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ORIGINAL PAPER

Effects of fresh and seawater ingestion on osmoregulation in Atlantic bottlenose dolphins (*Tursiops truncatus*)

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Abstract Bottlenose dolphins (*Tursiops truncatus*) are marine mammals with body water needs challenged by little access to fresh water and constant exposure to salt water. Osmoregulation has been studied in marine mammals for a century. Research assessing the effects of ingested fresh water or seawater in dolphins, however, has been limited to few animals and sampling times. Nine 16- to 25-h studies were conducted on eight adult dolphins to assess the hourly impact of fresh water, seawater, and seawater with protein ingestion on plasma and urine osmolality, urine flow rate (ufr), urinary and plasma solute concentrations, and solute clearance rates. Fresh water ingestion increased ufr. Fresh water ingestion also decreased plasma and urine osmolality, sodium and chloride urine concentrations, and solute excretion rates. Seawater ingestion resulted in increased ufr, sodium, chloride, and potassium urine concentrations, sodium excretion rates, and urine osmolality. Seawater with protein ingestion was associated with increased ufr, plasma osmolality, sodium excretion, and sodium, chloride, potassium, and urea urine concentrations. In conclusion, bottlenose dolphins appear to maintain water and plasma solute balance after ingesting fresh water or seawater by altering urine osmolality and solute clearance. Ingestion of protein with seawater appears to further push osmoregulation limits and urine solute concentrations in dolphins.

Keywords Dolphin · Marine mammal · Osmoregulation · Fresh water · Seawater

Abbreviations
HPTR High protein test ration
MMP Navy Marine Mammal Program

Introduction

Bottlenose dolphins (*Tursiops truncatus*) are secondary marine inhabitants with ancestors that lived in terrestrial environments (Shimamura et al. 1997; Thewissen and Madar 1999). As such, adaptations that dolphins and other marine mammals have developed to live in saltwater with little to no access to fresh water have been of interest to renal physiologists for a century. Marine mammals have been characterized as good osmoregulators, and bottlenose dolphin urine osmolality and urine to plasma osmolality ratio have been previously reported as 1,815 mOsm/kg and 5.3, respectively (Ortiz 2001; Malvin and Rayner 1968). The water concentrating capability of dolphins and other cetaceans appears to be better than humans and their close ancestral relatives, cattle, but not as effective as other mammals, including the domestic cat and hopping mouse (Vander 1995; Birukawa et al. 2005; Schmidt-Nielsen 1990).

Reports from the early twentieth century initially indicated that marine mammals did not ordinarily drink seawater (Fetcher 1939). More recent literature, however, has demonstrated that some marine mammals ingest small

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amounts of seawater, including Weddell seal pups, harp seals, and bottlenose dolphins (Tedman and Green 2009; How and Nordøy 2007; Hui 1981; Telfer et al. 1970). Sea otters routinely drink seawater (Costa 1982), and ingestion of both fresh and seawater has been reported in captive harp seals (Gales and Renouf 1993; Renouf et al. 1990). Coastal bottlenose dolphins may swim into fresh water rivers (Caldwell and Caldwell 1972).

While fresh water ingestion may be a rare concern for most wild dolphins, managed collections of dolphins may be more likely to be exposed to fresh water sources. Further, fresh water may be provided intentionally to dolphins for therapeutic purposes, including management of renal disease or response to suspected dehydration.

Limited studies have assessed the impact of fresh water, seawater, and protein ingestion on cetacean osmoregulation. After feeding tap water to an adult bottlenose dolphin, urine osmolality appeared to remain at a steady state for 2 h following ingestion, and diuresis did not occur (Malvin and Rayner 1968). Hyperosmotic fluid infusion via stomach tube, however, did demonstrate diuresis in bottlenose dolphins (Fetcher and Fetcher 1942). Estimated glomerular filtration rate was higher in dolphins that were recently fed compared to those that were fasted overnight (Venn-Watson et al. 2008), and Ortiz et al. (2009) recently reported no significant effect of feeding on plasma osmolality. In the Ortiz et al. study (2009), there were also no significant post-feeding changes in plasma sodium concentrations. Based upon dramatically higher plasma and urine urea levels in cetaceans compared to cattle, it has been hypothesized that cetacean osmoregulation mechanisms may be strongly associated with diet (Birukawa et al. 2005).

Extensive research has been conducted on seals and sea lions to characterize marine mammal osmoregulatory responses to fresh water and seawater challenges. Increased urine flow rate and decreased urine osmolality have been reported in seals and sea lions challenged with fresh water infusions, and increased flow rate and increased sodium and chloride excretion have been reported following hypertonic saline ingestion or infusions (Albrecht 1950; Bradley et al. 1954; Tarasoff and Toews 1972; Hong et al. 1982; Skog and Folkow 1994; Storeheier and Nordøy 2001; Ortiz et al. 2002). Both fresh and seawater infusions increased urine volume in harbor seals (Tarasoff and Toews 1972), and infusion of hypertonic saline in Northern elephant seals resulted in increased glomerular filtration rate and urine output (Ortiz et al. 2002, 2003).

To better assess the impact of fresh water, seawater, and seawater with protein ingestion on osmoregulation in dolphins, nine 16- to 25-h urine and blood collection studies were conducted on eight adult bottlenose dolphins. As demonstrated in other marine mammals, we hypothesized that ingestion of fresh water would increase urine flow rate and decrease urine osmolality and solute excretion; ingestion of seawater would increase flow rate, urine osmolality, and solute excretion; and addition of protein with seawater may have an additive impact on osmoregulation, including diuresis.

Materials and methods

The Navy Marine Mammal Program (MMP) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and adheres to the national standards of the United States Public Health Service Policy on the Humane Care and Use of Laboratory Animals and the Animal Welfare Act. As required by the Department of Defense, the MMP's animal care and use program is routinely reviewed by an Institutional Animal Care and Use Committee and the Navy Bureau of Medicine.

Experimental study

Eight dolphins employed in the study were all adult *Tursiops truncatus* that were healthy at the start of the study as determined by behavior, appetite, and clinical blood values (Ridgway et al. 1970). All animals were fasted 12 h overnight before the start of each study. The animals were taken from their seawater habitat in a fleece-lined transport sling (Fig. 1). They were placed on their sides on a soft rubber pad. An initial blood sample was taken from the central vessels of the fluke (Ridgway 1965). A standard 8–14 Fr clinical urinary catheter with inflatable cuff was inserted through the urethra into the urinary bladder. The cuff was inflated with 15–20 mL of sterile normal saline to retain the catheter in the bladder throughout the study. An initial

Fig. 1 The dolphin is lifted in a fleece-lined sling in preparation for the experiment

urine sample was taken and the collection end of the catheter was pulled through a small central hole in the transport sling and connected to a length of tubing. The dolphin was then lifted into a transport container that had been partially filled with seawater (34 parts per thousand salinity) from the dolphin's home pool. The level of seawater in the container was sufficient to keep the water surface just above the level of the dolphin's eyes (Fig. 2). During the experiment, seawater was poured or sponged over the dorsal portion of the animal to prevent drying. The catheter tubing was connected through a port in the dolphin transport container and inserted into a urine collection vessel.

Sample collection

Blood and urine sample collections were conducted every hour over a desired 24–25 h. In some cases, issues related to catheter placement or animal comfort did not enable studies to persist for the full 24–25 h; the shortest studies ($n = 3$) lasted 16–17 h. A description of the duration and challenge of each study is provided (Table 1). For blood collection, the dolphin's flukes were carefully lifted free of the water for obtaining blood samples of approximately 20 mL using a sterile 20 gauge disposable hypodermic needle. At hourly intervals, the urine collection vessel was emptied, measured, and an aliquot saved in a standard urinalysis vial.

Blood and urine diagnostics

Study plasma and urine variables included urea (mg/dl), sodium (mEq/L), chloride (mEq/L), potassium (mEq/L), and osmolality (mOsm/kg). Plasma and urine glucose (mEq/L) were measured during the fasting studies and the study involving seawater with protein ingestion. Specimens were stored at 4°C. Periodically, plasma was collected from each heparinized blood tube after cells had settled. Plasma and urine were transported to a clinical laboratory experienced with dolphin specimens (Bioscience Laboratories, Van Nuys, California) for analysis. The following methodologies were employed: plasma urea, Autoanalyzer (Technicon SMA-12, Technicon Corp. Ardaley, NY); urine urea, modified urease and Berthelot method; sodium and potassium, flame photometry (Instrumentation Laboratory, Inc., Waterman, MS.); chloride, Buchler–Cotlove chloridometer (Buchler Instruments, Inc., Fort Lee, NJ). Urine and plasma osmolality were measured by freezing point

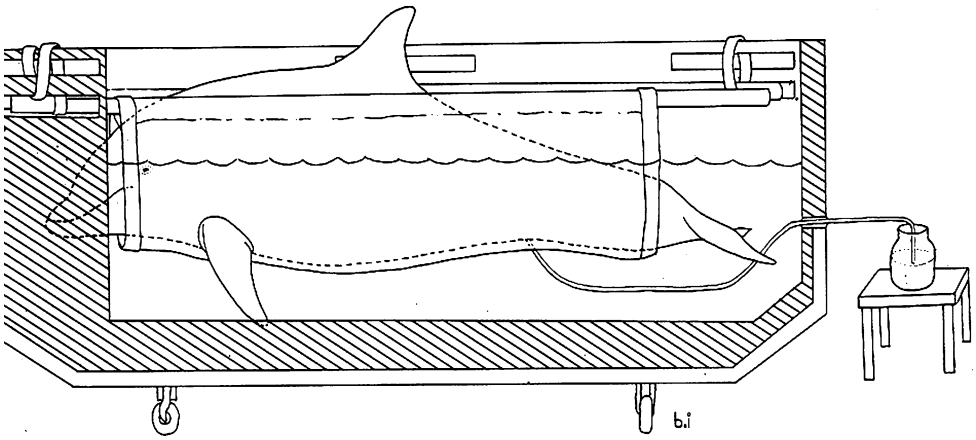

Fig. 2 During urine and blood collection, the dolphin rests in a container of sea water as depicted here

Table 1 Descriptions of nine studies among eight bottlenose dolphins (*Tursiops truncatus*)

Study number	Study type	Total hours	Study date	Animal ID	Plasma samples (#)	Urine samples (#)
1	Fasting	16	01 May	A	14	13
2	Fasting	24	25 November	B	16	15
3	Fresh water (2 L)	25	20 March	C	5	25
4	Fresh water (4 L)	25	20 March	D	5	25
5	Fresh water (4 L)	21	04 April	B	2	21
6	Sea water (3 L)	24	26 March	E	10	24
7	Sea water (2 L) + mackerel (10.5 lb)	22	26 March	F	9	20
8	Sea water + HPTR (4 L)	16	01 May	G	13	13
9	Sea water + HPTR (4 L)	17	01 May	H	13	16

depression with a Fiske osmometer (Advanced Instruments Inc., Norwood, MA).

Clearance rates, excretion rates, and urine to plasma osmolality ratios

Clearance and filtration rates were calculated using the following equations: hourly urine flow rate (mL/min) = (total mL urine/h)/60. Clearance rates for sodium, chloride, potassium and urea (mL/min) = [urine concentration of substance (mEq/L) × urine flow rate (mL/min)]/plasma concentration of substance (mEq/L). Hourly excretion rates of solutes were calculated as mEq/min = [total urinary solute (mEq)/60]. Urine to plasma osmolality ratio = urine osmolality/plasma osmolality.

Feeding trials

A total of nine feeding trials were conducted via stomach tube with eight adult bottlenose dolphins (Table 1). One dolphin (Animal B) participated in two feeding trials; these trials were conducted several months apart. The five feeding trial categories were fasting (last 16–23 h of a 36 h fasting period); 2–4 L deionized water; 3 L seawater; 2 L seawater with 4.8 kg mackerel; and 4 L seawater with 4 kg high protein test ration (HPTR). The HPTR was made in our laboratory and had been successfully fed to dolphins and sea lions (Van Dyke 1972). Each kilogram of HPTR consisted of the following ingredients: fish protein concentrate (100 g), Fish meal (100 g), gelatin (40 g), lard (65 g), cottonseed oil (15 g), sodium alginate (9 g), sodium tripolyphosphate (3 g), and 668 g water. Total protein, 17.6%, 68% water, carbohydrate content was negligible. By comparison mackerel contained 18.2% protein, 75% water and also negligible carbohydrate.

Statistics

Data were analyzed using SAS® software (Release 9.2; SAS Institute, Inc., Cary, NC). Mean and standard error of the mean (SEM) values were determined for urine flow rates, plasma and urine osmolalities, urine to plasma osmolality ratios, and urine concentrations, urine clearance rates, and serum levels for sodium, chloride, potassium, and urea. Within each study group, changes in urine and plasma values over time were assessed by comparing mean values among the following time categories: 0–5 h, >5–10 h, >10–15 h, and >15 h. An one-way analysis of variance with a general linear model was used with post hoc comparisons among each of the time categories (PROC GLM; CLASS TIMECATEGORY; MODEL [serum and urine variables] = TIMECATEGORY; MEANS TIMECATEGORY/SCHEFFE; BY STUDY_TYPE).

Results

Descriptive analyses

Maximum values recorded for urine flow rate, plasma osmolality, urine osmolality, and urine to plasma osmolality ratios were 8.2 mL/min, 378, 2,658 mOsm/kg, and 7.3, respectively. The maximum urine concentrations of sodium, chloride, potassium, and urea were 1,706, 2,222, 351, and 110 mEq/L, respectively. Maximum clearance rates for sodium, chloride, potassium, and urea were 24.1, 42.7, 292, and 200 mEq/min, respectively. Minimum and maximum sodium, chloride, potassium, and urea plasma levels were 141–168, 102–127, 2.8–4.9, and 27.5–65 mEq/L, respectively. In-house serum reference ranges for these variables are 152–158, 115–125, 3.4–4.1, and 36–59 mEq/L; it is important to note that these reference ranges are for serum, not plasma, and were established from routine samples submitted to a different reference laboratory using different equipment than that used in the present study.

Group comparisons and fasting

A comparison of mean ± SEM plasma and urine values among the five study groups is provided in Table 2. While these comparisons are interesting, interpretation is limited by the fact that only one to two animals were used for each study, and inter-animal variation could not be controlled. Comparisons of measured blood and urine values during the last 24 h of 36 h of fasting are provided in Table 3.

Fresh water ingestion

After ingestion of 2–4 L of fresh water, urine flow rate was significantly higher during the first 15 h after ingestion (2.5–2.9 mL/min) compared to more than 15 h after ingestion (1.6 ± 0.1 mL/min; $P = 0.0009$) (Table 4). Plasma osmolality was lowest 15 or more hours after ingestion (319 ± 4 mOsm/kg) compared to the first 5 h (341 ± 5 mOsm/kg) ($P = 0.01$), and decreases in urine osmolality were detected at 6–10 h as well as more than 15 h after ingestion (736 ± 32 and 747 ± 41 mOsm/kg, respectively; $P = 0.008$). No changes in the urine to plasma osmolality ratio were noted during 24 h post ingestion ($P = 0.21$).

There were no statistically significant changes in plasma sodium levels during 24 h post ingestion ($P = 0.3$). Plasma chloride was significantly lower 15 h or more post ingestion compared to the first 5 h (119 ± 2 and 106 ± 1 mEq/L, respectively; $P = 0.003$). At greater than 5 h post ingestion, both sodium and chloride mean plasma levels went below the normal reference ranges for this population (normal low limit of sodium = 152 mEq/L, normal low limit of chloride = 115 mEq/L, Venn-Watson et al. 2007).

Table 2 Comparisons of mean values ± SEM for selected blood and urine values of hourly samples from bottlenose dolphins (*Tursiops truncatus*) by study group (9 studies, 8 animals)

Variable	Mean value ± SEM				
	Fasted	Deionized water	Seawater	Mackerel + seawater	HPTR + seawater
Urine flow rate (mL/min)	0.8 ± 0.1	2.3 ± 0.2	1.8 ± 0.4	2.1 ± 0.4	3.1 ± 0.2
Plasma osmolality (mOsm/kg)	335 ± 2	328 ± 4	336 ± 2	346 ± 7	338 ± 2
Urine osmolality (mOsm/kg)	1,295 ± 53	813 ± 30	1,794 ± 48	1,670 ± 37	2,129 ± 50
Urine:plasma osmolality ratio	3.8 ± 0.2	2.8 ± 0.3	5.0 ± 0.3	5.0 ± 0.2	6.2 ± 0.1
Sodium					
Urine concentration (mEq/L)	44 ± 6	28 ± 8	370 ± 87	258 ± 78	710 ± 63
Plasma (mEq/L)	151 ± 1	150 ± 2	160 ± 1	161 ± 2	153 ± 1
Chloride					
Urine concentration (mEq/L)	61 ± 6	66 ± 11	567 ± 114	323 ± 77	915 ± 83
Plasma (mEq/L)	110 ± 1	113 ± 2	112 ± 1	112 ± 3	116 ± 1
Potassium					
Urine concentration (mEq/L)	34 ± 7	44 ± 6	52 ± 8	107 ± 14	213 ± 14
Plasma (mEq/L)	3.8 ± 0.03	3.5 ± 0.1	3.5 ± 0.2	3.7 ± 0.1	3.7 ± 0.1
Urea					
Urine concentration (mEq/L)	14 ± 2	18 ± 2	ND	35 ± 7	58 ± 5
Plasma (mEq/L)	43 ± 1	40 ± 3	44 ± 4	45 ± 2	39 ± 1
Glucose					
Urine concentration (mEq/L)	184 ± 22				12,382 ± 3,612
Plasma (mEq/L)	133 ± 2				170 ± 6

Prot-sea, 4L seawater with 4 kg HPTR; mack sea, 10.5 lb Spanish mackerel and 2 L seawater; fresh, 2–4 L deinonized water; sea, 3 L seawater; fast, last 16–23 h of a 36 h fast; ND, not determined or *P* value could not be calculated due to small numbers

Urine sodium and chloride concentrations decreased after 15 h post ingestion compared to the first 5 h (urine sodium 8 ± 2 vs. 83 ± 34 mEq/L, urine chloride 33 ± 6 vs. 140 ± 44 mEq/L; $P = 0.003$). Sodium and chloride excretion rates were higher during the first 5 h post ingestion compared to greater than 5–24 h (sodium excretion = 1.6 ± 0.3 vs. 0.6 ± 0.1, chloride excretion = 2.5 ± 0.4 vs. 1.0 ± 0.1; $P = 0.0001$).

Urine potassium concentration was lower 15 h or more post ingestion compared to the first 5 h (27 ± 5 and 74 ± 20 mEq/L, respectively; $P = 0.02$). There were no significant differences in potassium plasma levels or excretion rate during 24 h post ingestion. There were no differences in plasma or urine urea concentrations or urea excretion rate during 24 h post ingestion.

Sea water ingestion

After ingestion of 3 L of sea water, urine flow rate was higher during the first 5 h after ingestion (4.8 ± 0.9 mL/min) compared to more than 5 h after ingestion (0.8–1.6 mL/min; $P \leq 0.0001$) (Table 5). There were no changes in plasma osmolality during 24 h post ingestion ($P = 0.07$). Urine osmolality was higher 5 h or more post ingestion compared to the first 5 h (1,826–1,931 vs. 1,391 ± 76 mOsm/kg; $P \leq 0.0001$). Urine to plasma osmolality ratio was higher 15 h or more post ingestion compared to the first 5 h (5.8 ± 0.1 and 4.1 ± 0.3, respectively; $P = 0.005$).

There were no changes in plasma sodium or chloride levels during 24 h post ingestion ($P = 0.27$ and 0.16, respectively). Plasma sodium did exceed normal high levels, however, during the first 10 h post ingestion (normal high limit of sodium 159 mEq/L, Venn-Watson et al. 2007).

Urine sodium and chloride concentrations were highest during the first 5 h post ingestion compared to greater than 5 h (urine sodium 1,043 ± 232 vs. 108–359 mEq/L, urine chloride 1,419 ± 318 vs. 225–574 mEq/L; $P \leq 0.0001$). Sodium excretion rates were higher during the first 15 h post ingestion compared to more than 15 h (4.9–6.2 vs. 3.8 ± 0.2). Chloride excretion was higher during 6–10 h post ingestion compared to greater than 15 h (9.8 ± 0.2 and 7.9 ± 0.4, respectively; $P = 0.01$).

Urine potassium concentration was highest during the first 5 h post ingestion compared to more than 5 h (121 ± 15 vs. 26–46 mEq/L; $P < 0.0001$) (Table 4). Potassium excretion was highest 10 h or more post ingestion compared to the first 5 h (0.9–1.0 vs. 0.7 ± 0.05, $P = 0.0003$). There were no significant differences in potassium

Table 3 Comparisons of mean selected blood and urine values, by time after feeding challenge, in two bottlenose dolphins (*Tursiops truncatus*)

Variable	Fasting, mean value ± SEM				*P* value	Significant comparisons among time groups
	Time 1 >0–5 h (*n* = 8)	Time 2 >5–10 h (*n* = 10)	Time 3 >10–15 h (*n* = 10)	Time 4 >15 h (*n* = 9)		
Urine flow rate (mL/min)	0.8 ± 0.1	0.6 ± 0.1	1.1 ± 0.2	0.8 ± 0.2	0.12	
Plasma osmolality (mOsm/kg)	336 ± 4	333 ± 3	334 ± 3	339 ± 1	0.62	
Urine osmolality (mOsm/kg)	1,471 ± 113	1,100 ± 84	1,290 ± 67	1,390 ± 88	0.04	1 > 2
Urine:plasma osmolality ratio	4.2 ± 0.4	3.1 ± 0.3	3.9 ± 0.2	4.1 ± 0.2	0.11	
Sodium						
Urine concentration (mEq/L)	72 ± 16	19 ± 5	38 ± 5	53 ± 10	0.004	1 > 2
Excretion (mEq/min)	2.2 ± 0.3	0.8 ± 0.1	1.0 ± 0.2	2.3 ± 0.2	<0.0001	4, 1 > 3,2
Plasma (mEq/L)	153 ± 1	150 ± 2	149 ± 2	152 ± 1	0.39	
Chloride						
Urine concentration (mEq/L)	78 ± 13	33 ± 7	72 ± 8	66 ± 14	0.02	1 > 2
Excretion (mEq/min)	2.4 ± 0.2	1.6 ± 0.1	1.7 ± 0.2	2.8 ± 0.2	0.0002	4 > 3,2
Plasma (mEq/L)	109 ± 1	111 ± 1	112 ± 1	108 ± 2	0.13	
Potassium						
Urine concentration (mEq/L)	28 ± 9	20 ± 9	75 ± 20	13 ± 5	0.01	3 > 2,4
Excretion (mEq/min)	1.0 ± 0.3	1.4 ± 0.4	1.4 ± 0.3	0.5 ± 0.2	0.37	
Plasma (mEq/L)	3.8 ± 0.1	3.6 ± 0.1	3.7 ± 0.04	3.9 ± 0.03	0.06	
Urea						
Urine concentration (mEq/L)	13 ± 3	7 ± 1	21 ± 3	16 ± 5	0.02	3 > 2
Excretion (mEq/min)	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.64	
Plasma (mEq/L)	40 ± 0.5	42 ± 1	46 ± 2	43 ± 1	0.01	3 > 1
Glucose						
Urine concentration (mEq/L)	99 ± 23	173 ± 44	271 ± 44	245 ± 26	0.02	3 > 1
Excretion (mEq/min)	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.03	0.02	2 > 1
Plasma (mEq/L)	129 ± 4	142 ± 2	133 ± 2	127 ± 4	0.008	2 > 1,4

or urea plasma levels during 24 h post ingestion ($P = 0.3$ and 0.06, respectively). Urea excretion was higher more than 5 h after ingestion compared to the first 5 h (0.03–0.04 vs. 0.02 ± 0.005, $P < 0.0001$).

Sea water and mackerel ingestion

After ingestion of 2 L of sea water and 10.5 lb of mackerel, urine flow rate was higher during the first 5 h after ingestion (5.0 ± 0.5 mL/min) compared to more than 5 h after ingestion (0.8–1.6 mL/min; $P \leq 0.0001$) (Table 6). Plasma osmolality was highest during the first 5 h post ingestion (358 ± 2 mOsm/kg), followed by 6–10 h (337 mOsm/kg); the lowest plasma osmolality occurred after 15 h post ingestion (324 ± 0.3 mOsm/kg, $P = 0.0003$). There were no changes in urine osmolality or urine to plasma osmolality ratio during 22 h post ingestion ($P = 0.09$ and 0.57, respectively).

Plasma sodium levels exceeded normal high levels (normal high limit of sodium = 159 mEq/L) during the first 10 h post ingestion and were highest during the first 5 h compared to greater than 5 h (166 ± 0.6 and 159 ± 2 mEq/L, respectively; $P = 0.05$). There were no changes in plasma chloride levels during 22 h post ingestion ($P = 0.44$).

Urine sodium and chloride concentrations were highest during the first 5 h post ingestion compared to greater than 5 h (urine sodium 798 ± 107 vs. 41–158 mEq/L, urine chloride 823 ± 132 vs. 108–254 mEq/L; $P \leq 0.0001$). Sodium excretion rates were highest during the first 5 h post ingestion (4.4 ± 0.4), followed by 6–10 h post ingestion (2.4 ± 0.4), compared to more than 10 h (1.2 ± 0.1) ($P < 0.0001$). There were no differences in chloride excretion during 22 h post ingestion ($P = 0.08$).

Urine potassium and urea concentrations were highest during the first 5 h post ingestion compared to more than 5 h (urine potassium = 198 ± 22 vs. 68–84 mEq/L, urine urea = 80 ± 7 vs. 12–31 mEq/L; $P < 0.0001$). Plasma potassium was lowest after 15 h post ingestion (3.5 ± 0.1 mEq/L, $P = 0.02$). Potassium excretion was highest during 10–15 h post ingestion (2.9 ± 0.03 vs. 01.1–1.9, $P \leq 0.0001$). There were no differences in plasma urea during 22 h post ingestion ($P = 0.6$).

Table 4 Comparisons of mean selected blood and urine values, by time after feeding challenge, in two bottlenose dolphins (*Tursiops truncatus*)

Variable	Fresh water, mean value ± SEM				*P* value	Significant comparisons among time groups
	Time 1 >0–5 h (*n* = 10)	Time 2 >5–10 h (*n* = 10)	Time 3 >10–15 h (*n* = 10)	Time 4 >15 h (*n* = 16)		
Urine flow rate (mL/min)	2.9 ± 0.6	2.7 ± 0.4	2.5 ± 0.2	1.6 ± 0.1	0.009	1 > 4
Plasma osmolality (mOsm/kg)	341 ± 5	320 ± 2		319 ± 4	0.01	1 > 4
Urine osmolality (mOsm/kg)	996 ± 86	736 ± 32	820 ± 84	747 ± 41	0.008	1 > 2,4
Urine:plasma osmolality ratio	3.4 ± 0.5	2.1 ± 0.5		2.6 ± 0.3	0.21	
Sodium						
Urine concentration (mEq/L)	83 ± 34	19 ± 5	17 ± 5	8 ± 2	0.003	1 > 3,4
Excretion (mEq/min)	1.6 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.0001	1 > 2,3,4
Plasma (mEq/L)	153 ± 3	144 ± 2		150 ± 3	0.30	
Chloride						
Urine concentration (mEq/L)	140 ± 44	63 ± 14	54 ± 11	33 ± 6	0.003	1 > 4
Excretion (mEq/min)	2.5 ± 0.4	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	<0.0001	1 > 2,3,4
Plasma (mEq/L)	119 ± 2	113 ± 4		106 ± 1	0.003	1 > 4
Potassium						
Urine concentration (mEq/L)	74 ± 20	46 ± 10	40 ± 8	27 ± 5	0.02	1 > 4
Excretion (mEq/min)	1.3 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	0.69	
Plasma (mEq/L)	3.7 ± 0.3	3.2 ± 0.4		3.4 ± 0.2	0.47	
Urea						
Urine concentration (mEq/L)	29 ± 9	20 ± 4	20 ± 3	11 ± 2	0.09	
Excretion (mEq/min)	0.03 ± 0.004	0.02 ± 0.003	0.03 ± 0.004	0.02 ± 0.003	0.26	
Plasma (mEq/L)	42 ± 2	39 ± 1		38 ± 7	0.85	

Sea water and HPTR ingestion

After ingestion of 4 L of sea water and a high protein test ration (HPTR), urine flow rate was higher during the first 10 h after ingestion (3.2–3.9 mL/min) compared to more than 15 h after ingestion (1.4 ± 0.2 mL/min; $P = 0.001$) (Table 7). Plasma osmolality was higher during the first 5 h post ingestion (346 ± 5 mOsm/kg), compared to more than 10 h post ingestion (328 mOsm/kg, $P = 0.0003$). Urine osmolality was higher during greater than 5–10 h post ingestion (2,328 ± 62 mOsm/kg) compared to more than 15 h post ingestion (1,789 ± 34 mOsm/kg). There were no changes in urine to plasma osmolarity ratio during 16–17 h post ingestion ($P = 0.11$).

Plasma sodium did not change during 16–17 h post ingestion ($P = 0.09$), and plasma chloride was higher during 5–10 h post ingestion compared to greater than 10 h (121 ± 1 vs. 112–114 mEq/L, $P = 0.002$). Urine sodium concentrations were highest during the first 10 h post ingestion compared to greater than 15 h (763–950 vs. 177 ± 34 mEq/L; $P = 0.001$). Urine chloride levels were higher during 5–10 h post ingestion compared to greater than 15 h (1,237 ± 106 vs. 275 ± 50 mEq/L, $P = 0.005$). Sodium and chloride excretion rates were higher during the first 15 h post ingestion compared to more than 15 h (sodium excretion = 5.8–6.7 vs. 3.6 ± 0.3, $P = 0.0001$; chloride excretion = 7.8–8.7 vs. 5.5 ± 0.2, $P = 0.003$).

Urine potassium and urea concentrations were highest during 5–10 h post ingestion compared to more than 15 h (urine potassium = 272 ± 20 vs. 110 ± 27 mEq/L, $P = 0.001$; urine urea = 75 ± 5 vs. 19 ± 3 mEq/L; $P = 0.03$). There were no differences in plasma potassium or potassium or urea excretion during 16–17 h post ingestion ($P = 0.94$, 0.88, and 0.27, respectively).

Clearance rates

Clearance rates for sodium, chloride, potassium, and urea are provided in Table 8. Clearance rates for all solutes appeared lowest during fasting and highest after ingestion of seawater and HPTR.

Discussion

In our study, bottlenose dolphins demonstrated the ability to dilute and concentrate urine from 225 to 2,658 mOsm/kg, respectively. The upper limit identified in our study was higher than that previously reported in a study involving two bottlenose dolphins fed 25 lb raw fish (range 1,322–1,815

Table 5 Comparisons of mean selected blood and urine values, by time after feeding challenge, in two bottlenose dolphins (*Tursiops truncatus*)

Variable	Sea water, mean value ± SEM				*P* value	Significant comparisons among time groups
	Time 1 >0–5 h (*n* = 5)	Time 2 >5–10 h (*n* = 5)	Time 3 >10–15 h (*n* = 5)	Time 4 >15 h (*n* = 9)		
Urine flow rate (mL/min)	4.8 ± 0.9	1.6 ± 0.2	1.0 ± 0.04	0.8 ± 0.04	<0.0001	1 > 2,3,4
Plasma osmolality (mOsm/kg)	338 ± 3	342 ± 2		332 ± 1	0.07	
Urine osmolality (mOsm/kg)	1,391 ± 76	1,826 ± 67	1,931 ± 12	1,925 ± 10	<0.0001	1 < 2,3,4
Urine:plasma osmolality ratio	4.1 ± 0.3	5.1 ± 0.4		5.8 ± 0.1	0.005	4 > 1
Sodium						
Urine concentration (mEq/L)	1,043 ± 232	359 ± 49	178 ± 10	108 ± 10	<0.0001	1 > 2,3,4
Excretion (mEq/min)	5.9 ± 0.4	6.2 ± 0.2	4.9 ± 0.1	3.8 ± 0.2	<0.0001	4 < 1,2,3
Plasma (mEq/L)	162 ± 2	160 ± 1		158 ± 1	0.27	
Chloride						
Urine concentration (mEq/L)	1,419 ± 318	574 ± 72	324 ± 12	225 ± 18	<0.0001	1 > 2,3,4
Excretion (mEq/min)	7.9 ± 0.7	9.8 ± 0.2	9.0 ± 0.1	7.9 ± 0.4	0.01	2 > 4
Plasma (mEq/L)	112 ± 1	116 ± 1		110 ± 2	0.16	
Potassium						
Urine concentration (mEq/L)	121 ± 15	46 ± 5	35 ± 2	26 ± 1	<0.0001	1 > 2,3,4
Excretion (mEq/min)	0.7 ± 0.05	0.8 ± 0.04	1.0 ± 0.02	0.9 ± 0.02	0.0003	1 < 3,4
Plasma (mEq/L)	3.9 ± 0.4	3.3 ± 0.1		3.3 ± 0.1	0.30	
Urea						
Urine concentration (mEq/L)						
Excretion (mEq/min)	0.02 ± 0.005	0.03 ± 0.002	0.04 ± 0.001	0.04 ± 0.001	<0.0001	1 < 2,3,4
Plasma (mEq/L)	55 ± 7	40 ± 5		36 ± 2	0.06	

mOsm/kg, Malvin and Rayner 1968). While dolphins in our study demonstrated higher urine concentrations compared to humans (range of 50–1,400 mOsm/kg, Vander 1995), the maximum values recorded in our studies more closely resembled that of camels (2,800 mOsm/kg, Bengoumi et al. 1993) than the extreme osmoregulation capabilities of kangaroo rats (5,500 mOsm/kg, Schmidt-Nielsen 1990) or the Australian hopping mouse (9,400 mOsm/kg, Schmidt-Nielsen 1990). Given the dolphin's evolutionary relationship to ungulates, similarities in osmoregulation between camels and dolphins may not be surprising (Thewissen and Madar 1999). Our study was not designed to confirm, however, true osmoregulation limits of dolphins. As such, any comparisons regarding osmoregulation capabilities of dolphins with other species need to be made with caution.

High urine concentration abilities of the hopping mouse enable it to maintain water balance through dietary and metabolic water without the need to drink fresh water. Although dolphins are exposed to similar challenges, it does not appear that they have acquired the need or the ability to concentrate urine as well as the hopping mouse. Four hypotheses that may negate the need for dolphins to further concentrate urine in the marine environment are (1) the ability to hydrate by drinking seawater (Telfer et al. 1970), (2) selective movement of salt-free water across the skin (Hui 1981), and most likely, (3) efficient generation of metabolic water from fat (Ortiz 2001), and (4) sufficient water content of fish that make up the majority of food that the animals eat (Ortiz 2001).

Plasma osmolalities remained relatively constant (309–387 mOsm/kg) among study dolphins and were comparable to those previously reported in bottlenose dolphins (343 mOsm/kg, Malvin and Rayner 1968; 335 ± 14 mOsm/kg, Ridgway et al. 1970) and other cetaceans (327–353 mOsm/kg, Malvin and Rayner 1968). Dolphin plasma osmolalities were higher than findings in many other mammalian species, including humans (300 mOsm/kg ± 2–3%, Vander 1995) and elephant seals (302 mOsm/kg, Ortiz et al. 1996). Plasma sodium, chloride and potassium levels ranged from 141 to 168, 102 to 127, and 2.8 to 4.9 mEq/L, respectively. These ranges included values that were lower and higher than our in-house normal, healthy reference ranges for sodium, chloride, and potassium (Venn-Watson et al. 2007).

Impact of fresh water ingestion

The goal of osmoregulation during excess fresh water ingestion is to increase water excretion without losing solutes. As such, increased urine flow rate, decreased urine

Table 6 Comparisons of mean selected blood and urine values, by time after feeding challenge, in two bottlenose dolphins (*Tursiops truncatus*)

Variable	Mackerel and sea water, mean value ± SEM				*P* value	Significant comparisons among time groups
	Time 1 > 0–5 h *n* = 5	Time 2 >5–10 h *n* = 5	Time 3 >10–15 h *n* = 5	Time 4 >15 h *n* = 5		
Urine flow rate (mL/min)	5.0 ± 0.5	1.6 ± 0.4	0.8 ± 0.1	1.0 ± 0.3	<0.0001	1 > 2,3,4
Plasma osmolality (mOsm/kg)	358 ± 2	337		324 ± 0.3	0.0003	1 > 2 > 4
Urine osmolality (mOsm/kg)	1,730 ± 107	1,789 ± 71	1,581 ± 21	1,578 ± 35	0.09	
Urine:plasma osmolality ratio	4.8 ± 0.5	5.7		4.9 ± 0.2	0.57	
Sodium						
Urine concentration (mEq/L)	798 ± 107	158 ± 63	35 ± 6	41 ± 11	<0.0001	1 > 2,3,4
Excretion (mEq/min)	4.4 ± 0.4	2.4 ± 0.4	1.2 ± 0.1	1.2 ± 0.1	<0.0001	1 > 2 > 3,4
Plasma (mEq/L)	166 ± 0.6	161		159 ± 2	0.05	1 > 4
Chloride						
Urine concentration (mEq/L)	823 ± 132	254 ± 83	108 ± 16	105 ± 28	<0.0001	1 > 2,3,4
Excretion (mEq/min)	4.7 ± 0.8	4.2 ± 0.3	3.7 ± 0.1	3.0 ± 0.1	0.08	
Plasma (mEq/L)	114 ± 5	108		106 ± 3	0.44	
Potassium						
Urine concentration (mEq/L)	198 ± 22	76 ± 6	84 ± 11	68 ± 18	<0.0001	1 > 2,3,4
Excretion (mEq/min)	1.1 ± 0.1	1.6 ± 0.3	2.9 ± 0.03	1.9 ± 0.1	<0.0001	3 > 4,2 > 1
Plasma (mEq/L)	3.9 ± 0.03	4.0		3.5 ± 0.1	0.02	4 < 1,2
Urea						
Urine concentration (mEq/L)	80 ± 7	31 ± 10	12 ± 2	17 ± 5	<0.0001	1 > 2,3,4
Excretion (mEq/min)	0.04 ± 0.001	0.05 ± 0.002	0.04 ± 0.001	0.05 ± 0.001	0.02	2,4 > 1,3
Plasma (mEq/L)	48 ± 5	46		41 ± 4	0.60	

osmolality, and stable, low urinary solute excretion are expected. Our study demonstrated that, in dolphins that ingested 2–4 L of fresh water, urine flow rates were sustained for at least 15 h, and urine flow rate was highest for the first 5 h. Fresh water ingestion was also associated with relatively low mean plasma and urine osmolality, as well as plasma to urine osmolality.

Similar findings of increased urine flow rate and decreased urine osmolality have been reported in seals and sea lions challenged with fresh water infusions (Albrecht 1950; Bradley et al. 1954; Tarasoff and Toews 1972; Hong et al. 1982; Skog and Folkow 1994; Ortiz et al. 2002). Our results are inconsistent, however, with those previously reported by Malvin and Rayner (1968), in which urine osmolality in one bottlenose dolphin that ingested 4 L of tap water remained at a steady state (approximately 1,700–1,800 mOsm/kg) for 2 h following ingestion, and diuresis did not occur. Reasons for Malvin and Rayner's inconsistencies with our study may include their use of tap versus deionized water, use of only one test subject animal instead of multiple animals, and monitoring animals for 2 h instead of 24 h.

The present study demonstrated a decrease in sodium and chloride excretion 5 h after ingestion of fresh water that was sustained for at least 24 h. Mean sodium and chloride plasma levels reached values below that of in-house normal reference ranges indicating that, even with decreased solute excretion, a fresh water induced diuresis may cause a state of hypochloremia and hyponatremia in dolphins. Concerns regarding the potential for electrolyte imbalances, namely hyponatremia, have been raised for managed collections of pinnipeds housed in fresh water facilities (Geraci 1972). While the potential for lower plasma sodium and chloride may exist with fresh water challenges in bottlenose dolphins, it is interesting to note that hydration therapy involving daily infusion of 1–2 L of fresh water has been used routinely and successfully in our facility for treating renal nephrolithiasis in this species without evidence of electrolyte imbalances. This potential discrepancy may have to do with our population's access to saltwater and a high protein diet, which may positively influence water and solute balance in the face of routine fresh water infusions. Clinical research assessing the impact of long term hydration therapy on dolphins may include monitoring plasma and urine solute concentrations, along with hormonal regulators of sodium excretion or retention.

Impact of seawater ingestion

In most terrestrial mammals, ingestion of seawater leads to an increase in both water and solute excretion with a net

Table 7 Comparisons of mean selected blood and urine values, by time after feeding challenge, in two bottlenose dolphins (*Tursiops truncatus*)

Variable	HPTR and sea water, mean value ± SEM				*P* value	Significant comparisons among time groups
	Time 1 > 0–5 h *n* = 7	Time 2 >5–10 h *n* = 9	Time 3 >10–15 h *n* = 10	Time 4 >15 h *n* = 3		
Urine flow rate (mL/min)	3.2 ± 0.2	3.9 ± 0.3	2.7 ± 0.4	1.4 ± 0.2	0.001	4 < 1,2
Plasma osmolality (mOsm/kg)	346 ± 5	343 ± 3	332 ± 1	328 ± 1	0.003	1 > 3,4
Urine osmolality (mOsm/kg)	2,167 ± 112	2,328 ± 62	2,024 ± 69	1,789 ± 34	0.005	2 > 4
Urine:plasma osmolality ratio	6.3 ± 0.4	6.6 ± 0.2	6.1 ± 0.2	5.4 ± 0.1	0.11	
Sodium						
Urine concentration (mEq/L)	763 ± 57	950 ± 70	617 ± 116	177 ± 34	0.001	4 < 1,2
Excretion (mEq/min)	6.5 ± 0.1	6.7 ± 0.1	5.8 ± 0.4	3.6 ± 0.3	0.0001	4 < 1,2,3
Plasma (mEq/L)	157 ± 2	153 ± 3	150 ± 1	151 ± 0.7	0.09	
Chloride						
Urine concentration (mEq/L)	902 ± 77	1,237 ± 106	827 ± 161	275 ± 50	0.005	2 > 4
Excretion (mEq/min)	7.7 ± 0.2	8.7 ± 0.2	7.8 ± 0.5	5.5 ± 0.2	0.003	4 < 2,3
Plasma (mEq/L)	116 ± 2	121 ± 1	114 ± 1	112 ± 2	0.002	2 > 3,4
Potassium						
Urine concentration (mEq/L)	230 ± 24	272 ± 20	178 ± 19	110 ± 27	0.001	2 > 3,4
Excretion (mEq/min)	2.0 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	2.2 ± 0.4	0.88	
Plasma (mEq/L)	3.6 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.3	0.94	
Urea						
Urine concentration (mEq/L)	57 ± 6	75 ± 5	51 ± 10	19 ± 3	0.03	2 > 4
Excretion (mEq/min)	0.05 ± 0.005	0.05 ± 0.002	0.04 ± 0.002	0.04 ± 0.001	0.27	
Plasma (mEq/L)	42 ± 2	41 ± 1	35 ± 2	39 ± 1	0.009	1 > 3
Glucose						
Urine concentration (mEq/L)	13,894 ± 6,250	27,426 ± 5,330	983 ± 680	245 ± 24	0.003	2 > 3,4
Excretion (mEq/min)	11 ± 5	17 ± 2	0.7 ± 0.4	0.5 ± 0.1	0.002	2 > 3,4
Plasma (mEq/L)	181 ± 6	184 ± 3	156 ± 14	145 ± 8	0.002	2 > 3,4

Table 8 Clearance rates of electrolytes and urea among adult bottlenose dolphins (*Tursiops truncatus*) fasted or fed fresh water, seawater, and/or protein

Study type	Clearance rates (mEq/min)				
	Total hours	Sodium	Chloride	Potassium	Urea
Fasted (last 23 h of a 36 h fast)	23	0.4	0.7	1.9	26.5
Deionized water (2L)	25	1.9	5.6	102.0	138.1
Mackerel + seawater (2L)	20	6.4	11.7	129.7	163.7
Seawater (3L)	24	10.2	21.8	65.6	NM
Seawater + 50/50 HPTR (4L total)	17	14.7	25.6	205.5	200.2

NM not measured

loss in total body water and dehydration (Guyton and Hall 1996). Since the osmolality and electrolyte content of seawater is high, dehydration may be expected to occur in animals that are unable to concentrate urine to at least the same level as seawater.

Our study involving ingestion of 3 L seawater demonstrated marked increases in urine flow rate, urine osmolality (maximum of 1,968 mOsm/kg), and sodium and chloride excretion for 5 h. Hyperosmotic fluid infusion via stomach tube has previously demonstrated diuresis in bottlenose dolphins (Fetcher and Fetcher 1942) and has been associated with increased flow rate and increased sodium and chloride excretion in pinnipeds (Albrecht 1950; Bradley et al. 1954; Tarasoff and Toews 1972; Hong et al. 1982; Skog and Folkow 1994; Storeheier and Nordøy 2001; Ortiz et al. 2002).

While there were no significant changes in plasma osmolality after ingestion of seawater in the present study, animals did have higher plasma sodium levels up to 5 h after seawater ingestion, and there was evidence of animals

reaching a hypernatremic state (>159 mEq/L) within 10 h after ingestion of seawater. Along with the seawater and mackerel challenge, ingestion of seawater alone led to the highest plasma sodium levels among all five study groups. Hyperosmotic fluid challenges among pinnipeds have led to vomiting, diarrhea, and death (Albrecht 1950). While dolphins may have achieved higher plasma sodium levels after ingestion of seawater, animals in our study did not demonstrate outward clinical signs of seawater toxicosis.

The general consensus, based upon results of earlier studies involving infusion of hyperosmotic fluid via stomach tube in dolphins, is that cetaceans do not routinely ingest seawater (Fetcher and Fetcher 1942; Malvin and Rayner 1968). This hypothesis is supported by the lack of routine observation of urine osmolality and urine sodium and chloride concentrations matching or exceeding that of seawater (Ortiz 2001). In our study, dolphins were able to achieve urine osmolality above that of seawater, and mean urinary concentrations of sodium and chloride reached after ingestion of seawater were 370 and 567 mEq/L, respectively. Despite this response to seawater ingestion, sodium urine concentrations in the seawater ingestion study did not match that found in seawater (470 and 548 mEq/L, respectively), supporting earlier evidence that bottlenose dolphins may not routinely ingest large boluses of seawater. Interestingly, mean sodium and chloride urine concentrations following ingestion of HPTR and seawater led to mean urine concentrations of 710 and 915 mEq/L, respectively, in our study indicating that the dolphin is capable of concentrating sodium and chloride beyond levels found in seawater.

Ingestion of seawater by dolphins during the fasting state has been reported [ranging between 4.5 and 13 mL/(kg day)] (Telfer et al. 1970; Hui 1981). Further, mean postmortem urine osmolality among 286 fin whales (*Balaenoptera physalus*) was higher than seawater (>2,400 mOsm/L), demonstrating that at least some cetaceans can have consistently high urine concentrations (Kjeld 2001). One hypothesis that may resolve this discrepancy may be that dolphins ingest small amounts of seawater over time while ingesting high protein which does not elicit the marked response found after a single, large dose of 3 L. This hypothesis would make sense, given that wild dolphins likely routinely ingest saltwater incidentally when catching fish.

Our seawater ingestion studies demonstrate that marine mammals can tolerate disruption of ionic and osmotic homeostasis and restore balance relatively quickly, within 5 h. In comparison, sodium-infused dogs may take 3 days to recover (Bie 1977), and humans with water intoxication may take 24 h to restore plasma osmolality (Irving et al. 1991). The ability for marine mammals to rapidly restore osmotic balance likely evolved to enable their survival in a hyperosmotic environment. The consistent and rapid response of increased urine flow rates in all of our challenge studies indicate that GFR is an important means for dolphins to maintain osmotic balance. Further research is warranted to explain the mechanism of increased GFR following fresh and seawater ingestion among marine mammals.

Impact of seawater with protein

Perhaps the most interesting of all the groups in the present study were those involving seawater with protein. Similar to our findings with seawater ingestion, ingestion of 3 L of seawater with 10.5 lb of mackerel led to higher plasma sodium levels, urine flow rates, sodium excretion rates, and sodium and chloride urinary concentrations during the first 5 h post ingestion. Unlike seawater alone, addition of mackerel was associated with the highest plasma osmolality achieved among all five study groups, and high plasma osmolality lasted 10 h. Concurrent to high plasma osmolality was a high sodium excretion rate also lasting 10 h. Further, unlike seawater alone, addition of mackerel was associated with highest potassium and urea urine concentrations during the first 5 h after ingestion.

Pinnipeds that ingest high protein diets have demonstrated increased glomerular filtration rates after eating (Hiatt and Hiatt 1942). Positive associations between feeding and diuresis have also been reported among cetaceans, including bottlenose dolphins (Malvin and Rayner 1968; Venn-Watson et al. 2008). The cause of feeding-associated diuresis in dolphins and other marine mammals appears to be driven by nitrogen loading in the kidney (Hiatt and Hiatt 1942). This association is also found in terrestrial animals; in fact, immediate weight loss with ingestion of high protein, low carbohydrate diets is mainly attributed to a diet-driven diuresis in humans (Denke 2001).

Similar to our findings in dolphins, ingestion of fish among harbor seals was associated with increases in sodium, potassium, and urea urine concentrations (Schmidt-Nielsen et al. 1959). High protein diets can enhance renal concentrating abilities in rats (Hendrikx and Epstein 1958) and have been associated with increased urine osmolality independent of urine volume (Bouby et al. 1991). Protein-associated changes in renal function, including increased urine osmolality and hyperfiltration lasting up to 10 h post ingestion, appear to be partially attributed to alterations in vasoactive hormones, including plasma renin activity and aldosterone (Daniels and Hostetter 1990; Rosenberg et al. 1987). Given the confirmed presence of renin in bottlenose dolphins (Eichelberger et al. 1940; Malvin and Vander 1967; Malvin et al. 1978; Ortiz and Worthy 2000), it is likely that a similar association between high protein and high plasma renin exists in cetaceans. Interestingly, a recent study by Ortiz et al. (2009) did not

demonstrate significant changes in plasma osmolality in post-fed dolphins; this difference from our findings may be due to the combined effect of seawater with protein on plasma osmolality or differences in the dose or type of fish and protein fed.

As with the seawater alone study group, animals in the present study that ingested seawater with mackerel had high plasma sodium levels suggestive of hypernatremia for the first 5 h. Given the structure of this study, it was not possible to determine how much of an impact protein had on plasma sodium; there is a need to repeat urine and plasma studies on dolphins that ingested protein without seawater. Unlike the findings in our study, previously reported post-feeding dolphins did not exhibit significant changes in plasma sodium after 5, 11 or 24 h (Ortiz et al. 2009).

In animals that ingested a HPTR with seawater, a high mean flow rate lasted 10 h and remained at levels above the fasting state throughout the study. While plasma osmolality was highest during the first 5 h post infusion, urine osmolality and urine concentrations of sodium, chloride, potassium, and urea reached the highest levels among all study treatments at 5–10 h, maintaining a mean urine osmolality above 2,000 mOsm/L and high sodium and chloride excretion rates for 15 h. Interestingly, despite large shifts in osmoregulation after ingesting HPRT with seawater, animals did not become hypernatremic or hyperchloremic. These results indicate that high doses of protein may extend the duration of diuresis and higher urinary concentration of all measured solutes. The physiological response causing this protein-induced change peaked at 5–10 h. Our interpreted role of protein in osmoregulation has been reported previously by Birukawa et al. (2005), who demonstrated that cetaceans had significantly higher plasma and urine urea concentrations compared to cattle and concluded that cetacean osmoregulation may be driven by diet more than its terrestrial relatives.

There was a marked increase in mean glucose urine and plasma concentrations (2,743 and 1,048 mEq/L, respectively) as well as glucose excretion, after dolphins ingested HPRT with seawater compared to fasted dolphins (urine = 17 mEq/L, plasma = 142 mEq/L and excretion = 0.6); the greatest increases of these variables, similar to urine osmolality and solute concentrations, occurred at 5–10 h post infusion.

In rats, hyperglycemia induces diuresis by increasing nephron filtration rate and reducing proximal reabsorption (Blantz et al. 1983). A hyperglycemic crisis that occurs in humans with diabetes involves a hyperosmolar, hyperglycemic state that includes diuresis (Kitabchi et al. 2001). Thus, there is a need to study how high protein diets impact plasma glucose levels in dolphins and, in turn, how hyperglycemia may drive osmoregulation. Interestingly, unlike terrestrial mammalian kidneys, cetacean kidneys appear to have specialized glycogen stores in the proximal convoluted tubule epithelial cells (Pfeiffer 1997; Ortiz 2001). Given our study's findings, it would be beneficial to further understand the role of glycogen stores in dolphin kidneys.

The present study had several limitations, including the use of eight different adult bottlenose dolphins for nine studies; while dolphins may have responded differently to the five different challenges, changes in urine and plasma levels were tracked over time in individual animals, allowing animals to serve as their own controls. Interpretation of intergroup comparisons was limited by the fact that only one to two animals were used for each study, and inter-animal variation could not be controlled. Plasma solutes were measured using an outside laboratory not used for routine serum chemistries at our institution. As such, comparisons of the study population's values with that of our current normal reference ranges may be inaccurate. The duration of hourly measurements varied from 16 to 25 h post infusion, which may have limited detectable and comparable impacts of water and protein challenges after 16 h. Most significant results, however, involved the first 5–10 h post infusion.

In conclusion, bottlenose dolphins appear to diurese and conserve solutes in response to fresh water ingestion, concentrate urinary solutes in response to seawater ingestion, and diurese and concentrate solutes to extreme levels after ingesting high protein meals. Abnormal plasma values appear possible, including hyponatremia after freshwater ingestion and hypernatremia following seawater ingestion, indicating that dolphins do not routinely drink large boluses of fresh or seawater. Protein, however, may play an important role in maintaining water and solute balance in dolphins ingesting low levels of fresh water or seawater. Additional studies are needed to better characterize the impact of high protein diets on dolphin metabolism.

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Conflict of interest statement None.

References

Albrecht CB (1950) Toxicity of sea water in mammals. Am J Physiol 163:370–385

Bengoumi M, Riad F, Giry J, de la Farge F, Safwate A, Davicco MJ, Barlet JP (1993) Hormonal control of water and sodium in plasma and urine of camels during dehydration and rehydration. Gen Comp Endocrinol 89:378–386

Bie P (1977) Sustained water diuresis in anesthetized dogs: antidiuresis in response to intravenous and bilateral introcarotid infusion of hyper-osmolar solutions of sodium chloride. Act Physiol Scand 101:446–457

Birukawa N, Ando H, Goto M, Kanda N, Pastene LA, Nakatsuji H, Hata H, Urano A (2005) Plasma and urine levels of electrolytes, urea, and steroid hormones involved in osmoregulation of cetaceans. Zool Sci 22:1245–1257

Blantz RC, Tucker BJ, Gushwa L, Peterson OW (1983) Mechanism of diuresis following acute modest hyperglycemia in the rat. Am J Physiol Renal Physiol 244:F185–F194

Bouby N, Trinh-Trang-Tan MM, Coutaud C, Bankir L (1991) Vasopressin is involved in renal effects of high-protein diet: study in homozygous Brattleboro rats. Am J Physiol Renal Physiol 260:96–100

Bradley SE, Mudge GH, Blake WD (1954) The renal excretion of sodium, potassium and water by the harbor seal (*Phoce vitulina* L.): effect of apnea; sodium, potassium and; pitressin; and mercurial diuresis. J Cell Comp Physiol 43:1–22

Caldwell DK, Caldwell MC (1972) World of the bottlenose dolphin. J. B. Lippincott, Co., Philadelphia

Costa DP (1982) Energy, nitrogen, electrolyte flux and sea water drinking in the sea otter *Enhydra lutris*. Physiol Zool 55:35–44

Daniels BS, Hostetter TH (1990) Effects of dietary protein intake on vasoactive hormones. Am J Phys Regul Integr Comp Physiol 258:R1095–R1100

Denke M (2001) Metabolic effects of high-protein low-carbohydrate diets. Am J Cardiol 88:59–61

Eichelberger L, Leiter L, Geiling EMK (1940) Water and electrolyte content of dolphin kidney and extraction of pressor substance (renin). Proc Soc Exp Biol Med 44:356–359

Fetcher ES Jr (1939) The water balance in marine mammals. Q Rev Biol 14:451–459

Fetcher ES Jr, Fetcher GW (1942) Experiments on the osmotic regulation of dolphins. J Cell Comp Physiol 19:123–130

Gales R, Renouf D (1993) Detecting and measuring food and water intake in captive seals using temperature telemetry. J Wildl Manage 57:514–519

Geraci JR (1972) Hyponatremia and the need for dietary salt supplementation in captive pinnipeds. J Am Vet Med Assoc 161:618–623

Guyton AC, Hall JE (eds) (1996) Textbook of medical physiology, 9th edn. W. B. Saunders Company, Philadelphia

Hendrikx A, Epstein FH (1958) Effect of feeding protein and urea on renal concentrating ability in the rat. Am J Physiol 195:539–542

Hiatt EP, Hiatt RB (1942) The effect of food on the glomerular filtration rate and renal blood flow in the harbor seal (*Phoca vitulina* L.). J Cell Comp Physiol 19:221–227

Hong SK, Elsner R, Claybaugh JR, Ronald K (1982) Renal functions of the Baikal seal *Pusa sibirica* and ringed seal *Pusa hispida*. Physiol Zool 55:289–299

How OJ, Nordøy ES (2007) Seawater drinking restores water balance in dehydrated harp seals. J Comp Physiol B Biochem Syst Environ Physiol 177:535–542

Hui CA (1981) Seawater consumption and water flux in the common dolphin *Delphinus delphi*. Physiol Zool 54:430–440

Irving RA, Noakes TD, Buck R, van Zyl Smit R, Raine E, Godlonton J, Norman RJ (1991) Evaluation of renal function and fluid homeostasis during recovery from exercise-induced hyponatremia. J Appl Physiol 70:342–348

Kitabchi AE, Umpierrez GE, Murphy MB, Barrett EJ, Kreisberg RA, Malone JI, Wall BM (2001) Management of hyperglycemic crises in patients with diabetes. Diabetes Care 24:131–153

Kjeld M (2001) Concentrations of electrolytes, hormones, and other constituents in fresh postmortem blood and urine of fin whales (*Balaenoptera physalus*). Can J Zool 79:438–446

Malvin RL, Rayner M (1968) Renal function and blood chemistry in cetacea. Am J Physiology 214:187–191

Malvin RL, Vander AJ (1967) Plasma renin activity in marine teleosts and Cetacea. Am J Physiol 213:1582–1584

Malvin RL, Ridgway S, Cornell L (1978) Renin and aldosterone levels in dolphins and sea lions. Proc Soc Exp Biol Med 157:665–668

Ortiz RM (2001) Osmoregulation in marine mammals. J Exp Biol 204:1831–1844

Ortiz RM, Worthy GAJ (2000) Effects of capture on adrenal steroid and vasopressin concentrations in free-ranging bottlenose dolphins (*Tursiops truncatus*). Comp Biochem Physiol Part A Mol Integr Physiol 125:317–324

Ortiz RM, Adams SH, Costa DP, Ortiz CL (1996) Plasma vasopressin levels and water conservation in fasting, postweaned northern elephant seal pups (*Mirounga angustirostris*). Mar Mamm Sci 12:99–106

Ortiz RM, Wade CE, Costa DP, Ortiz CL (2002) Renal responses to plasma volume expansion and hyperosmolality in fasting seal pups. Am J Phys Regul Integr Comp Physiol 282:R805–R817

Ortiz RM, Wade CE, Ortiz CL (2003) Body water handling in response to hypertonic-saline induced dieresis in fasting northern elephant seal pups (*Mirounga angustriostris*). Comp Biochem Physiol A Mol Integr Physiol 134:423–428

Ortiz RM, Long B, Casper D, Ortiz L, Williams TM (2009) Biochemical and hormonal changes during acute fasting and re-feeding in bottlenose dolphins (*Tursiops truncatus*). Mar Mamm Sci. doi:10.111/j.1748-7692.2009.0009.x

Pfeiffer CJ (1997) Renal cellular and tissue specializations in the bottlenose dolphin (*Tursiops truncatus*) and beluga whale (*Dephinapterus leucas*). Aquat Mammal 23:75–84

Renouf D, Noseworthy E, Scott MC (1990) Daily fresh water consumption by captive harp seals (*Phoca groenlandica*). Mar Mamm Sci 6:253–257

Ridgway SH (1972) Homeostasis in the aquatic environment. In: Ridgway S (ed) Mammals of the sea: biology and medicine. Charles C. Thomas, Springfield, pp 590–747

Ridgway SH, Simpson JG, Patton GS, Gilmartin WG (1970) Hematologic findings in certain small cetaceans. J Am Vet Med Assoc 157:566–575

Rosenberg ME, Swanson JE, Thomas BL, Hostetter TH (1987) Glomerular and hormal responses to dietary protein intake in human renal disease. Am J Physiol Renal Physiol 253:F1083–F1090

Schmidt-Nielsen K (1990) Animal physiology: adaptation and environment, 4th edn. Cambridge University Press, New York

Schmidt-Nielsen B, Murdaugh HV, O'Dell R, Bacsanyi J (1959) Urea excretion and diving in the seal (*Phoca vitulina* L.). J Cell Comp Physiol 53:393–411

Shimamura MH, Yasue H, Ohshima K et al (1997) Molecular evidence from retroposons that whales form a clade within even-toed ungulates. Nature 388:666–670

Skog EB, Folkow LP (1994) Nasal heat and wter exchange is not an effector mechanism for water balance regulation in grey seals. Acta Physiol Scand 151:233–240

Storeheier PV, Nordøy ES (2001) Physiological effects of seawater intake in adult harp seals during phase I of fasting. Comp Biochem Physiol Part A Mol Integr Physiol 128:307–315

Tarasoff F, Toews D (1972) The osmotic and ionic regulatory capacities of the kidney of the harbor seal, *Phoca vitulina*. J Comp Physiol 81:121–132

Tedman R, Green B (2009) Water and sodium fluxes and lactational energetic in suckling pups of Weddell seals (*Leptonychotes weddellii*). J Zool 212:29–42

Telfer N, Cornell LH, Prescott JH (1970) Do dolphins drink water? J Am Vet Med Assoc 157:555–558

Thewissen JGM, Madar SI (1999) Ankle morphology of the earliest cetaceans and its implications for the phylogenetic relations among ungulates. Syst Biol 48:21–30

Van Dyke D (1972) Contingency ration for California sea lions (*Zalophus californianus*). Naval Undersea Center, Technical Report No 317. San Diego

Vander AJ (1995) Renal physiology, 5th edn. McGraw-Hill, Inc., New York

Venn-Watson S, Jensen ED, Ridgway SH (2007) Effects of age and sex on clinicopathologic reference ranges in a healthy managed Atlantic bottlenose dolphin population. J Am Vet Med Assoc 231:596–601

Venn-Watson S, Smith CR, Dold C, Ridgway SH (2008) Use of a serum-based glomerular filtration rate prediction equation to assess renal function by age, sex, fasting, and health status in bottlenose dolphins (*Tursiops truncatus*). Mar Mamm Sci 24:71–80