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Dietary cation–anion difference may explain why ammonium urate nephrolithiasis occurs more frequently in common bottlenose dolphins (*Tursiops truncatus*) under human care than in free-ranging common bottlenose dolphins[1]

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ABSTRACT: Ammonium urate nephrolithiasis frequently develops in common bottlenose dolphins (*Tursiops truncatus*) managed under human care but is rare in free-ranging common bottlenose dolphins. In other species, the dietary cation–anion difference (DCAD) can affect ammonium urate urolith formation by increasing proton excretion as ammonium ions. Therefore, differences in diet between the 2 dolphin populations could affect urolith formation, but the DCAD of most species consumed by free-ranging and managed dolphins is unknown. To compare the nutrient composition of diets consumed by free-ranging and managed bottlenose dolphins, samples ($n = 5$) of the 8 species of fish commonly consumed by free-ranging bottlenose dolphins in Sarasota Bay, FL, and the 7 species of fish and squid commonly fed to managed bottlenose dolphins were analyzed for nutrient content. Metabolizable energy was calculated using Atwater factors; the DCAD was calculated using 4 equations commonly used in people and animals that use different absorption coefficients. The nutrient composition of individual species was used to predict the DCAD of 2 model diets typically fed to managed common bottlenose dolphins and a model diet typically consumed by common bottlenose dolphins in Sarasota Bay. To mimic differences in postmortem handling of fish for the 2 populations of bottlenose dolphins, "free-ranging" samples were immediately frozen at −80°C and minimally thawed before analysis, whereas "managed" samples were frozen for 6 to 9 mo at −18°C and completely thawed. "Free-ranging" species contained more Ca and P and less Na and Cl than "managed" fish and squid species. As a consequence, the DCAD of both model managed dolphin diets obtained using 3 of the 4 equations was much more negative than the DCAD of the model free-ranging bottlenose dolphin diet ($P < 0.05$). The results imply that managed bottlenose dolphins must excrete more protons in urine than free-ranging bottlenose dolphins, which will promote nephrolith formation. The nutrient composition of the free-ranging bottlenose dolphin diet, determined for the first time here, can be used as a guide for feeding managed bottlenose dolphins, but research in vivo is warranted to determine whether adding more cations to the diet will prevent urolith formation in managed dolphins.

Key words: ammonium urate uroliths, bottlenose dolphins, diet nutrient analysis, dietary cation–anion difference, fish, *Tursiops truncatus*

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INTRODUCTION

Ammonium urate nephroliths, which can cause azotemia and renal failure, frequently develop in common bottlenose dolphins (*Tursiops truncatus*) managed under human care but rarely occur in free-ranging common bottlenose dolphins (Venn-Watson et al., 2010; Smith et al., 2013). Managed dolphins are mostly fed cold-water fish and squid that are frozen, stored, and thawed before feeding, whereas free-ranging dolphins consume a variety of live, temperate-water fish (Venn-Watson et al., 2011; Wells et al., 2013). Nutrient content varies with species, sex, and age of fish; season and location where fish are caught; and storage conditions (Bernard and Allen, 2002; Åsli and Mørkøre, 2012).

Ammonium urate uroliths are more likely to form when urine pH decreases and concentrations of ammonium and urate ions increase in urine (Werness et al., 1985; Osborne et al., 1995). In other mammals, changes in diet influence ammonium urate urolith formation because consumption of more anions (Cl^-, phosphate, and sulfate) relative to cations (Ca^{+2}, Mg^{+2}, K^+, and Na^+) increases proton and ammonium ion excretion and decreases urine pH (Halperin et al., 1990; Asplin et al., 1998; Bartges et al., 1999; Soble et al., 1999). Therefore, the dietary cation–anion difference (**DCAD**) can affect the risk of ammonium urate urolith formation (Block, 1994; Remer and Manz, 1995b).

We hypothesized that diet differences between the 2 dolphin populations might explain why nephrolithiasis is more common in managed dolphins. The nutrient composition of fish consumed by free-ranging dolphins and the DCAD of fish fed to managed dolphins have not been determined (Bernard and Allen, 2002; Slifka et al., 2013). Therefore, the purpose of this study was to analyze the nutrient composition of species commonly consumed by bottlenose dolphins to determine whether differences in the DCAD between model diets consumed by the 2 populations could explain why nephroliths are more common in managed bottlenose dolphins.

MATERIALS AND METHODS

Fish samples were collected by the Chicago Zoological Society's Sarasota Dolphin Research Program under approvals by the Mote Marine Laboratory and University of Florida (**UF**) Institutional Animal Care and Use Committees.

Sample Collection and Processing

The 8 fish species most commonly consumed by free-ranging bottlenose dolphins residing in Sarasota Bay, FL ("free-ranging species"), were selected to represent the free-ranging bottlenose dolphin diet (Barros and Odell, 1990; Wells et al., 2004; Berens McCabe et al., 2010). Samples of these fish were caught between May and September 2013 from the waters off the west coast of Florida using a rod and reel, crab trap, or cast net or with a purse-seine net in Sarasota Bay. To mimic the rapid death of fish consumed by wild bottlenose dolphins as closely as possible, fish were humanely euthanized by immersion in a bath containing 500 mg/L tricaine methanesulfonate (MS 222; Western Chemical Inc., Ferndale, WA). When death was confirmed by cessation of opercular movement for 10 min, fish were weighed and their length was measured. Fish were then individually bagged and transported in dry ice to the UF laboratory (Gainesville, FL) where fish were stored at −80°C for a maximum of 6 mo before further processing.

Boxes of each of 6 fish species and 1 species of squid (Appendix 1) commonly fed to bottlenose dolphins under human care ("managed species") were supplied by 2 dolphin management facilities. Fish and squid had been caught during one commercial fishing season (Appendix 1), wrapped in plastic, and frozen at −18°C. These lots of fish and squid were tested for spoilage by the management facilities and then shipped overnight on dry ice to the UF laboratory where they were stored at −20°C. The total frozen storage time was 6 to 9 mo, depending on when lots were supplied to each management facility. The variation in duration of frozen storage time is typical for fish fed to managed dolphins at these facilities.

Free-ranging fish species were thawed the minimum amount needed to allow grinding, whereas managed diet fish species were thawed more completely to mimic the standard operating procedure of one bottlenose dolphin management facility. Free-ranging fish species were air thawed in a temperature-controlled cold room (11–12°C) for approximately 1 h, until fish thawed to a firm, slightly malleable texture. Managed diet species were air thawed in the cold room (11–12°C), wrapped in plastic, for approximately 20 h. Then, individuals of each species were removed from their plastic bags and rinsed with cold water (approximately 16°C).

Five separate samples of each species were analyzed. A minimum of 300 g of ground fish was needed to perform all nutrient analyses on every sample. Therefore, at least 2 individual fish (or squid) were included in each sample, but the number of individual fish (or squid) included in each sample varied depending on the size of the species so that each sample of smaller species contained more individuals than samples of large species. The 5 samples of each species were individually ground using commercial meat grinders with 4.5- and 10-mm plates (Biro 6642 [Biro Manufacturing Co., Lakeside Marblehead, OH] and 1.5 HP [LEM

Products, West Chester, OH]). Both minimally and well-thawed fish or squid were transported to the grinder in a cooler containing ice. Grinder equipment was thoroughly rinsed with water between each sample. Ground samples were homogenized by hand and stored in sample bags (Whirl-Pak bags; Nasco, Fort Atkinson, WI) at −80°C until shipped overnight on dry ice to each laboratory for analysis.

Nutrient Analysis

Gross energy and nutrient analyses were performed by a commercial laboratory (Dairy One Cooperative, Inc., Ithaca, NY). Gross energy density (kcal/g) was measured using a bomb calorimeter (IKA C2000 basic Calorimeter System; IKA Works, Inc., Wilmington, NC). Crude protein (**CP**)was measured with a N/protein analyzer (method 992.15; AOAC International, 1999; Leco FP-528 [Leco Corporation, St. Joseph, MI]). Crude fat (**CF**) was measured by ether extraction (method 2003.05; AOAC International, 1999), ash was measured by combustion (method 942.05; AOAC International, 1999), and moisture was measured by an oven drying (method 930.15; AOAC International, 1999). When these macronutrients were added together, the total obtained was greater than 100% of the analyzed sample in all species. This suggested that the method used to measure CP overestimated protein content. The AOAC International method (992.15; AOAC International, 1999) for CP measures N content and then calculates the protein content, assuming protein contains 16% N, by multiplying N content by 6.25. Fish protein may contain 17% N (Mariotti et al., 2008). Using a factor of 5.9 instead of 6.25 gave an average N-free extract content by difference of 0.5% (as-fed basis), of which about half was crude fiber. This suggests that fish contained negligible amounts of carbohydrate, so protein content was calculated by difference from 100% and carbohydrate content was assumed to be zero. Calcium, P, Mg, Na, K, and S were determined using an Inductively Coupled Plasma Radial Spectrometer (Thermo iCAP 6300; Fisher Scientific, Waltham, MA) after microwave digestion in a Microwave Accelerated Reaction System (CEM Corp., Matthews, NC). Chloride (Cl^-) was measured by potentiometric titration with silver nitrate and a silver electrode (Brinkmann Metrohm 716 Titrino Titration Unit; Metrohm USA, Riverview, FL). The laboratory was blind to the source of each sample duplicate. Analyses were repeated when duplicate sample analyses differed by more than 10%.

The ME density of each fish species was calculated using Atwater factors (Ardente and Hill, 2015), and then the nutrient content of each fish was calculated relative to ME. Total water (**TW**) content was calculated by adding moisture in food to metabolic water, calculated as the sum of 0.41 mL/g protein and 1.09 mL/g fat (NRC, 2006). Ratios (vol/wt) of TW to protein and Na content were also calculated.

Table 1. Mineral molecular weights, valences, and absorption coefficients used to calculate dietary cation–anion differences

Mineral	Molecular weight, g/mol	Valence	Absorption coefficient	
			Human[1]	Cat[2]
Na	22.990	1+	0.95	0.95
K	39.098	1+	0.8	0.95
Ca	40.078	2+	0.25	0.20
Mg	24.305	2+	0.32	0.25
Cl	35.450	1−	0.95	0.95
S	32.060	2−	0.91[3]	0.91[3]
P	30.974	1.8−	0.63	0.35

[1]Absorption coefficients are those used by Remer and Manz (1995b) to estimate the potential renal acid load in human beings, with the exception of S.

[2]Absorption coefficients were based on mineral absorption in domestic cats reported by NRC (2006) for Na, K, Mg, and Cl and by Mack et al. (2015) for Ca and P.

[3]Absorption coefficient for S was assumed to be 91%, which is the digestibility of protein predicated by Atwater factors (Ardente and Hill, 2015).

The DCAD (mEq/Mcal) was calculated using 4 equations (Table 1). The first 2 equations, $DCAD_{short}$ and $DCAD_{long}$, have been found to have utility in several species but do not take account of differences in absorption of protein and minerals (Kienzle et al., 1991; Block, 1994; Frassetto et al., 1998). A third equation, $DCAD_{human}$, which uses absorption coefficients derived from human studies, has been used to estimate the potential renal acid load in people. This equation was modified slightly because the S in the fish and squid species analyzed was measured directly rather than being estimated from the S-containing AA content of the diet (Remer and Manz, 1995b). Protein, from which most S is probably derived, appears to be well digested by marine mammals, which is why Atwater factors were used to calculate ME (Ardente and Hill, 2015). The absorption of S was assumed, therefore, to be the same (91%), as the digestibility of protein on which the Atwater factor for protein is based (NRC, 2006). Human beings are not pure carnivores like bottlenose dolphins, however, so a fourth equation was used to calculate $DCAD_{cat}$, using values for the apparent absorption of minerals obtained from studies of another pure carnivore, the domestic cat. Specifically, absorption of Na, K, and Cl are reported to be greater than 90% in adult cats and 95% in human beings, so an absorption coefficient of 95% was used for these minerals (Remer and Manz, 1995b; NRC, 2006). Absorption of S was again assumed to be 91%. The absorption coefficients of 25% for Ca and 35% for P were obtained from linear regression equations correlating the concentration of dietary Ca and P to absorption in adult cats (Mack et al., 2015). The Mg absorption coefficient was assumed

to be 25% because the Ca content of whole bony fish is high and Mg absorption decreases in adult cats from 40% to 25% with increasing Ca in the diet (NRC, 2006).

Model Bottlenose Dolphin Diets

Two model managed bottlenose dolphin diets were formulated based on the relative proportions of fish and squid species fed by 2 dolphin management facilities (Table 2). Facilities vary the total amount of fish or squid fed to bottlenose dolphins to provide enough calories to maintain BW or growth rate, but wet weight of fish determines the relative proportion of each species that makes up the total diet (Ardente and Hill, 2015). Therefore, to determine the proportion of the total ME of the diet provided by each species, the percent weight (as-fed basis; g/100 g of diet) of each species was multiplied by the average "as fed" ME density of that species. The ME contribution of each species was then calculated as a percent of the total megacalories provided by all the species in 100 g of diet.

A model free-ranging bottlenose dolphin diet (Table 2) was derived from the proportions of fish species reported to be consumed by bottlenose dolphins in Sarasota Bay, FL (Wells et al., 2013). Investigators have reported the number of each fish species or family group of fish as a percentage of the total number of fish consumed based on stomach content analyses, observation, and prey abundance studies conducted over more than 20 yr (Wells et al., 2013). Such reports have grouped some fish species into families. For example, Atlantic threadfin herring and menhaden have been grouped together as the Clupeid family, and pinfish and sheepshead have been grouped under the Sparid family. For the model diet, Atlantic threadfin herring and menhaden were assumed to contribute equally to the total number of Clupeid fish consumed, whereas 35% of pinfish and 3.1% of sheepshead were assumed to make up the total Sparid fish consumed because 11 times more pinfish are generally consumed than sheepshead (Barros and Wells, 1998; Berens McCabe et al., 2010; Dunshea et al., 2013). In addition, percentages of all of the fish species added together gave a total of only 85%. The additional 15% probably represents other unnamed species in the diet. For the model diet, the percent of each documented fish species was proportionately increased so that the total percent of all of the fish was 100. The ME provided by each fish species to the diet was then calculated by multiplying the percentage of each species in the diet by the average weight (g) of that fish species caught for this study and by the average ME density of that fish species (Mcal/kg, as-fed basis). The ME provided by each fish species was then calculated as a percentage of the total ME provided by all of the fish.

Table 2. Proportions of fish and squid species in model managed and free-ranging common bottlenose dolphin diets

Fish and squid species	Percent[1]	Percent Mcal ME
Managed diet number 1		
Icelandic capelin (*Mallotus villosus*)	60	54.0
Pacific herring (*Clupea pallasii*)	20	31.9
Pacific mackerel (*Scomber japonicus*)	10	8.7
West coast Loligo squid (*Loligo opalescens*)	10	5.4
Managed diet number 2		
Canadian capelin (*Mallotus villosus*)	60	47.4
Atlantic herring (*Clupea harengus*)	10	15.2
Pacific herring (*Clupea pallasii*)	10	17.2
Pacific mackerel (*Scomber japonicus*)	10	9.4
Pacific sardine (*Sardinops sagax*)	10	10.8
Free-ranging diet		
Pinfish (*Lagodon rhomboides*)	41	27.1
Gulf toadfish (*Opsanus beta*)	40	24.0
Sheepshead (*Archosargus probatocephalus*)	4	9.2
Spot (*Leiostomus xanthurus*)	3	11.4
Pigfish (*Orthopristis chrysoptera*)	3	1.6
Mullet (*Mugil cephalus*)	2	19.3
Ladyfish (*Elops saurus*)	2	2.9
Spotted sea trout (*Cynoscion nebulosus*)	2	2.6
Menhaden[2]	1	1.9
Atlantic threadfin herring[2]	1	0.2

[1]Percent as-fed weight for managed diet species, and percent of individual fish for free-ranging diet species.

[2]Genus and species not specified by Wells et al. (2013).

Statistical Analysis

Statistical comparisons were performed using statistical software (SAS for Windows software version 9.4; SAS Inst. Inc., Cary, NC). The distributions of nutrient concentrations within species were visually assessed and assessed for normality using the Shapiro–Wilk test. Concentrations that were not normally distributed or with widely different variances were log transformed before being compared. Nutrient concentrations were compared among fish species nested within either managed or free-ranging groups using a general linear model design (SAS procedure GLIMMIX). Multiple comparisons were performed with a Tukey–Kramer correction. Least squares means of nutrient contents were compared among model diets (SAS procedure LSMESTIMATE).

RESULTS

Macro- and micro-nutrients and DCAD differed ($P \leq 0.05$) among fish species (Tables 3, 4 and 5) and among model diets (Table 6). Species and model diets consumed by free-ranging common bottlenose dolphins contained more DM, protein, and ash and less fat ($P \leq 0.05$) than species and model diets fed to common bottle-

Table 3. Energy and macronutrient content of fish and squid commonly consumed by free-ranging and managed bottlenose dolphins[1]

Fish and squid species	GE, Mcal/kg, as-fed basis	ME,[3] Mcal/kg, as-fed basis	DM,[3] g/kg, as-fed basis	Protein,[3] g/Mcal ME	Fat,[4] g/Mcal ME	Ash,[3] g/Mcal ME
Free-ranging diet[2]						
Pinfish	1.49 (0.07)^{c}	1.24 (0.07)^{d}	286 (5)^{cd}	136 (9)^{e}	51 (4)^{d}	46 (6)^{bc}
Gulf toadfish	0.87 (0.04)^{f}	0.66 (0.03)^{g}	201 (14)^{fgh}	218 (6)^{a}	14 (3)^{h}	68 (12)^{ab}
Mullet	1.89 (0.08)^{b}	1.67 (0.08)^{bc}	334 (8)^{a}	101 (6)^{f}	66 (3)^{c}	33 (3)^{de}
Spot	2.26 (0.12)^{a}	2.06 (0.08)^{a}	348 (11)^{a}	73 (3.5)^{h}	79 (2)^{a}	18 (1)^{gh}
Sheepshead	1.17 (0.13)^{de}	0.96 (0.10)^{ef}	272 (15)^{cde}	174 (16)^{cd}	34 (7)^{ef}	82 (11)^{a}
Ladyfish	1.15 (0.12)^{de}	0.89 (0.11)^{ef}	238 (15)^{ef}	208 (21)^{abc}	19 (9)^{fgh}	38 (4)^{cd}
Spotted sea trout	1.01 (0.05)^{e}	0.78 (0.05)^{f}	216 (12)^{fg}	214 (7)^{ab}	16 (3)^{gh}	44 (6)^{bcd}
Pigfish	1.42 (0.12)^{cd}	1.18 (0.12)^{de}	259 (14)^{de}	134 (11)^{e}	52 (5)^{d}	34 (4)^{cde}
All species	1.41 (0.02)	1.18 (0.01)	269 (2)	157 (1.8)	41 (0.8)	45 (1)
Managed diet						
Canadian capelin	1.10 (0.021)^{e}	0.83 (0.01)^{f}	190 (3)^{h}	156 (0.7)^{d}	42 (0.3)^{e}	28 (0.8)^{ef}
Icelandic capelin	1.27 (0.05)^{d}	1.02 (0.05)^{e}	201 (4)^{gh}	122 (3)^{e}	57 (2)^{d}	19 (1)^{g}
Pacific herring	2.11 (0.02)^{a}	1.82 (0.04)^{b}	313 (4)^{b}	85 (3)^{g}	73 (1)^{b}	12 (0.3)^{j}
Atlantic herring	1.89 (0.02)^{b}	1.61 (0.01)^{c}	291 (2)^{c}	96 (2)^{f}	69 (1)^{c}	16 (0.7^{h}
Pacific mackerel	1.34 (0.02)^{d}	0.99 (0.01)^{e}	246 (1)^{e}	188 (5)^{c}	28 (2)^{f}	28 (1)^{ef}
Pacific sardine	1.49 (0.04)^{c}	1.14 (0.06)^{de}	265 (8)^{de}	168 (7)^{cd}	37 (3)^{ef}	26 (2)^{f}
Loligo squid	0.81 (0.03)^{f}	0.61 (0.01)^{g}	150 (1)^{i}	206 (2)^{bc}	20 (0.9)^{fg}	14 (0.4)^{i}
All species	1.43 (0.005)	1.15 (0.005)	237 (0.7)	146 (0.7)	46 (0.3)	20 (0.2)

$^{a–j}$Nutrient concentrations within a column with different superscripts differ ($P \leq 0.05$) among species.

[1]Values are means (1 SD) for each species ($n = 5$) or means (1 SE) for all species within each diet group ($n = 40$ for all free-ranging diet species and $n = 35$ for all managed diet species).

[2]Free-ranging diet species are listed in order of greatest to least percent contribution to the total energy content of the diet.

[3]Energy content or nutrient concentrations were greater for free-ranging diet species than for managed diet species ($P \leq 0.05$).

[4]Nutrient concentrations were greater for managed diet species than free-ranging diet species ($P \leq 0.05$).

nose dolphins under human care. Spot and Pacific herring provided up to 3-fold more "as fed" ME, up to 5-fold more CF, and up to 3-fold less protein relative to ME than other species. Gulf toadfish and Loligo squid were the least energy dense and contained 5-fold less CF and 3-fold more protein relative to energy than other species.

All mineral concentrations, except S, significantly differed ($P \leq 0.0001$) between the 2 groups of fish species (Table 4) and among model diets (Table 6). The Ca and P concentrations were 4 and 3 times greater, respectively, in free-ranging diet fish species than in managed diet fish species. Managed diet species contained 60% more Cl and 20% more Na than free-ranging species. In particularly, Canadian capelin contained up to 3-fold more Na and up to 8-fold more Cl compared with other managed and free-ranging species.

The managed diet species provided more water relative to energy and CP compared with the free-ranging diet species, whereas the free-ranging diet species (Table 5) and model diets (Table 6) provided more water relative to Na ($P \leq 0.05$). Total water relative to ME, protein, and Na also widely varied among individual species ($P \leq 0.05$; Table 5). Specifically, Loligo squid and Gulf toadfish provided up to 4 times more water per megacalorie ME than other species, and spot, mullet, and Atlantic herring provided the least amount of water per megacalorie ME. Mullet and spotted sea trout provided approximately twice the amount of water relative to Na than was provided by Atlantic herring and Canadian capelin.

The DCAD calculated using the $DCAD_{short}$, $DCAD_{long}$, and $DCAD_{cat}$ equations was more positive in free-ranging diet species than in managed diet species, whereas DCADhuman was more positive for the managed diet species than for the free-ranging diet species ($P \leq 0.05$; Table 5). The DCAD also widely varied among fish species within groups depending on the equation used, but $DCAD_{long}$ was notable for being positive for all but one free-ranging diet fish species and negative for all managed diet species (Table 5).

Gross energy and ME differed by less than 6% among managed and free-ranging model diets. The free-ranging model diet contained 8 to 25% more protein and 8 to 22% less fat ($P \leq 0.05$) than the managed model diets (Table 6). The free-ranging model diet also contained up to 500% more Ca and up to 250% more P than the model managed diets. On the other hand, "Managed diet number 2" contained approximately 60% more Na and Cl than the other managed diet and 40% more Na and 100% more Cl than the free-ranging model diet. Managed diet number 2 also had 20 to 28%

Table 4. Mineral concentrations of fish and squid consumed by free-ranging and managed bottlenose dolphins[1]

Fish and squid species	Ca[3] g/Mcal ME	P[3] g/Mcal ME	Mg[3] g/Mcal ME	K[3] g/Mcal ME	Na[4] g/Mcal ME	Cl[4] g/Mcal ME	S g/Mcal ME
Free-ranging diet[2]							
Pinfish	11.2 (0.9)^{bc}	6.9 (0.5)^{bc}	0.43 (0.03)^{c}	2.5 (0.2)^{cd}	1.4 (0.2)^{cd}	1.8 (0.2)^{d}	2.1 (0.1)^{c}
Gulf toadfish	17.1 (3.2)^{ab}	10.5 (2.0)^{ab}	0.69 (0.05)^{a}	3.7 (0.3)^{ab}	2.6 (0.3)^{ab}	3.5 (0.7)^{bc}	3.0 (0.1)^{b}
Mullet	6.6 (0.7)^{d}	4.1 (0.5)^{de}	0.24 (0.02)^{ef}	1.5 (0.1)^{e}	0.7 (0.63)^{e}	0.7 (0.2)^{ef}	1.3 (0.06)^{ef}
Spot	4.3 (0.6)^{ef}	2.8 (0.3)^{fg}	0.19 (0.02)^{fg}	1.2 (0.07)^{f}	0.7 (0.05)^{e}	0.3 (0.09)^{f}	1.0 (0.05)^{g}
Sheepshead	22.7 (3.0)^{a}	12.3 (1.9)^{a}	0.61 (0.08)^{ab}	2.9 (0.2)^{bc}	1.9 (0.3)^{bc}	2.4 (0.3)^{cd}	2.6 (0.2)^{bc}
Ladyfish	9.0 (1.3)^{cd}	6.8 (0.9)^{bc}	0.46 (0.05)^{bc}	3.8 (0.5)^{ab}	1.5 (0.3)^{cd}	2.2 (0.4)^{cd}	3.2 (0.6)^{b}
Spotted sea trout	10.3 (1.9)^{bcd}	7.7 (1.0)^{bc}	0.45 (0.02)^{bc}	4.1 (0.4)^{a}	1.7 (0.07)^{c}	2.5 (0.05)^{c}	2.9 (0.2)^{b}
Pigfish	9.4 (0.9)^{cd}	5.8 (0.6)^{c}	0.36 (0.04)^{c}	2.5 (0.4)^{cd}	1.5 (0.2)^{cd}	2.2 (0.2)^{cd}	2.0 (0.3)^{cd}
All species	11.3 (0.3)	7.1 (0.2)	0.43 (0.0007)	2.8 (0.05)	1.5 (0.03)	2.0 (0.06)	2.3 (0.04)
Managed diet							
Canadian capelin	3.6 (0.2)^{f}	3.7 (0.07)^{e}	0.50 (0.01)^{b}	2.3 (0.06)^{d}	3.1 (0.1)^{a}	5.8 (0.1)^{a}	2.1 (0.07)^{c}
Icelandic capelin	2.6 (0.2)^{g}	2.9 (0.1)^{f}	0.26 (0.01)^{e}	2.3 (0.08)^{d}	1.5 (0.4)^{cd}	2.7 (0.06)^{c}	1.6 (0.06)^{d}
Pacific herring	2.0 (0.1)^{h}	2.4 (0.01)^{g}	0.16 (0.01)^{g}	1.7 (0.05)^{e}	0.7 (0.02)^{e}	0.9 (0.04)^{e}	1.2 (0.04)^{f}
Atlantic herring	2.4 (0.2)^{g}	2.4 (0.1)^{g}	0.28 (0.01)^{d}	1.7 (0.07)^{e}	1.7 (0.06)^{c}	2.6 (0.1)^{c}	1.3 (0.03)^{e}
Pacific mackerel	4.0 (0.1)^{ef}	4.4 (0.1)^{d}	0.45 (0.01)^{bc}	3.3 (0.08)^{b}	1.7 (0.03)^{c}	3.4 (0.04)^{bc}	2.5 (0.06)^{bc}
Pacific sardine	4.5 (0.4)^{e}	4.2 (0.2)^{d}	0.37 (0.02)^{c}	2.7 (0.06)^{c}	1.4 (0.09)^{d}	2.5 (0.2)^{c}	2.2 (0.07)^{bc}
Loligo squid	0.3 (0.03)^{i}	2.9 (0.2)^{f}	0.44 (0.05)^{bc}	2.5 (0.15)^{cd}	2.4 (0.08)^{b}	4.4 (0.7)^{b}	4.5 (0.3)^{a}
All species	2.8 (0.03)	3.3 (0.02)	0.35 (0.004)	2.4 (0.01)	1.8 (0.01)	3.2 (0.05)	2.2 (0.02)

[a–i]Nutrient concentrations within a column with different superscripts significantly differ ($P \leq 0.05$) among species.

[1]Values are means (1 SD) for each species ($n = 5$) or means (1 SE) for all species within each diet group ($n = 40$ for all free-ranging diet species and $n = 35$ for all managed diet species).

[2]Free-ranging diet species are listed in order of greatest to least contribution to the total energy content of the diet.

[3]Nutrient concentrations are significantly greater in free-ranging diet species than in managed diet species ($P \leq 0.05$).

[4]Nutrient concentrations are significantly greater in managed diet species than in free-ranging diet species ($P \leq 0.05$).

less TW relative to Na than the other 2 diets, and the model free-ranging diet had 7 to 15% less TW relative to protein than the model managed diets.

The DCAD of model diets varied depending on the equation used (Table 6). The $DCAD_{long}$ was strongly positive for the model free-ranging bottlenose dolphin diet but strongly negative for both model managed diets. All other DCAD equations gave negative DCAD values for both managed and free-ranging diets, but $DCAD_{cat}$ was 14 to 30% less negative for the model free-ranging diet compared with the model managed diets and $DCAD_{short}$ was 26% less negative for the free-ranging diet than Managed diet number 2 ($P \leq 0.05$). On the other hand, $DCAD_{human}$ was 9 to 30% more negative for the model free-ranging diet compared with the model managed diets.

DISCUSSION

To our knowledge, this study is the first to compare the nutrient content and the DCAD of the free-ranging bottlenose dolphin diet with diets commonly fed to bottlenose dolphins under human care. Previous studies have compared the nutrient content of only a few individual fish consumed by each group of dolphins, have not measured the DCAD, and have not taken into account the relative proportions of each fish species in the total diet (Bernard and Allen, 2002; Slifka et al., 2013). We measured the nutrient composition and the DCAD of 5 samples of a wider range of species that encompass almost all (85%) of the fish commonly consumed by bottlenose dolphins in Sarasota Bay, FL. We also measured the nutrient composition and the DCAD of all the fish and squid species that are fed to 2 large groups of bottlenose dolphins under human care. This allowed us to evaluate whether differences in nutrient content and the DCAD among model diets consumed by the 2 populations of dolphins could explain why ammonium urate nephrolithiasis is more prevalent in managed bottlenose dolphins than in free-ranging bottlenose dolphins.

The tendency for ammonia and urate to complex and precipitate as ammonium urate crystals is determined by the relative concentrations of ammonium and urate ions in urine, the presence of other solutes, and urine pH (Werness et al., 1985; Osborne et al., 1995; Moran, 2003). Given enough time and appropriate conditions, crystals may aggregate to form stones (Werness et al., 1985). Uric acid is a product of purine metabolism, and whole fish, which make up the bulk of the dolphin diet, are purine rich (Choi et al., 2005). Ammonium ions are produced by the action of glutaminase on glutamine. Ammonium ions provide a mechanism by which

Table 5. Total water (TW) relative to energy, crude protein (CP), and Na and dietary cation–anion differences (DCAD) among fish and squid species[1]

Fish/squid species	TW, mL/Mcal ME[2]	TW:CP ratio, mL:g[2]	TW:Na ratio, mL:g[3]	$DCAD_{short}$[3,4]	$DCAD_{long}$[3,4]	$DCAD_{human}$[2,4]	$DCAD_{cat}$[3,4]
Free-ranging diet[5]							
Pinfish	586 (39)^{e}	4.3 (0.15)^{de}	416 (35)^{c}	−59 (1)^{de}	136 (13)^{b}	−161 (8)^{ef}	−70 (8)^{cd}
Gulf toadfish	1,227 (77)^{b}	5.6 (0.26)^{c}	477 (73)^{abc}	−76 (24)^{def}	223 (33)^{ab}	−232 (29)^{gh}	−94 (25)^{def}
Mullet	413 (25)^{fg}	4.1 (0.05)^{ef}	620 (35)^{a}	−31 (0.6)^{bc}	75 (5)^{c}	−96 (2)^{bc}	−40 (1)^{b}
Spot	329 (17)^{h}	4.5 (0.25)^{de}	468 (34)^{bc}	−13 (2)^{a}	53 (9)^{c}	−60 (6)^{a}	−21 (5)^{a}
Sheepshead	776 (95)^{de}	4.5 (0.15)^{de}	399 (20)^{c}	−71 (13)^{def}	400 (16)^{a}	−221 (15)^{fg}	−71 (13)^{cde}
Ladyfish	877 (116)^{cd}	4.2 (0.16)^{ef}	592 (46)^{a}	−101 (3)^{ef}	−12 (14)^{d}	−228 (5)^{fgh}	−128 (4)^{ef}
Spotted sea trout	1,019 (83)^{bc}	4.8 (0.24)^{d}	615 (59)^{a}	−72 (18)^{def}	32 (76)^{cd}	−218 (32)^{fg}	−106 (20)^{ef}
Pigfish	645 (85)^{de}	4.8 (0.26)^{d}	423 (20)^{c}	−61 (3)^{de}	102 (5)^{bc}	−147 (3)^{de}	−69 (3)^{cd}
All species	730 (10)	4.6 (0.03)	501 (7)	−60 (2)	126 (4)	−170 (4)	−75 (2)
Managed diet							
Canadian capelin	983 (17)^{c}	6.3 (0.13)^{b}	317 (10)^{d}	−96 (7)^{ef}	−92 (19)^{fg}	−173 (17)^{efg}	−116 (9)^{ef}
Icelandic capelin	792 (44)^{d}	6.5 (0.33)^{ab}	521 (20)^{ab}	−48 (24)^{d}	−62 (21)^{e}	−116 (40)^{cd}	−68 (27)^{cd}
Pacific herring	392 (10)^{g}	4.6 (0.08)^{d}	586 (7)^{a}	−27 (10)^{b}	−54 (59)^{e}	−88 (59)^{b}	−49 (14)^{bc}
Atlantic herring	454 (4)^{f}	4.7 (0.10)^{d}	263 (8)^{e}	−39 (5)^{c}	−33 (14)^{d}	−90 (7)^{b}	−52 (5)^{c}
Pacific mackerel	773 (11)^{de}	4.1 (0.09)^{ef}	466 (7)^{c}	−101 (3)^{f}	−123 (15)^{g}	−203 (11)^{fg}	−131 (5)^{f}
Pacific sardine	660 (42)^{de}	3.9 (0.14)^{f}	490 (6)^{ab}	−79 (2)^{ef}	−69 (41)^{ef}	−169 (11)^{ef}	−103 (4)^{ef}
Loligo squid	1,398 (40)^{a}	6.8 (0.21)^{a}	576 (8)^{a}	−236 (4)^{cd}	−352 (11)^{h}	−312 (10)^{h}	−259 (5)^{g}
All species	780 (5)	5.3 (0.03)	460 (2)	−90 (2)	−112 (3)	−165 (2)	−111 (2)

$^{a–h}$Nutrient concentrations within a column with different superscripts significantly differ ($P \leq 0.05$) among species.

[1]Values are means (1 SD) for each species ($n = 5$) or means (1 SE) for all species within each diet group ($n = 40$ for all free-ranging diet species and $n = 35$ for all managed diet species).

[2]Nutrient concentrations are significantly greater, or DCAD are more positive, in managed diet species than in free-ranging diet species ($P \leq 0.05$).

[3]Nutrient concentrations are significantly greater, or DCAD are more positive, in free-ranging diet species than in managed diet species ($P \leq 0.05$).

[4]DCAD calculated using 4 equations: $DCAD_{short} = (Na + K) − (Cl + S)$; $DCAD_{long} = (Na + K + Ca + Mg) − (Cl + S + P)$; $DCAD_{cat} = (0.95Na + 0.95K + 0.2Ca + 0.25Mg) − (0.95Cl + 0.35P + 0.91S)$; and $DCAD_{human} = (0.95Na + 0.8K + 0.25Ca + 0.32Mg) − (0.95Cl + 0.63P + 0.91S)$, in which Na, K, Ca, Mg, Cl, S, and P represent the milliequivalents per megacalories ME of Na, K, Ca, Mg, Cl, S, and P, respectively.

[5]Free-ranging diet species are listed in order of greatest to least contribution to the total energy content of the diet.

protons are excreted by the kidney, and the kidney excretes greater amounts of ammonia in the urine during acidosis (Halperin et al., 1990; Curthoys and Watford, 1995). Proton excretion is influenced by the relative proportions of dietary anions and cations excreted in the urine. Absorption and excretion of positively charged cations, such as Na^{+}, K^{+}, Ca^{+2}, and Mg^{+2}, make urine more alkaline, whereas excretion of negatively charged anions, such as $Cl^{−}$, phosphate, and sulfate, make urine more acidic (Halperin et al., 1990; Asplin et al., 1998). This relative difference in concentrations of dietary anions and cations in the diet (the DCAD) has been used to predict how changes in the diet will affect the average blood and urine pH, excretion of ammonium ions, and risk of forming uroliths in dairy cows, cats, dogs, and people (Ender and Dishington, 1970; Kealy et al., 1993; Block, 1994; Remer and Manz, 1995b).

The free-ranging bottlenose dolphin model diet provided more protein and less fat than both managed bottlenose dolphin model diets because it comprised more lean fish species, such as pinfish, and fewer higher-fat fish species, such as spot and sheepshead. If macronutrients are considered without the DCAD, the free-ranging diet would be expected to result in excretion of more sulfate ions from protein and thus more ammonium ions (Breslau et al., 1988; Remer and Manz, 1995a). Taking mineral composition and the DCAD into account, however, suggests that consumption of the managed dolphin model diets would result in more proton and ammonium ion excretion in urine than the free-ranging model diet. Concentrations of Ca and P were greater and concentrations of Na and Cl were lower for free-ranging species than for managed diet species. The differences in Ca, P, and Na content are similar to differences previously reported when single samples of pinfish, pigfish, and mullet were compared with capelin and herring (Slifka et al., 2013), but Cl was not measured in that study, so differences in the DCAD could not be assessed. Free-ranging fish species tend to be bonier and have teeth, which would contribute to their greater Ca and P concentrations when compared with managed diet species. The greater Na and Cl content of managed diet species are likely caused by application of a brine solution (Slifka et al., 2013). The composition of this brine solution, and the concentration in which it is applied, varies depending on the fishery but generally contains sodium chloride

Table 6. Energy and nutrient content, nutrient ratios, and dietary cation–anion differences (DCAD) for model managed and free-ranging diets[1]

Nutrient	Managed model diet number 1	Managed model diet number 2	Free-ranging model diet
per kg, as-fed basis			
GE, Mcal	1.52 (0.01)[a]	1.46 (0.01)[b]	1.45 (0.01)[b]
ME, Mcal	1.25 (0.01)[a]	1.17 (0.01)[b]	1.23 (0.01)[a]
DM, g	238 (1)[a]	240 (0.9)[a]	276 (2)[b]
per Mcal ME			
TW, L	0.70 (0.01)[a]	0.75 (0.04)[b]	0.72 (0.01)[ab]
Protein, g	120 (0.9)[a]	139 (0.5)[b]	150 (1.5)[c]
Fat, g	58 (0.4)[a]	49 (0.2)[b]	45 (0.7)[c]
Ash, g	17 (0.3)[a]	23 (0.2)[b]	48 (1.5)[c]
Ca, g	2.4 (0.04)[a]	3.3 (0.04)[b]	11.9 (0.4)[c]
P, g	2.9 (0.03)[a]	3.4 (0.02)[b]	7.3 (0.2)[c]
Mg, g	0.25 (0.003)[a]	0.39 (0.003)[b]	0.44 (0.008)[c]
K, g	2.2 (0.02)[a]	2.3 (0.01)[a]	2.6 (0.04)[b]
Na, g	1.3 (0.01)[a]	2.1 (0.03)[b]	1.5 (0.04)[c]
Cl, g	2.3 (0.02)[a]	3.8 (0.03)[b]	1.9 (0.09)[c]
S, g	1.7 (0.02)[a]	1.9 (0.02)[b]	2.1 (0.02)[c]
TW:protein ratio, mL:g	5.7 (0.08)[a]	5.3 (0.03)[b]	4.6 (0.04)[c]
TW:Na ratio, mL:g	540 (5)[a]	388 (2)[b]	487 (10)[c]
mEq/Mcal ME[2]			
$DCAD_{short}$	−56 (1)[a]	−74 (2)[b]	−55 (2)[a]
$DCAD_{long}$	−80 (3)[a]	−77 (3)[a]	152 (9)[b]
$DCAD_{human}$	−125 (2)[a]	−149 (2)[b]	−163 (4)[c]
$DCAD_{cat}$	−78 (1)[a]	−95 (2)[b]	−67 (2)[c]

[a–c]Nutrient concentrations across rows with different superscripts differ ($P \leq 0.05$) among model diets.

[1]Values are means (SE). TW = total water.

[2]DCAD calculated using 4 equations: $DCAD_{short}$ = (Na + K) − (Cl + S); $DCAD_{long}$ = (Na + K + Ca + Mg) − (Cl + S + P); $DCAD_{cat}$ = (0.95Na + 0.95K + 0.2Ca + 0.25Mg) − (0.95Cl + 0.35P + 0.91S); and $DCAD_{human}$ = (0.95Na + 0.8K + 0.25Ca + 0.32Mg) − (0.95Cl + 0.63P + 0.91S), in which Na, K, Ca, Mg, Cl, S, and P represent the milliequivalents per megacalories ME of Na, K, Ca, Mg, Cl, S, and P, respectively.

at a concentration up to 25% (wt/vol; Duerr and Dyer, 1952; Gallart-Jornet et al., 2007). The greater Na and Cl concentrations of the managed model diets are accompanied by greater TW concentrations. For example, a large percentage of Managed diet number 2 is made up of Canadian capelin, which is the species with the greatest TW content and Na and Cl contents compared with all other species. Therefore, the second managed model diet would generate an enhanced postprandial diuresis as additional Na, Cl, and water are excreted in the urine, which may help to prevent ammonium urate nephrolith formation (Ridgway and Venn-Watson, 2010).

The effect of these mineral differences on DCAD and, subsequently, urine pH, ammonia excretion, and risk of nephrolith formation depends on the relative absorption of each mineral from the diet. The relative absorption of dietary minerals by dolphins is unknown, so we used 4 equations to calculate the DCAD, each of which assumes different relative absorptions of each mineral. The longest equation, $DCAD_{long}$, assumes 100% apparent absorption of *all* minerals, whereas the shortest equation assumes 100% absorption of Na, K, Cl, and S but does not account for absorption of Ca, Mg, and P. It is unlikely, however, that minerals are either completely absorbed or that Ca, Mg and P are not absorbed, so we also evaluated 2 additional equations: the $DCAD_{human}$ equation, which uses human mineral absorption coefficients, and a $DCAD_{cat}$ equation based on mineral absorption in adult domestic cats, which are obligate carnivores like dolphins (Remer and Manz, 1995b; NRC, 2006; Mack et al., 2015). Several authors have developed alternative equations for predicting the urine pH of cats by regressing dietary mineral and AA concentrations in the diet against urine pH (Yamka et al., 2006; Pires et al., 2011). These alternative cat equations were not used because the coefficients imply more than 100% absorption of some minerals, and the cat diets used in these studies include absorbable sources of S and P that are added to the diet to lower urine pH.

Three of the equations predict that both of the model managed bottlenose dolphin diets would produce a more acidic urine than the model free-ranging diet. A more acidic urine would result in more ammonium ion excretion and help to explain why managed dolphins form ammonium urate nephroliths. The $DCAD_{long}$ provides the most striking difference because it was strongly positive for all but one of the free-ranging diet fish species, and the model diet was strongly negative for *all* of the managed diet species and both model managed diets (Table 1). This is partly because capelin, Pacific mackerel, and sardines fed to managed dolphins contain much more Cl than Na compared with the free-ranging species. More importantly, however, there was more Ca and P in the free-ranging fish, and the Ca:P ratio was about 1.6:1 for the free-ranging fish species but only 1:1 in the managed species. The $DCAD_{long}$ reflects these differences because it assumes complete absorption of both Ca and P, whereas the other 3 equations reduce the effect of the increased Ca relative to P in the diet because they assume Ca and P are either not absorbed or only partly absorbed. Although $DCAD_{long}$ correlates well with urine pH in cats fed some feline diets (Kienzle et al., 1991), DCADlong suggests that 225 mEq/Mcal ME more cations than anions must be added to the managed model diets to match the free-ranging model diet $DCAD_{long}$. In contrast, the $DCAD_{short}$ and $DCAD_{cat}$ equations suggest that a more reasonable addition of 10 to 30 mEq/Mcal ME of cations relative to anions would be sufficient achieve a similar DCAD among managed and free-ranging model diets. The $DCAD_{human}$ suggests the opposite (the DCAD is more negative in the free-ranging diet), because the $DCAD_{human}$ assumes that intestinal absorption of P is 3 times greater than Ca absorption.

When combined with the increased amount of both Ca and P in the fish consumed by free-ranging bottlenose dolphins, the contribution of phosphate anion to the diet is strongly favored over the Ca^{+2} cation contribution. Unfortunately, only measuring mineral absorption in the intestine of bottlenose dolphins under human care or measuring the total urinary excretion of minerals and urine pH over 24 h during a controlled feeding trial will decide which of these DCAD equations best represents the effect of dietary acid–base balance on urine pH.

The study has several limitations. The nutrient content of fish depends on the location where fish are caught as well as the species, catch season, and frozen storage time. Within a given season, the protein and fat composition of fish also changes with water temperatures and spawning cycles (Henderson et al., 1984; Vollenweider et al., 2011). Due to financial constraints, nutrient analyses were performed only on fish caught during one season. Season was determined by practical considerations for free-ranging species collection and when commercial fisheries are active. The 2 managed bottlenose dolphin model diets are relatively standard among management facilities, but nutrient analysis was limited to 1 lot, or 1 catch date, of each type of fish also because of financial constraints. Therefore, differences between fish lots caught at different times within a commercial catch season could not be determined, and this study did not account for seasonal variations in fish body composition. Furthermore, frozen storage time was set at 6 to 9 mo for managed diet fish species. This is the average length of time fish are stored frozen before fed to managed bottlenose dolphins but varied within the 6 to 9 mo time frame due to commercial fish stock availability. Frozen storage has been well documented to affect the nutrient content of fish, particularly with respect to fatty acid oxidation and water loss; therefore, it is possible that storage times less than 6 mo or greater than 9 mo may have yielded different results for managed diet fish composition (Ackman et al., 1969; Nunes et al., 1992).

The free-ranging model diet also made assumptions regarding the species and relative proportions that are consumed by free-ranging bottlenose dolphins. The free-ranging model diet was inferred from previously reported data because it is impractical to measure the actual intake of free-ranging bottlenose dolphins, but it is specific to inshore bottlenose dolphins residing in Sarasota Bay, FL (Berens McCabe et al., 2010; Wells et al., 2013). This population of bottlenose dolphins was chosen as an example of a free-ranging population because they have been studied for more than 45 yr, and there are more published reports of the fish consumed by these bottlenose dolphins than any other free-ranging population. Nevertheless, this model diet does not account for individual variation based on age, sex, reproductive status, or prey preference, and other populations of free-ranging bottlenose dolphins may consume diets with a different composition. It is also possible that the fish caught for this study were not representative of fish consumed by bottlenose dolphins at different times of year or that bottlenose dolphins may positively select certain species to maintain acid–base homeostasis, such as cats (Cook et al., 1996). Nevertheless, all fish lengths fell within the reported range (50–300 mm, up to 1,027 mm) for fish consumed by free-ranging bottlenose dolphins (Allen et al., 2001; Berens McCabe et al., 2010).

The model diets comparisons also assume an equal caloric intake among bottlenose dolphin populations, whereas preliminary data suggest that free-ranging bottlenose dolphins may have higher energy requirements than managed bottlenose dolphins. An average 160-kg free-ranging bottlenose dolphin in Sarasota Bay, FL, has an average daily energy requirement (measured using the double labeled water method) ranging from approximately 16 Mcal/d in the winter to 22 Mcal/d in the summer (Costa et al., 2013). Among bottlenose dolphins under human care at one facility, however, nonpregnant, nonlactating adults have been reported to consume approximately 8.5 to 12 Mcal/d and growing male and female bottlenose dolphins have been reported to consume approximately 8.5 to 16 Mcal/d (Reddy et al., 1994). These differences in energy requirements are likely a consequence of different activity levels, water temperatures, and reproductive status. Nutrient intake is affected by the amount of food consumed as well as the nutrient composition of the diet, so free-ranging bottlenose dolphins may be consuming, metabolizing, and excreting more of some nutrients than some managed bottlenose dolphins even when the managed diet contains less of those nutrients on an equal caloric basis. This would not affect the relative proportions of nutrients that are used to calculate the DCAD, however, unless mineral absorption differs with intake.

In conclusion, this study showed that more cations relative to anions are present in model diets consumed by free-ranging common bottlenose dolphins than in model diets fed to common bottlenose dolphins managed under human care. The more negative DCAD of the managed dolphin diets likely contributes to the development of ammonium urate nephrolithiasis in managed bottlenose dolphins. By feeding fish, such as mullet, with a more positive DCAD, in place of fish, such as capelin, with a negative DCAD, it may be possible to reduce the prevalence of ammonium urate nephroliths in managed dolphins. Nevertheless, in vivo studies are warranted to determine the extent to which altering the DCAD (adding cations) or altering fish species in the managed dolphin diet affects solute excretion and saturation, urine pH, and ammonium urate nephrolith development.

LITERATURE CITED

Ackman, R. G., P. J. Ke, W. A. MacCallum, and D. R. Adams. 1969. Newfoundland capelin lipids: Fatty acid composition and alterations during frozen storage. J. Fish. Res. Board Can. 26:2037–2060. doi:10.1139/f69-191

Allen, M. C., A. J. Read, J. Gaudet, and L. S. Sayigh. 2001. Fine-scale habitat selection of foraging bottlenose dolphins *Tursiops truncatus* near Clearwater, Florida. Mar. Ecol. Prog. Ser. 222:253–264. doi:10.3354/meps222253

Ardente, A. J., and R. C. Hill. 2015. The nutrient composition of the diet of bottlenose dolphins (*Tursiops truncatus*) is better assessed relative to metabolizable energy than dry matter. J. Zoo Wildl. Med. 46:198–204. doi:10.1638/2014-0064R1.1

AOAC International. 1999. Official Methods of Analysis of AOAC International, 16th edition, 5th revision. AOAC International. Gaithersburg, MD

Åsli, M., and T. Mørkøre. 2012. Brines added sodium bicarbonate improve liquid retention and sensory attributes of lightly salted Atlantic cod. Lebensm. Wiss. Technol. 46:196–202. doi:10.1016/j.lwt.2011.10.007

Asplin, J., J. Parks, J. Lingeman, R. Kahnoski, H. Mardis, S. Lacey, D. Goldfarb, M. Grasso, and F. Coe. 1998. Supersaturation and stone composition in a network of dispersed treatment sites. J. Urol. 159:1821–1825. doi:10.1016/S0022-5347(01)63164-1

Barros, N. B., and D. K. Odell. 1990. Food habits of bottlenose dolphins in the southeastern United States. In: S. Leatherwood and R. Reeves, editors, The bottlenose dolphin. Academic Press, San Diego, CA. p. 309–328.

Barros, N. B., and R. S. Wells. 1998. Prey and feeding patterns of resident bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. J. Mammal. 79:1045–1059. doi:10.2307/1383114

Bartges, J. W., C. A. Osborne, J. P. Lulich, J. M. Kruger, S. L. Sanderson, L. A. Koehler, and L. K. Ulrich. 1999. Canine urate urolithiasis: Etiopathogenesis, diagnosis, and management. Vet. Clin. North Am.: Small Anim. Pract. 29:161–191. doi:10.1016/S0195-5616(99)50010-7

Berens McCabe, E. J., D. P. Gannon, N. B. Barros, and R. S. Wells. 2010. Prey selection by resident common bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Mar. Biol. 157:931–942. doi:10.1007/s00227-009-1371-2

Bernard, J. B., and M. E. Allen. 2002. Feeding captive piscivorous animals: Nutritional aspects of fish as food. Nutrition Advisory Group, http://nagonline.net/wp-content/uploads/2014/01/NAG-FS005-97-Fish-JONI-FEB-24-2002-MODIFIED.pdf Fact sheet 005:1-12, Association of Zoos and Aquariums Nutrition Advisory Group, NAG online.

Block, E. 1994. Manipulation of dietary cation-anion difference on nutritionally related production diseases, productivity, and metabolic responses of dairy cows. J. Dairy Sci. 77:1437–1450. doi:10.3168/jds.S0022-0302(94)77082-X

Breslau, N. A., L. Brinkley, K. D. Hill, and C. Y. Pak. 1988. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. J. Clin. Endocrinol. Metab. 66:140–146. doi:10.1210/jcem-66-1-140

Choi, H. K., S. M. Liu, and G. Curhan. 2005. Intake of purine-rich foods, protein, and dairy products and relationship to serum levels of uric acid – The Third National Health and Nutrition Examination Survey. Arthritis Rheum. 52:283–289. doi:10.1002/art.20761

Cook, N. E., Q. R. Rogers, and J. G. Morris. 1996. Acid-base balance affects dietary choice in cats. Appetite 26:175–192. doi:10.1006/appe.1996.0014

Costa, D., G. A. Worthy, R. S. Wells, A. J. Read, D. Waples, and M. D. Scott. 2013. Patterns of seasonal metabolic rate variation for bottlenose dolphins in Sarasota Bay, Florida. In: Society for Marine Mammalogy 20th Biennial Conference, Dunedin, New Zealand. p. 49.

Curthoys, N. P., and M. Watford. 1995. Regulation of glutaminase activity and glutamine metabolism. Annu. Rev. Nutr. 15:133–159. doi:10.1146/annurev.nu.15.070195.001025

Duerr, J. D., and W. J. Dyer. 1952. Proteins in fish muscle, IV. Denaturation by salt. J. Fish. Res. Board Can. 8c:325–331. doi:10.1139/f50-021

Dunshea, G., N. B. Barros, E. J. Berens McCabe, N. J. Gales, M. A. Hindell, S. N. Jarman, and R. S. Wells. 2013. Stranded dolphin stomach contents represent the free-ranging population's diet. Biol. Lett. 9(3):20121036.

Ender, F., and I. W. Dishington. 1970. Etiology and prevention of paresis puerperalis in dairy cows. In: J. Anderson, editor, Parturient hypocalcemia. Academic Press, New York, NY. p. 71–79.

Frassetto, L. A., K. M. Todd, R. C. Morris, and A. Sebastian. 1998. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am. J. Clin. Nutr. 68:576–583.

Gallart-Jornet, L., J. M. Barat, T. Rustad, U. Erikson, I. Escriche, and P. Fito. 2007. A comparative study of brine salting of Altantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*). J. Food Eng. 79:261–270. doi:10.1016/j.jfoodeng.2006.01.053

Halperin, M. L., J. H. Ethier, and K. S. Kamel. 1990. The excretion of ammonium ions and acid base balance. Clin. Biochem. 23:185–188. doi:10.1016/0009-9120(90)90575-F

Henderson, R. J., J. R. Sargent, and C. C. E. Hopkins. 1984. Changes in the content and fatty acid composition of lipid in an isolated population of the capelin Mallotus villosus during sexual maturation and spawning. Mar. Biol. 78:255–263. doi:10.1007/BF00393011

Kealy, R. D., D. F. Lawler, K. L. Monti, D. Biery, R. W. Helms, G. Lust, S. E. Olsson, and G. K. Smith. 1993. Effects of dietary electrolyte balance on subluxation of the femoral head in growing dogs. Am. J. Vet. Res. 54:555–562.

Kienzle, E., A. Schuknecht, and H. Meyer. 1991. Influence of food composition on the urine pH in cats. J. Nutr. 121:S87–S88.

Mack, J. K., L. G. Alexander, P. J. Morris, B. Dobenecker, and E. Kienzle. 2015. Demonstration of uniformity of calcium absorption in adult dogs and cats. J. Anim. Physiol. Anim. Nutr. 99:801–809. doi:10.1111/jpn.12294

Mariotti, F., D. Tomé, and P. P. Mirand. 2008. Converting nitrogen into protein – Beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. Nutr. 48:177–184. doi:10.1080/10408390701279749

Moran, M. E. 2003. Uric acid stone disease. Front. Biosci. 8:s1339–s1355. doi:10.2741/1178

NRC. 2006. Nutrient requirements of dogs and cats. Natl. Acad. Press, Washington, DC.

Nunes, M. L., I. Batista, and R. Morao de Campos. 1992. Physical, chemical, and sensory analysis of sardine (*Sardina pilchardus*) stored in ice. J. Sci. Food Agric. 59:37–43. doi:10.1002/jsfa.2740590106

Osborne, C. A., J. P. Lulich, J. W. Bartges, L. K. Unger, R. Thumchai, L. A. Koehler, K. A. Bird, and L. J. Felice. 1995. Canine and feline urolithiasis: Relationship of etiopathogenesis to treatment and prevention. In: C. A. Osborne and D. R. Finco, editors, Canine and feline nephrology and urology. Lea & Febiger, Philadelphia, PA. p. 798–888.

Pires, C. P., F. M. de Oliveira Borges Saad, A. C. Carciofi, and J. P. F. Santos. 2011. Inter-relação entre o balanço cátion-aniônico do alimento e o pH urinário de gatos. (In Portuguese.) Arch. Vet. Sci. 16(3).76-86. doi:10.5380/avs.v16i3.20966

Reddy, M., T. Kamolnick, C. Curry, and D. Skaar. 1994. Energy requirements for the bottlenose dolphin (*Tursiops truncatus*) in relation to sex, age, and reproductive status. Mar. Mamm.: Public Disp. Res. 1:26–31.

Remer, T., and F. Manz. 1995a. Dietary protein as a modulator of the renal net acid excretion capacity: Evidence that an increased protein intake improves the capability of the kidney to excrete ammonium. J. Nutr. Biochem. 6:431–437. doi:10.1016/0955-2863(95)00064-7

Remer, T., and F. Manz. 1995b. Potential renal acid load of foods and its influence on urine pH. J. Am. Diet. Assoc. 95:791–797. doi:10.1016/S0002-8223(95)00219-7

Ridgway, S., and S. Venn-Watson. 2010. Effects of fresh and seawater ingestion on osmoregulation in Atlantic bottlenose dolphins (*Tursiops truncatus*). J. Comp. Physiol. B 180:563–576. doi:10.1007/s00360-009-0439-0

Slifka, K. A., R. S. Wells, A. J. Ardente, and S. Crissey. 2013. Comparative diet analysis of fish species commonly consumed by managed and free-ranging bottlenose dolphins (*Tursiops truncatus*). Int. J. Vet. Med. 10:1. doi:10.1638/2014-0064R1.1

Smith, C. R., S. Venn-Watson, R. S. Wells, S. P. Johnson, N. Maffeo, B. C. Balmer, E. D. Jensen, F. I. Townsend, and K. Sakhaee. 2013. Comparison of nephrolithiasis prevalence in two bottlenose dolphin (*Tursiops truncatus*) populations. Front. Endocrinol. 4:145. doi:10.3389/fendo.2013.00145

Soble, J. J., B. D. Hamilton, and S. B. Streem. 1999. Ammonium acid urate calculi: A reevaluation of risk factors. J. Urol. 161:869–873. doi:10.1016/S0022-5347(01)61794-4

Venn-Watson, S., C. R. Smith, F. Gomez, and E. D. Jensen. 2011. Physiology of aging among healthy, older bottlenose dolphins (*Tursiops truncatus*): Comparisons with aging humans. J. Comp. Physiol. B 181:667–680. doi:10.1007/s00360-011-0549-3

Venn-Watson, S., C. R. Smith, S. Johnson, R. Daniels, and F. Townsend. 2010. Clinical relevance of urate nephrolithiasis in bottlenose dolphins, *Tursiops truncatus.* Dis. Aquat. Organ. 89:167–177. doi:10.3354/dao02187

Vollenweider, J. J., R. A. Heintz, L. Schaufler, and R. Bradshaw. 2011. Seasonal cycles in whole-body proximate composition and energy content of forage fish vary with water depth. Mar. Biol. 158:413–427. doi:10.1007/s00227-010-1569-3

Wells, R. S., K. A. McHugh, D. C. Douglas, S. Shippee, E. Berens McCabe, N. B. Barros, and G. T. Phillips. 2013. Evaluation of potential protective factors against metabolic syndrome in bottlenose dolphins: Feeding and activity patterns of dolphins in Sarasota Bay, FL. Front. Endocrinol. 4:139. doi:10.3389/fendo.2013.00139

Wells, R. S., H. L. Rhinehart, L. J. Hansen, J. C. Sweeney, F. I. Townsend, R. Stone, D. R. Casper, M. D. Scott, A. A. Hohn, and T. K. Rowles. 2004. Bottlenose dolphins as marine ecosystem sentinels: Developing a health monitoring system. EcoHealth 1:246–254. doi:10.1007/s10393-004-0094-6

Werness, P. G., C. M. Brown, L. H. Smith, and B. Finlayson. 1985. EQUIL 2: A basic computer program for the calculation of urinary saturation. J. Urol. 134:1242–1244.

Yamka, R. M., K. G. Friesen, and H. Schakenraad. 2006. The prediction of urine pH using dietary cations and anions in cats fed dry and wet foods. Int. J. Appl. Res. Vet. Med. 4:58–66.

APPENDIX 1. Fish species commonly consumed by free-ranging dolphins and fed to dolphins under human care, the location where fish were caught, the month and year when fish were caught, and the wet weights and lengths of free-ranging fish species caught

Fish and squid species	Catch location	Catch month, year	Wet weight (g)[1]	Length (mm)[1]
Free-ranging diet fish species				
Abundant species				
Pinfish	Sarasota Bay, FL	May-September, 2013	70 (7-174)	143 (68-209)[2]
Striped mullet	Sarasota Bay, FL Roberts Bay, FL Gulf of Mexico, FL	May-September, 2013	615 (195-875)	333 (242-400)[2]
Sheepshead	Sarasota Bay, FL	May-September, 2013	310 (165-560)	236 (188-294)[2]
Ladyfish	Sarasota Bay, FL Gulf of Mexico, FL	May-September, 2013	285 (134-919)	339 (253-600)[2]
Soniforous species				
Pigfish	Sarasota Bay, FL	May-September, 2013	65 (3-171)	143 (65-220)[2]
Spot croaker	Sarasota Bay, FL Gulf of Mexico, FL	May-September, 2013	200 (132-310)	224 (202-260)[2]
Spotted sea trout	Sarasota Bay, FL Gulf of Mexico, FL	May-September, 2013	293 (40-670)	313 (158-440)[3]
Gulf toadfish	Sarasota Bay, FL Gulf of Mexico, FL	May-September, 2013	119 (8-520)	157 (85-300)[3]
Managed diet fish and squid species				
Pacific herring	Pacific coast, USA	December, 2013		
Atlantic herring	East coast, USA	November, 2013		
Icelandic capelin	Iceland	March, 2014		
Canadian capelin	East coast, Canada	June, 2014		
Pacific mackerel	Pacific coast, USA	April, 2014		
Pacific sardine	Pacific coast, USA	October, 2013		
West coast Loligo squid	Pacific coast, USA	October, 2013		

[1]Values are medians with ranges in parentheses.

[2]Fork length measured from most anterior point of head to the deepest notch in tail fin. Measurements not performed on managed diet species.

[3]Straight length measured from most anterior point of head to most caudal point of tail fin. Measurements not performed on managed diet species.