Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 6 Issue 2 February 2024

Research Article

Urinalysis Reference Intervals between Rough-Toothed Dolphins (*Steno bredanensis*) and Common Bottlenose Dolphins (*Tursiops truncatus*) Kept Under Human Care

Cristina V Valenzuela-Venegas[1], Lesly J Cabrias-Contreras[1], Julie M Cavin[2], Bert Rivera-Marchand[3], Roberto Sánchez-Okrucky[4] and Antonio A Mignucci-Giannoni[1,5]*

[1]Caribbean Manatee Conservation Center, Inter American University of Puerto Rico, Bayamón, Puerto Rico

[2]Gulf World Marine Park, Panama City Beach, Florida, USA

[3]Department of Natural Sciences and Mathematics, Inter American University of Puerto Rico, Bayamón, Puerto Rico

[4]The Dolphin Company, Cancún, Quintana Roo, Mexico

[5]Center for Conservation Medicine and Ecosystem Health, Ross University School of Veterinary Medicine, Basseterre, St. Kitts

***Corresponding Author:** Antonio A Mignucci-Giannoni, Caribbean Manatee Conservation Center, Inter American University of Puerto Rico, Bayamón, Puerto Rico and Center for Conservation Medicine and Ecosystem Health, Ross University School of Veterinary Medicine, Basseterre, St. Kitts.

Received: December 19, 2023

Published: January 16, 2024

Abstract

Rough-toothed dolphins (*Steno bredanensis*) are a little-known species, distinguished by their particular rostrum. At the time of the study, only six individuals were under human care in North America at the Gulf World Marine Park without any urinalysis reference. This facility has a pod of sixteen common bottlenose dolphins (*Tursiops truncatus*). Urinalysis is a rapid, easy, and inexpensive laboratory tool that presents essential information to veterinarians about a patient's overall health status. Also, it is possible to diagnose health diseases before showing symptoms. Urine samples from both species were collected and analyzed to determine baseline parameters in the rough-toothed dolphins for the first time and compare them with those of the common bottlenose dolphins. Urinalysis was performed by voluntary voided under medical operant conditions and analyzed for all 22 dolphins for gross, chemical, and microscopic findings. The values obtained were used to establish the standard parameters for urinalysis to help veterinarians and dolphin facilities to perform better daily physical routine examinations of rough-toothed dolphins. In addition, a comparison between rough-toothed and common bottlenose dolphins showed no significant differences in urine values. These results help to understand the similarity between these two species in terms of renal function and to develop better care procedures in rescue and rehabilitation facilities that maintain dolphins under human care.

Keywords: Urinalysis; Clinical diagnosis; Rough-toothed dolphins; Common bottlenose dolphins

Introduction

The rough-toothed dolphin (*Steno bredanensis*) is distributed worldwide in tropical and subtropical oceanic waters [1]. It is easily distinguished from other dolphins by its long snout with a smoothly sloping melon, white lips, white tip of the rostrum, and its marked dark cape on its dorsum [2]. Rough-toothed dolphins have not been kept under human care until recently; thus, little is known about this species' health compared to other dolphin species, particularly the common bottlenose dolphin (*Tursiops truncatus*) [1-4].

The common bottlenose dolphin is distributed worldwide in tropical, subtropical, and temperate waters [5,6], and it is one of the best-known marine mammals because they have been in managed care in aquaria and zoos since 1883 [6]. Biological, behavioral, and medical research have also been conducted on common bottlenose dolphins for more than six decades [7-10]. Diagnostic methods for common bottlenose dolphins have been developed, particularly hematology, blood chemistry, gastric sampling, and fecal cytology [11]. Nevertheless, it was not until 2019 that the baseline parameter of urinalysis was published for the first time for the common bottlenose dolphins, as urinalysis was rarely used as a diagnostic tool in these and other cetaceans [12].

With the increase of rough-toothed dolphins under human care, we seek to establish urinalysis reference intervals for this species and compare them with those of the common bottlenose dolphins living under human care.

Objectives of the Study

The objectives of the study are (1) to establish for the first-time normal urine reference values in rough-toothed dolphins, particularly physical, chemical, and microscopic parameters; and (2) compare obtained values in rough-toothed dolphins to those of common bottlenose dolphins both held under captive care.

Materials and Methods

Ethical statement

This research was conducted under the authorization of the Inter American University's Institutional Animal Care and Use Committees (IACUC) and The Dolphin Company's Research Committee Standards and Guidelines.

Study plan and subjects

The study was conducted at the Gulf World Marine Park in Panama City Beach, Florida, in November 2019, where both species are kept alongside each other. The facility had six *Steno bredanensis* (three females, three males) and 16 *Tursiops truncatus* (five females, 11 males). The rough-toothed dolphins were wild-born and rescued from a mass stranding in Florida's Gulf of Mexico. At the time of the study, all were recovered and maintained as non-releasable animals. Five of the common bottlenose dolphins were wild-born (one captured in 1989 and four rescued from stranding), and eleven were born under human care.

All dolphins in this study were clinically healthy according to hematological and chemical analysis by physical veterinary examination and blood work. Both species were fed capelin (*Mallotus villosus*), Atlantic herring (*Clupea harengus*), and squid (*Illex illecebrosus*) daily, hydrated with filtered water once a week, and all received gelatin daily for additional hydration. The dolphins were maintained in a saltwater habitat with multiple divided pools, allowing the separation or mixing of individuals at various times. The habitat had a depth of 4.6 m, with a salinity of 29-31 ppt, and kept at water temperature between 22.8°C and 26.1°C.

Sample collection

All 22 dolphins were trained to provide urine on command using positive operant conditioning, as described in Jiménez-Zucchet., *et al*. [12]. The specific medical behavior steps were station, roll-over, maintenance of the genital slit above the water level, cleaning and drying the genital slit, and massage of the pubic area (Figure 1). After these steps, urination was produced within a few minutes. The samples were collected as an inverted "free-catch" with a sterile syringe and placed in a clear sterile 118-ml specimen container (Dinarex, Orangeburg, New York), labeled with the dolphin's name and the time of collection. The samples were placed in a closed ice cooler and transported to the on-site laboratory for analysis, as in Gunn-Christie., *et al*. [13]. Physical, chemical, and microscopic examinations were conducted in the laboratory within one hour after collection.

Figure 1: Urine sampling from a rough-toothed dolphin through operant conditioning.

Urinalysis

The urinalysis consisted of three tests: physical, chemical, and microscopic.

- **Physical examination:** The volume in ml of urine, color, appearance (clarity/turbidity), and odor were evaluated. The appearance was described as clear, hazy, or slightly cloudy by placing the sample on a white surface. The color was described as colorless, pale-yellow, yellow, dark-yellow, dark-brown-yellow, pale-green-yellow, green-yellow, dark-green-yellow, straw, ochre (orange), red-brown, or red. Finally, the odor was described as none (no odor), urinoid, fruity/sweet, fruity/fishy, fishy, pungent, sulfuric, aromatic, fecal, or ammoniacal.
- **Chemical examination:** The urine sample was transferred to a 15-ml falcon centrifuge tube (Greiner Bio-One, Monroe, North Carolina). Following, a URS-10 urine reagent strip (Teco Diagnostic, Anaheim, California), a pH-specific dipstick (HealthyWiser, Cheyenne, Wyoming), and a veterinary refractometer for determination of specific gravity were used for the examination. Jiménez-Zucchet., *et al.* [12] demonstrated that in dolphins, the Aim-Strip 10-SG (Germain Laboratories, San Antonio, Texas) urine strip presented elevated acidic pH levels and that the HealthWiser dipstick results were more accurate. The URS-10 was inserted into the tube to measure glucose, bilirubin, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leukocyte. The dipstick was removed quickly, and the excess urine was dried by tapping the strip on a square gauze. Once the recommended time had elapsed, the strip was analyzed using the manufacturer's standards for color (positive or negative and values, if applicable).
- **Microscopic examination:** The Falcon tube containing the urine sample was centrifuged for 5 minutes at 1,500 rpm in a Zeny 80-2 Scientific Variable Speed Digital Centrifuge (Zeny, Fontana, California) [14]. Once the sediment concentrated in the bottom of the tube as a pellet, the supernatant was discarded. A sterile 3-ml transfer pipette was used to collect the sediment, transfer it to a microscope slide, and cover it with a coverslip. To minimize technical errors, the sample was examined under 10x and 40x in 10 fields. The presence of white blood cells, red blood cells, epithelial cells (tubular, squamous, or transitional), casts (red blood cells, WBC, epithelial, granular, waxy, fatty, hyaline, bacterial, or mixed cellular), bacteria (cocci, rods or other), crystals (ammonium (bi) urate, amorphous, bilirubin, cysteine, calcium carbonate, calcium oxalate dehydrate, calcium oxalate monohydrate, calcium phosphate, cholesterol, ciprofloxacin, sulfonamide, leucine, melamine, struvite, tyrosine, uric acid, xanthine or drug-associated), yeast, spermatozoa, parasite, and mucus were identified in the low-power field (LPF) and high-power field (HPF), and indicated on the urinalysis data sheet as none (0), rare (1-5), few (6-10), some (11-19), or many (>20). If casts, bacteria, or crystals were present, each type was also noted on the datasheet. Finally, a confirmatory test was performed, staining the sample with a JorVet stain for cell differentiation (Jorgensen Laboratories, Loveland, Colorado) to re-evaluate the urine sediment.

Statistical analysis

Descriptive statistics were used to determine differences between values in rough-toothed dolphins and common bottlenose dolphins. Differences in continuous data were evaluated with a student's t-test. The numerical values were consistent with Birukawa., *et al*. [15] and Daniels., *et al*. [16]; this included the standard deviation, the high as the mean, and the normal for low values. The SigmaStat program performed all statistical analyses for this study, and significance levels for the statistics were established at $p \leq 0.05$.

Results

Urine samples were requested through trained medical behavior and obtained by inverted free catch from 6 rough-toothed dolphins and 16 common bottlenose dolphins. Different volumes of urine samples were obtained, ranging from 4 to 8.5 ml for rough-toothed dolphins ($\bar{x}$ = 5.9 ml) and from 0.6 ml to 10 ml ($\bar{x}$ = 4.3 ml) for common bottlenose dolphins.

Physical examination

Urine physical characteristics for both species of dolphins were assessed in terms of color, appearance, and odor (Table 1). The urine of most of the rough-toothed dolphins was most often yellow (50.0%), followed by pale yellow (33.3%) and colorless (16.7%) (Figure 2). Urine samples for most of the common bottlenose dolphins were most commonly pale yellow (43.8%), followed by yellow (31.2%), pale green-yellow (12.4%), and dark yellow and colorless (6.3% each) (Figure 2). The appearance of the urine for most rough-toothed dolphins was clear (83.3%), except for one that was hazy (16.7%), while for common bottlenose dolphins, most urine was clear (87.4%), with fewer appearing hazy and slightly cloudy (6.3% each). Most rough-toothed dolphin urine was characterized by a urinoid odor (50.0%), followed by a fishy odor (33.3%), and only one with no perceivable odor (16.7%), while in the common bottlenose dolphin, urine odor was odorless primarily (50.0%), followed by urinoid (25.0%), fruity/sweet (12.4%), and fruity/fishy and fishy (6.3% each).

Physical characteristic	Rough-toothed dolphins	Common bottlenose dolphins
Color		
Colorless	16.7	6.3
Pale yellow	33.3	43.8
Yellow (normal)	50.0	31.2
Dark yellow	0	6.3
Dark brown, yellow	0	0
Pale green, yellow	0	12.4
Green, yellow	0	0
Dark green, yellow	0	0
Straw	0	0
Ochre (orange)	0	0
Red brown	0	0
Red	0	0
Appearance		
Clear	83.3	87.4
Hazy	16.7	6.3
Slightly cloudy	0	6.3
Odor		
None	16.7	50.0
Urinoid	50.0	25.0
Fruity/sweet	0	12.4
Fruity/fishy	0	6.3
Fishy	33.3	6.3
Pungent	0	0
Sulfuric	0	0
Aromatic	0	0
Fecal	0	0
Ammoniacal	0	0

Table 1: Physical examination of urinalysis in rough-toothed dolphins (n = 6) and common bottlenose dolphins (n = 16) under human care. Values in percent.

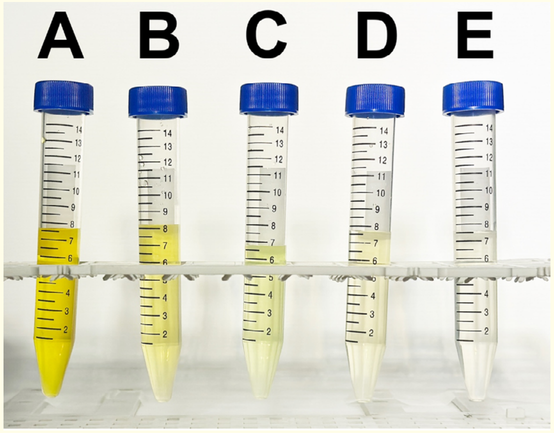

Figure 2: Colors found in urine collected from rough-toothed dolphin and common bottlenose dolphins; dark yellow (A), yellow (B), pale green yellow (C), pale yellow (D), and colorless (E).

Chemical examination

Biochemical characteristics of urine for both dolphin species were assessed as positive or negative for glucose, bilirubin, ketone, nitrite, protein, blood, and leukocytes (Table 2). Additionally, the pH and specific gravity were measured for the urine of both species. Most parameters were negative, except for protein and blood in rough-toothed dolphins and bilirubin, protein, blood, and leukocytes in common bottlenose dolphins (Table 2). Urine pH was 6.5 in all rough-toothed dolphins ranging from 5.0 to 6.5 (x̄ = 6.34 ± 0.39) for common bottlenose dolphins (Table 2). Specific gravity ranged from 1.019 to 1.039 (x̄ 1.030 ± 0.005) for rough-toothed dolphins, and from 1.019 to 1.035 (x̄ = 1.028 ± 0.005) for common bottlenose dolphins (Table 2).

	Rough-toothed dolphins							Common bottlenose dolphins					
	Positive	Negative	Min	Max		SD		Positive	Negative	Min	Max		SD
Glucose	0.0%	100%	0.00	0.00	0.00	0.00		0.0%	100.0%	-	-	-	-
Bilirubin	0.0%	100%	0.00	0.00	0.00	0.00		18.8%	81.2%	0.00	1.00	0.18	0.40
Ketone	0.0%	100%	0.00	0.00	0.00	0.00		0.0%	100.0%	-	-	-	-
Nitrite	0.0%	100%	0.00	0.00	0.00	0.00		0.0%	100.0%	-	-	-	-
Protein	66.7%	33.3%	0.00	0.30	0.10	0.11		81.2%	18.8%	0.00	0.30	0.09	0.07
Blood	16.8%	83.2%	0.00	80.00	13.33	32.66		18.8%	81.2%	0.00	25.00	3.12	7.27
Leukocyte	0.0%	100%	0.00	0.00	0.00	0.00		12.5%	87.5%	0.00	125.00	8.75	31.22
pH	-	-	6.50	6.50	6.50	0.00		-	-	5.00	6.50	6.34	0.39
Specific Gravity	-	-	1.019	1.039	1.030	0.008		-	-	1.019	1.035	1.028	0.005

Table 2: Biochemistry parameters of urinalysis in rough-toothed dolphins (n = 6) and common bottlenose dolphins (n = 16) under human care. Dash (-) = not applicable or cannot be measure since results were negative.

Microscopic examination

Microscopic urine sediment values in both species of dolphins for minimum and maximum in the high-power field (HPF- 400x) were determined by counting a minimum of 10 fields (Table 3), and observations for presence were described as none, rare, few, some, or many (Table 4). White blood cells were rare in the urine of rough-toothed dolphins (x̄ = 2.17 ± 0.75) and none or rare in common bottlenose dolphins (x̄ = 1.18 ± 1.22). In contrast, red blood cells were most common in both species; few to none in rough-toothed dolphins (x̄ = 1.83 ± 2.23) and many to none in common bottlenose dolphins (x̄ = 1.14 ± 2.66). Epithelial cells were found to be from some to rare in both species, but higher on average in *Steno* (x̄ = 7.67 ± 4.28) than in *Tursiops* (x̄ = 4.28± 2.64). Crystals were not found in rough-toothed dolphins but were either few or none in bottlenose dolphins. Similarly, sperm cells were rare or not present in both species, although with a higher average in rough-tooth dolphins (x̄ = 1.5 ± 2.5) than in common bottlenose dolphins (x̄ = 0.07 ± 0.27). Bacteria (cocci) were few or rare in rough-toothed dolphins and either few, rare, or many in common bottlenose dolphins. On rare occasions, mucus was found in common bottlenose

	Rough-toothed dolphins					Common bottlenose dolphins			
	Min	Max		SD		Min	Max		SD
White blood cells	1	3	2.17	0.75		0	3	1.18	1.22
Red blood cells	0	6	1.83	2.23		0	9	1.14	2.66
Epithelial cells	3	15	7.67	4.28		2	12	4.28	2.64
Casts	-	-	-	-		-	-	-	-
Crystals	-	-	-	-		-	-	-	-
Yeasts	-	-	-	-		-	-	-	-
Spermatozoa	0	6	1.50	2.50		0	1	0.07	0.27
Parasite/ova	-	-	-	-		-	-	-	-
Bacteria	-	-	-	-		-	-	-	-
Mucus	-	-	-	-		-	-	-	-

Table 3: Microscopy parameters of urinalysis in rough-toothed dolphins (n = 6) and common bottlenose dolphins (n = 16) under human care. Dash (-) = not applicable or cannot be measure since results were negative.

	Rough-toothed dolphins						Common bottlenose dolphins				
	% None	% Rare	% Few	% Some	% Many		% None	% Rare	% Few	% Some	% Many
White blood cells	0.0	100.0	0.0	0.0	0.0		43.8	56.2	0.0	0.0	0.0
Red blood cells	33.3	50.0	16.7	0.0	0.0		68.8	12.5	0.0	6.2	12.5
Epithelial cells	0.0	50.0	16.7	33.3	0.0		0.0	87.4	6.3	6.3	0.0
Casts	100.0	0.0	0.0	0.0	0.0		100.0	0.0	0.0	0.0	0.0
Crystals	100.0	0.0	0.0	0.0	0.0		93.7	0.0	6.3	0.0	0.0
Yeasts	100.0	0.0	0.0	0.0	0.0		100.0	0.0	0.0	0.0	0.0
Spermatozoa	66.7	33.3	0.0	0.0	0.0		81.3	12.4	0.0	0.0	6.3
Parasite/ova	100.0	0.0	0.0	0.0	0.0		100.0	0.0	0.0	0.0	0.0
Bacteria	0.0	83.3	16.7	0.0	0.0		6.3	75.0	12.4	0.0	6.3
Mucus	100.0	0.0	0.0	0.0	0.0		75.0	12.5	12.5	0.0	0.0

Table 4: Microscopy parameters of urinalysis in percentages of none, rare, few, some and many, in rough-toothed dolphins (n = 6) and common bottlenose dolphins (n = 16) under human care.

dolphins, but it was mostly not found in both species. Cast, yeast, and parasites were not found in either species.

Comparative analysis

No significant differences in urine parameters of biochemistry and microscopic examinations were found between rough-toothed and common bottlenose dolphins.

Discussion

Urinalysis is an inexpensive, rapid, and easy routine laboratory test that provides veterinarians with significant data. This procedure can give insight into hydration status, renal health, systemic disease, as well as toxic insults and reveal conditions that do not present signs or symptoms [14,17,18]. In addition, urinalysis can help with prompt intervention or prevention of some patients' underlying diseases [19]. Finally, this test should be noticed as it provides a large amount of data and can give the veterinarians documentation of abnormalities in the urine. For all these reasons, urinalysis should be part of routine diagnostics for animals in human care to monitor their health status [20], regardless of whether the animal is aquatic or terrestrial.

Various medical behavior training techniques have been conducted in husbandry for years in dolphinariums, zoos, and aquariums. Training for medical behaviors is essential since the animal, in this case, dolphins, needs to adapt to the presence of veterinarians or assistants and become comfortable with accepting and performing the commands. In addition, positive reinforcement will reduce the stress of medical interventions [11]. For example, collecting urine with a catheter inserted into the bladder, as in Malvin and Rayner [21], can be stressful and invasive for dolphins; however, for this study, like in Jiménez-Zucchet., *et al*. [12], urine collection was by trained "free catch." This method can be considered an effective low, or non-stressful, and non-invasive method that can be used as part of the routine physical examination of dolphins in human care. The daily obtained samples will help maintain the medical history and prevent or early diagnose the animal's renal condition.

Physical Examination

- **Color:** Urine color in most animals presents as colorless, yellow, or amber depending on the concentrations of urobilin and urochrome [14]. The sample volume collected, the sample collection method, medication, and diet can all cause a change in color and turbidity [14,22]. In addition, animals with some diseases, like urinary tract infections can present changes in urine color [23]. In this study, the color of the urine of rough-toothed dolphins ranged from colorless to pale yellow to yellow (normal). This colorless or clear appearance may be normal or indicate a polyureic state associated with a recent water intake [14]. On the other hand, common bottlenose dolphins presented urine ranging in color from colorless to pale yellow to dark yellow or pale green, yellow. A green appearance in urine may be due to the ingestion of some marine fish containing a protein tetrapyrrole known as biliverdin, which has a blue-green color [24].
- **Appearance:** The appearance (turbidity) of the urine as clear, hazy, or slightly cloudy may vary depending on the diet and health of the animal and is also associated with the specific

gravity of urine [14,22]. An increase in turbidity may also be due to crystals, mucus, epithelial cells, cast, proteinuria (high preset of protein), or semen, among other factors [18,25-26]. If animal urine samples are left still for some time, they may become hazy or cloudy due to salt precipitation or white due to phosphate and carbonates [17]. In some cases, as in horses, the appearance is cloudy and thicker due to mucus and crystals in the urine; nevertheless, the appearance of cloudy urine may not be considered a sign of ill health [19]. Rough-toothed dolphin urine appeared clear and hazy; Common bottlenose dolphin samples presented as clear, hazy, and slightly cloudy. The hazy or slightly cloudy appearance was due to sediment in all but one common bottlenose dolphin, which had many spermatozoa in the sample.

- **Odor:** Normal urine odor is reported as urinoid. However, some diseases, infections, bacteria, medication, metabolic supplements, and diet can influence urine odor. An ammonia odor is due to bacteria in the urea turning into urease from retention. Ketoacidosis is presented as a fruity or sweet odor [18,23,27-28]. A fishy smell is because a non-infective organism produces a hepatic metabolic enzyme called trimethylaminuria [29]. Dolphins' diet and daily supplements can also influence the odor and make the urine smell fishy or fruity [12]. In this study, rough-toothed dolphins' urine odor was none, urinoid and fishy. Common bottlenose dolphin urine odor was none, urinoid, fruity/sweet, fruity/fishy, and fishy. Results for fishy odor in both species may be due to spermatozoa in one rough-toothed dolphin and one common bottlenose dolphin. One rough-toothed dolphin presented normal values but trace protein. The presence of protein may result from a high-protein diet. One common bottlenose dolphin presented a fruity/fishy odor showing protein and was positive for small amounts of bilirubin in the chemical examination. Further testing for this dolphin should be done to confirm the presence or absence of bilirubin. Only two common bottlenose dolphins showed a fruity/sweet odor; one was completely normal in all values, and the other presented spermatozoa.

Chemical examination

Biochemical findings using a dipstick is a commonly used semi-quantitative test and should be performed according to the manufacturer's instructions [25] and tested for the presence or absence (positive, negative, and scale numbers) of glucose, bilirubin, ketone, blood, protein, nitrite, leukocytes, pH, urobilinogen, and specific gravity in the urine. This test can help determine some diseases in conjunction with the gross and microscopic examination [12]. However, specific gravity results from a dipstick are not considered completely trustworthy and must be measured by a veterinary refractometer. In addition, urine components such as leukocytes and blood need to be analyzed by microscopic examination to confirm all the chemical data.

- **Glucose:** Expected glucose values should be normal or negative for a healthy patient. However, if glucose is present in urine, known as glycosuria or glucosuria, it is a sign of potential disease [18,25]. Glucose is a molecule that passes through the glomerulus and is then reabsorbed in the renal tubules (kidney tubules). The test results depend on the amount of glucose in the blood levels and the filtration in the glomerulus. Normal animals do not present glucose in the urine; if it appears and blood glucose levels are higher, this can be pathological evidence of disorders such as Fanconi's syndrome, diabetes mellitus, kidney failure, or urinary tract obstruction. Nevertheless, some cases are non-pathological in animals with stress [18,19,28]. Also, false positives can occur with sample oxidizing agents. In this study, all rough-toothed and common bottlenose dolphins were negative (non-presence) for glucose in the urine.
- **Bilirubin:** Expected bilirubin values should be negative with trace amounts in some cases. Bilirubin can be produced by hemolysis, obstruction, or failure to release bilirubin. However, studies in some male dogs have demonstrated that, depending on the specific gravity, bilirubin can be found in small quantities and classified as normal in the urine [30]. Bilirubin is created by degrading hemoglobin; the hem portion becomes bilirubin and then is conjugate (water-soluble) in the liver to be eliminated as bile or, after having been filtered by the glomerulus, expelled in the urine. Excess to bilirubin is known as bilirubinuria and can be due to hepatic disease, diabetes mellitus, infections, and anemias [14,19,25]. Intestinal bacteria can cause false positives; false negatives can be caused by ascorbic acid [28]. In this study, all rough-toothed dolphins were negative for the test. However, three common bottlenose dolphins showed bilirubin in positive-small amounts, and the rest were negative. Those three dolphins presented normal values for the remainder of the examinations and did not present any medical conditions at the time of the study.
- **Ketone:** Expected ketone values should be negative. It is the secondary form of lipid excess mobility and is filtered by the glomerulus. In abnormal cases, ketone in urine is known as ketonuria [18,19], the product of an abnormal increase in glucose metabolism. Pathological diseases such as uncontrolled diabetes mellitus, liver disease, and some types of glycogen diseases [31] can produce these cases. It may

also be found in animals recovering from or going through starvation [32]. False positives and negatives can also alter results [28]. In this study, all rough-toothed and common bottlenose dolphins were negative.

- **Nitrite:** Expected nitrite values in urine should be negative. Nitrite in urine is caused by bacteria that can reduce nitrate to nitrite and may produce a urinary tract infection. Although this is very common in humans, in the veterinary field, there is no evidence of nitrite in the urine of animals [28,31-32]. In this study, all dolphins of both species were negative for nitrite.

- **Protein:** Expected values for protein should be negative to trace. Trace amounts are associated with protein secreted by plasma, tubular epithelial cells, urogenital tract, and albumin. It can also be present in acid and alkaline urine due to different conditions [28]. An excess of protein in the urine, called proteinuria, can be prerenal (a condition that increases protein levels before getting into the kidney), renal (glomerular disease, tubular disease, or both), and postrenal (proteins attached to urine by bacteria, fungi, blood trauma, inflammation, a large number of spermatozoa, among others) [14,28]. False positive and negative results can interfere with the analysis [28]. However, non-pathological cases can present protein due to high-protein diets, increased exercise, and stress [19]. The rough-toothed and common bottlenose dolphins presented both negative and positive in this study. Most of the rough-toothed dolphins were positive-trace except for one (previously mentioned) with a past urinary tract infection that was positive (0.3). Most of the common bottlenose dolphins presented positive trace except for one that showed many spermatozoa marking positive (0.3). Because dolphins are piscivores and consume a high-protein diet, this might influence the positive-trace results [12].
- **Blood:** Expected values for blood are negative; however, trace amounts of hemoglobin can appear as a positive marker on sensitive urine dipsticks [28,31]. A positive test can be a sign of hematuria, hemoglobinuria, and myoglobinuria. The presence of blood should always be confirmed in the microscopic urine examination. These pathological disorders can be due to skeletal muscle damage, intravascular hemolysis, urinary tract injury or infections, tumors, glomerular diseases, tubular disorders, stones, and hypercalciuria. False positives and false negatives also occur [25,28,31]. In this study, both dolphin species presented both negative and positive results. The one positive rough-toothed dolphin that was positive had a urinary tract infection two weeks prior to the study. Still, at the time of the study, all the treatment medications had been completed, and the rest of the examination presented normal values except for blood and protein. The three common bottlenose dolphins that showed positive for blood had medical histories and were in medical treatment, with one having kidney stones.
- **Leukocytes:** Expected values for leukocytes in urine are negative. High leukocyte values can be a sign of urinary infection [18]. False positive and false negative results occurred depending on the species [27]. In this study, all rough-toothed dolphins were negative for leukocytes in urine, but common bottlenose dolphins were negative (14 of 16) and positive (2 of 16). The latter may have been caused by external contamination since the microscope results for white blood cells were normal.
- **pH:** Results in urine pH vary depending on diet, medication, and illness [14]. It is used to indicate overall acid-base levels, but external and some internal conditions may interfere with the results [25]. It is recommended to use a specific pH meter to confirm results since some studies have presented evidence of different values between urine strips and a pH meter-specific strip [12,28]. Dolphin pH is influenced by habitat, urine retention, and diet. Since dolphins are piscivores, urine pH can be from acid to neutral. Alkalinity pH changes in carnivores could indicate urinary tract disease, obstruction, or kidney tubular disease [12,19]. In this study, a urine strip and a pH meter corroborated results in urine pH; both tests presented the same values. The statistical analysis made with the urinalysis dipstick resulted in a mean of 6.5 ± 0.0 for rough-toothed dolphins and 6.34 ± 0.4 for common bottlenose dolphins. According to Renner [33] and Jiménez-Zucchet., *et al*. [12], values for common bottlenose dolphins were within the parameters, and values for rough-toothed dolphins were consistent with preview studies in other marine mammals under human care.
- **Urobilinogen:** Expected urobilinogen values are to be within normal range. Urobilinogen comes from conjugated bilirubin that goes through the intestines and then becomes urobilinogen. It is reabsorbed in the blood port, and a small amount is excreted in the urine [14,28,32]. An increase in urobilinogen levels is known as hyperbilirubinemia, which may indicate hemolysis, hepatocellular disease, and biliary obstruction [14,18]. According to the manufacturer's instructions, all dolphins from both species were in the normal range (0.2-3.2 normal).
- **Specific Gravity:** Expected results for specific gravity depend on the diet, hydration, temperature, and timing of sample collection. It is recommended to take the sample, if possible, from the first urination of the day to see the correct concentration [27]. This test is used to determine the ability of the kidney to concentrate the sediment in the urine and the status of hydration [18,27]. Dolphins and other marine mammals have renal organ adaptation to living in hyperosmotic conditions; they are capable of osmoregulation and conserving water from their prey [34,35]. However, if the dolphin presents renal dis-

ease, the specific gravity test should not be used until the end of the illness; preventive medicine should be used to support the renal system [36]. Specific gravity in dolphins is expected to be higher than in other mammals since the primary dolphin diet is protein. According to Jiménez-Zucchet., *et al*. [12], common bottlenose dolphins' specific gravity is 1.035 ± 0.008, higher than other mammals' results due to the ability of the reticulate kidney to concentrate urine. Also, specific gravity in some animals from the order of Cetartiodactyla, like mule deer (1.036 ± 0.006), sheep, goats, and cattle (1.035 ± 0.009) are higher as well [19,37]. Results from a urine strip cannot be trusted for veterinary use; therefore, a veterinary refractometer was used. In this study, values from the refractometer were as expected for rough-toothed dolphins (1.030 ± 0.008), and common bottlenose dolphins were within the parameters (1.028 ± 0.005).

Microscopic examination

The last essential step in urinalysis is to observe the urine sediment components under the microscope to identify the presence of white blood cells, red blood cells, epithelial cells, casts, bacteria, crystals, yeast, spermatozoa, parasites, and mucus. This identification will help to determine if the animal has any pathological conditions [18]. An examination should be a minimum of 10 fields with the 10x (low power field) objective to find epithelial cells, mucus, crystal, casts, and parasites and the objective 40x (high power field) for finding white blood cells, red blood cells, bacteria, yeast, and spermatozoa [14,38].

- **White blood cells:** Normal white blood cell values are less than 5/HPF. An increase in white blood cells, depending on the leukocyte morphology (neutrophils, macrophages, eosinophils, lymphocytes, and monocytes), can be an indicator of the disease, inflammation, or infection in the renal system [14,25,28]. The values for rough-toothed dolphins were between 1 and 3; for common bottlenose dolphins, the values were between 0 and 3, demonstrating no significant changes between species.
- **Red blood cells:** Red blood cells can be present in the urine in small amounts (less than 5/HPF); however, in some dogs and cats, it can be in quantities categorized as few [14,32]. Samples may be contaminated with renal tract erythrocytes due to a hemorrhage in any part of the renal tract [14,25]. Rough-toothed dolphins presented neither rare and few amounts of red blood cells. The maximum value presented for rough-toothed dolphins was 6/HPF, while the maximum for common bottlenose dolphins was 9/HPF. These values can be considered within the normal range. In contrast, common bottlenose dolphins presented none, rare, few, and many in the number of red blood cells. The dolphins, with results of few and of many, had chronic medical conditions such as kidney stones. No significant differences were found between species.
- **Epithelial cells:** Different types of epithelial cells are found in urine sediment from throughout the urinary tract [32]. In a "free catch" urinalysis, the most common epithelial cell to be found is squamous. Neoplastic transitional cells can be found in the urine of some animals with cell carcinoma [14]. Rough-toothed dolphins presented rare, few, and some. Common bottlenose dolphins presented rare, few, some, and many. All dolphins from both species showed squamous cells. Dolphins with few, and some may be due to cells from the urinary tract since the animals were clinically normal. One of the common bottlenose dolphins that showed many have a history of kidney stones and presented with many red blood cells. The minimum value for rough-toothed dolphins was 3, and the maximum was 15. The minimum value for 14 common bottlenose dolphins was 2, and the maximum value was 12. This demonstrates no significant differences between species.
- **Casts:** Urine samples are expected to have none to rare (1-2 LPF) for the presence of casts in dogs and cats. Casts are formed of mucoprotein by the epithelium from the renal tubules [32]. Different casts can result from renal tubular disease, inflammation, or metabolic disorders [14]. Hyaline and granular casts are considered normal in small amounts. Increased casts are considered cylindruria, and urinalysis can detect early abnormalities by detecting casts [14]. Casts were not found in the urine of either species.
- **Crystals:** It is common to find some crystals in the urine of animals, known as crystalluria, but the presence of many may still not be non-pathological [25,27]. To identify if the crystals are pathological, a microscopic analysis should be done. Metabolic diseases can form crystals and renal infections [14,28]. Calcium oxalate is the most commonly found crystal in urine and can be amorphous. This type of crystal is related to ethylene glycol toxicity. However, it is normal to find calcium oxalate in dogs and cats. Dolphin and other cetacean studies have demonstrated that calcium oxalate and struvite crystals can be found in the urine, causing hypocitraturia and nephrolithiasis [35-36,39]. All rough-toothed dolphins were negative for crystals. Only one common bottlenose dolphin presented a few amorphous crystals, but the rest of his analysis was normal, and there was no medical history of nephrolithiasis. The presence of this crystalluria may be due to the dolphin's diet.
- **Yeast or fungi:** Microscopically, identifying yeast under the microscope can be difficult as their peculiar shape is ovoid instead of round. Yeast varies in size and are usually colorless. If they are present, it may indicate a severe infection [28]. All urine samples for both species had no signs of yeast or fungi.

- **Spermatozoa:** Spermatozoa can be observed in the urine of some male dolphins, either with or without motility [28,38]. The shape is an oval head with a flagellum attached. However, sperm was observed from none to rare in most rough-toothed dolphins' urine samples and were rare in two male common bottlenose dolphins, and one had many. Spermatogenesis and the amount of sperm present depend on the maturity of the dolphins and exposure or proximity to females in heat.
- **Parasites/ova:** Parasites can be observed in some urine samples but are likely from fecal contamination or parasitic infection [28]. Therefore, the urine for both species analyzed was categorized as none.
- **Bacteria:** A small number of bacteria from the lower urinary tract can be found in the urine if the sample is taken by "free catch" [14]. Commonly found bacteria are rods, bacilli, or cocci. The rough-toothed dolphins presented rare and few. The animal with few bacteria was the same animal with past urinary infections. All common bottlenose dolphins were analyzed for bacteria showing none, rare, few, and many. Since the dolphins with few and many presented normal bacteria values in the study, it is possible that there was some contamination factor in the process, such as from the skin of the dolphin during collection.
- **Mucus:** Mucus can only be viewed with the bright light of the microscope, and it can be confused with hyaline casts. The shape is irregular and a product of the epithelial cells of the urinary tract [28]. It is normal to find mucus in urine, but an excess can result from inflammation in the urinary tract [38]. All rough-toothed dolphins presented none for mucus, while common bottlenose dolphins showed none or were rare for mucus.

Conclusions

It is recognized that rough-toothed dolphins and common bottlenose dolphins are two distinct species based on their morphology and genetic makeup. Even yet, they have been regarded as sister species [40], and there have been a number of hybrids between them [41]. The similarities of the reference values for urinalysis contribute to the understanding of the tight relationship between *Steno* and *Tursiops*.

Urinalysis is an important test that can provide significant amounts of data for evaluating a patient's overall health and should, therefore, be noticed. This test consists of three connected parts and should be interpreted together for a better diagnosis. The test should be part of a routine physical examination to help improve the lives of dolphins under human care. This study is the first to provide urine references for rough-toothed dolphins and compare urine values between species under human care. The result of this study provides baseline parameters for healthy rough-toothed dolphins for gross, chemical, and microscopic examination and comparison with common bottlenose dolphins' parameters (Table 5). Since these two dolphin species showed similar urinalysis results, dolphins from the Delphinidae may likely have similar urine values due to evolutionary reasons and living in similar environments. Further studies with other dolphin-like odontocetes, particularly under human care, should be conducted to determine if they can be treated equally or similarly in urinalysis tests.

Physical characteristic	Rough-toothed dolphins	Common bottlenose dolphins
Gross examination		
Color	Pale yellow - Yellow	Pale yellow - Yellow
Appearance	Clear	Clear
Odor	Urinoid or fishy	None, urinoid or fishy
Chemical examination		
Glucose	Negative	Negative
Bilirubin	Negative	Negative
Ketone	Negative	Negative
Nitrite	Negative	Negative
Protein	Negative	Negative
Blood	Negative	Negative
Leukocyte	Negative	Negative
pH	6.5	6.3-6.5
Urobilinogen	0.2-3.2	0.2-3.2
Specific gravity	1.020-1.036	1.020-1.035
Microscopic examination		
White blood cells	0-2	0-2
Red blood cells	0-2	0-2
Epithelial cells	None-rare	None-rare
Casts	None	None
Crystals	None	None
Bacteria	None-rare	None-rare
Yeast	None	None
Spermatozoa		
Males	None-rare	None-rare
Females	None	None
Parasites/ova	None	None
Mucus	None	None

Table 5: Urinalysis reference values for rough-toothed dolphins in comparison with common bottlenose dolphins under human care.

Bibliography

1. Jefferson TA. "Rough-toothed dolphin, *Steno bredanensis*". In: Wursig B, Thewissen JGM, Kovacs K. Editors". *Encyclopedia of Marine Mammals*. Associated Press: San Diego (2018): 838-840.
2. West KL., *et al.* "*Steno bredanensis*". *Mammalian Species* 43 (2011): 177-189.
3. Davis RW., *et al.* "Physical habitat of cetaceans along the continental slope in the north-central and western Gulf of Mexico". *Marine Mammal Science* 14 (1998): 490-507.
4. Manire CA., *et al.* "Blood chemistry and hematology values in healthy and rehabilitate rough-toothed dolphins (*Steno bredanensis*)". *Journal of Wildlife Diseases* 54 (2018): 1-13.
5. Baird RW., *et al.* "Site fidelity and association patterns in a deep-water dolphin: Rough-toothed dolphins (*Steno bredanensis*) in the Hawaiian Archipelago". *Marine Mammal Science* 24 (2008): 535-553.
6. Wells RS and Scott MD. "Bottlenose dolphin, *Tursiops truncatus*, Common bottlenose dolphin". In: Wursig B, *et al.* Editors". *Encyclopedia of Marine Mammals*. Associated Press: San Diego (2017): 118-125.
7. Caballero S., *et al.* "Phylogeography, genetic diversity and population structure of common bottlenose dolphins in the Wider Caribbean inferred from analyses of mitochondrial DNA control region sequences and microsatellite loci: Conservation and management implications". *Animal Conservation* 15 (2012): 95-112.
8. Leatherwood S and Reeves RR. "*The Bottlenose Dolphin"*. Academic Press: San Diego (1990).
9. Mignucci-Giannoni AA. "Marine mammal captivity in the northeastern Caribbean, with notes on the rehabilitation of stranded whales, dolphins and manatees". *Caribbean Journal of Science* 34 (1998): 191-203.
10. Shane SH., *et al.* "Ecology, behavior and social organization of the bottlenose: A review". *Marine Mammal Science* 2 (1986): 34-63.
11. Ramírez K. "Marine mammal training: The history of training animals for medical behaviors and keys to their success". *Veterinary Clinics of North America Exotic Animal Practice* 15 (2012): 413-423.
12. Jiménez-Zucchet N., *et al.* "Baseline urinalysis values in common bottlenose dolphins under human care in the Caribbean". *Journal of Veterinary Diagnostic Investigation* 31 (2019): 426-433.
13. Gunn-Christie RG., *et al.* "ASVCP quality assurance guidelines: control of preanalytical, analytical, and postanalytical factors for urinalysis, cytology, and clinical chemistry in veterinary laboratories". *Veterinary Clinical Pathology* 4 (2012): 18-26.
14. Callens AJ and Bartges JW. "Urinalysis". *Veterinary Clinics of North America Small Animal Practice* 45 (2015): 621-637.
15. Birukawa N., *et al.* "Plasma and urine levels of electrolytes, urea and steroid hormones involved in osmoregulation of cetaceans". *Zoological Science* 22 (2005): 1245-1257.
16. Daniels RL., *et al.* "Effects of freeze-thaw cycle on urine values from bottlenose dolphins (*Tursiops truncatus*)". *Aquatic Mammals* 39 (2013): 330-334.
17. Pantoja TMA., *et al.* "Amazonian manatee urinalysis: Conservation applications". In: Povilitis T. Editor. *Topics in Conservation Biology*, IntechOpen: Rijeka, Croatia (2012): 57-80.
18. Simerville JA., *et al.* "Urinalysis: A comprehensive review". *American Family Physician* 71 (2005): 1156-1162.
19. Parrah JD., *et al.* "Importance of urinalysis in veterinary practice-a review". *Veterinary World* 6 (2013): 640-646.
20. Pantoja TMA., *et al.* "Urinary parameters of *Trichechus inunguis* (Mammalia, Sirenia): Reference values for the Amazonian manatee". *Brazilian Journal of Biology* 70 (2010): 607-615.
21. Malvin RL and Rayner M. "Renal function and blood chemistry in Cetacea". *American Journal of Physiology* 214 (1968): 187-191.
22. McCutcheon H., *et al.* "Health status and translocation success of wild and rehabilitated possums". In: *National Wildlife Rehabilitation Conference Proceedings 2007*, Fremantle, Western Australia (2007).
23. Barsanti JA and Finco DR. "Laboratory findings in urinary tract infections". *Veterinary Clinics of North America Small Animal Practice* 9 (1979): 729-748.
24. Fang LS and Bada JL. "The blue-green blood plasma of marine fish". *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 97 (1990): 37-45.

25. Piech TL and Wycislo KL. "Importance of urinalysis". *Veterinary Clinics of North America Small Animal Practice* 49 (2019): 233-245.

26. Sweeney JC and Ridgeway SH. "Procedures for the clinical management of small cetaceans". *Journal of the American Veterinary Medical Association* 167 (1975): 540-545.

27. Martini-Johnson L. "Urinalysis". In: Hendrix CM and Sirois M. Editors. *Laboratory Procedures for Veterinary Technicians*. Mosby Elsevier: St. Louis (2007): 151-180.

28. Sink CA and Weinstein NM. "Practical Veterinary Urinalysis". Wiley-Blackwell: Ames (2012).

29. Aguilar-Shea AL., *et al.* "Síndrome de olor a pescado (Trimetilaminuria), la dieta es importante". *Revista Española de Nutrición Humana y Dietética* 20 (2016): 254-257.

30. Wamsley HL and Alleman AR. "Physical and chemical aspects of urinalysis". In: Cowell RL Editor. *Veterinary Clinical Pathology Secrets*, Elsevier Mosby: St. Louis (2004): 146-154.

31. Patel HP. "The abnormal urinalysis". *Pediatrics Clinics of North America* 53 (2006): 325-337.

32. Reine NJ and Langston CE. "Urinalysis interpretation: how to squeeze out the maximum information from a small sample". *Clinical Techniques in Small Animal Practice* 20 (2005): 2-10.

33. Renner MS. "Urinary indices in the captive killer whale (*Orcinus orca*)." In: *Proceedings of the IAAM 33rd Annual Conference*, International Association for Aquatic Animal Medicine, Albufeira, Portugal (2002).

34. Ortíz RM. "Osmoregulation in marine mammals". *Journal of Experimental Biology* 204 (2001): 1831-1844.

35. Venn-Watson SK., *et al.* "Clinical relevance of urate nephrolithiasis in bottlenose dolphins *Tursiops truncatus*". *Diseases of Aquatic Organisms* 89 (2010): 167-177.

36. Venn-Watson SK., *et al.* "Hypocitraturia in common bottlenose dolphins (*Tursiops truncatus*): Assessing a potential risk factor for urate nephrolithiasis". *Comparative Medicine* 60 (2010): 149-153.

37. DelGiudice GD, *et al.*, "Blood and urinary profiles of free-ranging desert mule deer in Arizona". *Journal of Wildlife Diseases* 26 (1990): 83-89.

38. Chew DJ and DiBartola SP. "Interpretation of Canine and Feline Urinalysis". The Gloyd Group: Wilmington (1998).

39. McFee WE and Osborne CA. "Struvite calculus in the vagina of a bottlenose dolphin (*Tursiops truncatus*)". *Journal of Wildlife Diseases* 40 (2004): 125-128.

40. Cuha HA., *et al.* "Phylogenetic status and timescale for the diversification of *Steno* and *Sotalia* dolphins". *PLOS ONE* 6 (2011): e28297.

41. Dohl TP., *et al.* "A porpoise hybrid: *Tursiops* x *Steno*". *Journal of Mammalogy* 55 (1974): 217-221.