type of crystal was recorded in urine with a pH suitable to its appearance.

Crystal	Urinary pH
Phosphate amorphous	Alkaline
Triple phosphate	Alkaline, neutral or slightly acid
Amorphous urate	Acid
Calcium oxalate	Acid, neutral or alkaline

Table 2. Crystals observed in Amazonian manatee urine and urinary directly related to their occurrence (Adapted from Benjamin (1976)).

Amorphous phosphate crystals actually consist of clusters of fine granules which, although being very small, could be distinguished from bacteria by their refringence. An increase in number in urine may be due to retention of urine in the bladder, enlarged prostate and chronic pyelitis. Triple phosphate crystals (or struvite crystals) emerge from the alkaline fermentation of urine, which can occur both before and after urination. When found in urine freshly collected it may be indicative of urine retention in the bladder (as in chronic cystitis), paraplegia, enlarge of prostate size or chronic pyelitis.

These crystals can also be observed in feline urologic syndrome, or may indicate the presence of a struvite urolithiasis (Kantek Garcia-Navarro, 1996). They are also commonly found in the urine of horses (M. S. Matos & P. F. Matos, 1995). There are records of incidental findings of these crystals in ringed seal (*Pusa hispida*), elephant seal (*Mirounga angustirostris*), Weddell seal (*Leptonychotes weddellii*), *T. truncatus* and humpback whale (*Megaptera novaeangliae*) by some authors (Gulland et al., 2001). The triple phosphate crystals can present the form of short or elongated prisms, resembling at times the cover of a coffin box or the roof of a house. They were observed in the form of a leaf or fern (penalty) (Kantek Garcia-Navarro, 1996). In urine samples of the Amazonian manatee, they presented themselves in the form of box coffin cover. The observation of phosphate crystals in the amorphous and triple phosphate tests did not follow any pattern, but their record may be used as a basis for future studies.

Amorphous urate crystals appear as clusters of small yellow-brown granules, which may also suffer crystallization and appear as colourless fine needles, arranged in the form of pulleys or sheaves (M. S. Matos & P. F. Matos, 1995; Kantek Garcia-Navarro, 1996). By observation of amorphous urate crystals in few samples of both males and females, it was noted that they are hardly found in *T. inunguis* urine, which is mainly due to the fact that the urine of these animals is normally alkaline, and these crystals are evidenced in acidic urine. In this study, the few tests at those amorphous urate crystals were detected had pH 6.0.

Easily identified by taking the classic "envelope letter" form, the crystals of calcium oxalate are considered a normal finding in urine, especially after eating tomatoes, garlic, oranges and asparagus. They are also commonly found in the urine of horses (M. S. Matos & P. F. Matos, 1995). However when observed in increased numbers in the urine they may be related to diabetes mellitus, liver disease, heart, or lungs (Kantek Garcia-Navarro, 1996). The occurrence of this type of crystal was recorded by De Monte & Pilleri (1972) in the urine of *I. geoffrensis* and *P. gangetica.* As just four samples of Amazonian manatees showed to have these crystals, it seems that they are not normally found in the urine of this species. These rare cases were probably due to factors related to ingestion of tomatoes, which was part of the diet of these animals in the days before the exams, since the animals did not show any apparent symptoms of above conditions.

3.2.3.5 Blood cells (RBCs)

In most microscopic examinations of both males (n=105) as females (n=68), red blood cells were not seen. However, these were present in some samples (Fig. 9). Unusually, the tests of an adult female (Cambá) showed 80-100 red cells per field in July and more than 10 red cells per field in the months of August and December.

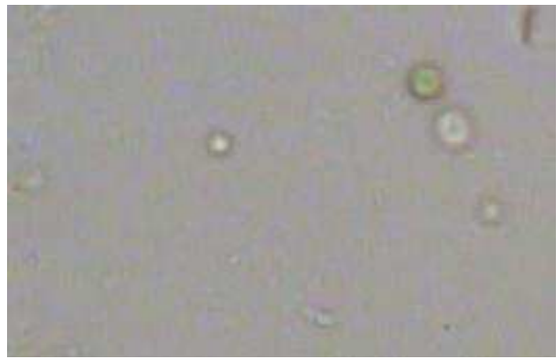

Fig. 9. Red blood cells found in urine samples of male *Trichechus inunguis*.

As previously mentioned, the results obtained qualitatively by reactive strips were discarded and the study of red blood cells by microscopic analysis of the sediment was considered the most efficient method. In females, the appearance of red blood cells in urine, if any, was in very small quantities, and even in samples where the number of red blood cells was found to be slightly higher (15 to 20 erythrocytes per field), these findings do not represent a worrying result, since their occurrence was not accompanied by symptoms related to disease previously described as causes of haematuria. Moreover, the presence of red blood cells in these samples failed to give a reddish-brown color to the samples as expected for samples containing higher amounts of this cell type (Kantek Garcia-Navarro, 1996). In males, when there was detection of red blood cells, the cellular amounts of this element were too small serve as diagnosis of a disorder in these animals.

3.2.3.6. Hyphae

In most tests, both in males (n=87) and females (n=58), hyphae were not observed microscopically. In samples that contained the hyphae they appeared in small quantities (Fig. 10).

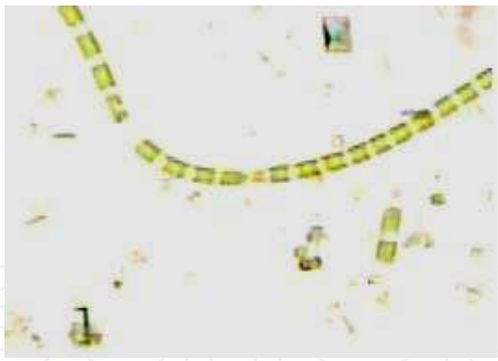

Fig. 10. Hyphae found in urine samples of male *Trichechus inunguis*.

In general, hyphae are the result of material contamination. However, true infections have been recorded in domestic animals (Kantek Garcia-Navarro, 1996). None of the Amazonian manatee's urine samples showed high enough amounts of hyphae to suggest a serious infection by fungi. Even samples that contained hyphae showed very small amounts, which may have been due to contamination of the sample during urine collection and/or analysis.

4. Conclusions

The qualitative analysis by reactive strips proved to be useful for urinalysis in the Amazonian manatee in captivity for the parameters pH, density, bilirubin, urobilinogen, ketones, protein and glucose. On the other hand, the analysis of: blood (erythrocytes), nitrite and leukocytes needs to be confirmed by microscopic analysis of the sediment.

Given the constancy of the results of glucose, bilirubin, urobilinogen, ketones and reactive protein measured by the strips, they appear to be the minimum expected normal values of these substances in the urine of *T. inunguis*. Despite the qualitative nature of results obtained by reactive strips, from a practical standpoint, they can be applied routinely to monitor these parameters.

The urinary sediment showed no cylinders, suggesting that the animals analyzed had no illnesses related to their presence. The observed elements that could indicate some pathology, such as leukocytes or bacteria, were present just in small quantities, or accompanied by casts (in the case of leukocytes) or pyuria (in the case of bacteria), ruling out the possibility of disease in sampled animals. The temporal record of the results of other elements of urinary sediment, as well as physical aspects of color and appearance will be the basis for future comparative analysis on urinalysis animal.

Results reported in this study may help monitor the health situation of *T. inunguis* both in captivity and in the wild, as well as being referential data for urinary parameters in related species. This investigation, by furnishing physiological knowledge to assess the health condition of these animals, provides basis for management and conservation of this vulnerable species.

Additionally, knowledge of urinary compounds levels, as well as their patterns, provided by this study underlies actions for Amazonian manatee conservation, such as the

reintroduction of rehabilitated animals after stranding or bycatch, since it enhances the possibility of success of such procedures.

Finally, we emphasize the need for future research on variations in some urinary components levels in response to experimentally induced physiological stress. These results can be compared with those obtained in this study in order to try to understand the mechanisms of homeostatic self-regulation of this currently threatened species.

5. Acknowledgments

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Conservation biology is called a "crisis discipline." In a world undergoing rapid change, this science informs us about research, technologies, management practices, and policies that can help protect the earth's naturally-occurring biological diversity. The six chapters of this book provide insightful analysis on managing protected areas (Middle East), conserving biochemical and genetic diversity of carob tree (Tunisia) and wild pear (Japan), determining the health status of Amazon manatee, manipulating sex ratios to benefit wildlife, and narrowing the gap between religion and conservation. The authors approach threats to biological diversity from varied angles, reflecting the interdisciplinary nature of the field. This book offers room for reflection on the definition and utility of the word 'natural' on a planet now overwhelmingly dominated by people.

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