Osmoregulation in Wild and Captive West Indian Manatees (*Trichechus manatus*)

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ABSTRACT

The ability of West Indian manatees (*Trichechus manatus latirostris* and *Trichechus manatus manatus*) to inhabit both freshwater and marine habitats presents an interesting model to study osmoregulation in sirenians. Blood samples were analyzed from manatees held in fresh- and saltwater and from wild animals captured in fresh-, brackish, and saltwater for concentrations of aldosterone, arginine vasopressin, plasma renin activity, Na^+, K^+, Cl^-, and osmolality. Two separate experiments were also conducted on captive animals to evaluate osmoregulatory responses to acute saltwater exposure and freshwater deprivation. Spurious differences were observed in plasma electrolyte and osmolality among the captive and wild groups. Wild brackish water animals exhibited the highest vasopressin concentrations, while wild freshwater manatees had the highest aldosterone levels. A significant correlation between mean vasopressin and osmolality was demonstrated for captive and wild animals. When freshwater animals were acutely exposed to saltwater, osmolality, Na^+, and Cl^- increased 5.5%, 8.0%, and 14%, respectively, while aldosterone decreased 82.6%. Saltwater animals deprived of freshwater exhibited an almost twofold increase in aldosterone during the deprivation period and a fourfold decrease when freshwater was again provided. Within this group, osmolality increased significantly by 3.4% over the course of the study; however, electrolytes did not change. The lack of consistent differences in electrolyte and osmolality among wild and captive groups suggests that manatees are good osmoregulators regardless of the environment. The high aldosterone levels in wild freshwater animals may indicate a need to conserve Na^+, while the high vasopressin levels in wild brackish-water manatees suggest an antidiuretic state to conserve water. Vasopressin levels appear to be osmotically mediated in manatees as in other mammals.

Introduction

Although West Indian manatees (*Trichechus manatus*) are primarily found in freshwater, they can inhabit regions with salinity levels as high as 35‰ (Husar 1977). The observation of two West Indian manatees near the Dry Tortugas Islands (a location not normally considered to be part of the species' range and far from freshwater) provides evidence of their ability to tolerate the osmotic pressures induced by marine habitats from the ingestion of salt-laden sea grasses and marine algae (Reynolds and Ferguson 1984; Maluf 1989). Marine vertebrates must possess renal and/or extrarenal structures and endocrine mechanisms necessary to tolerate a hyperosmotic habitat. Unlike manatees, other marine mammals have lobulate kidneys (Hedges et al. 1979; Hoover and Tyler 1986), which allow them to drink saltwater (mariposia) and to concentrate their urine while maintaining water balance and a constant plasma osmolality (Kooyman and Drabek 1968; Tarasoff and Toews 1972). Manatees and dugongs (Order: Sirenia) do, however, possess renal structures that are indicative of an ability to conserve water via the concentration of urine, which therefore suggests that these animals have the ability to inhabit marine environments (Osman-Hill 1945; Batrawi 1957; Hill and Reynolds 1989; Maluf 1989).

The present study was conducted to determine the osmoregulatory mechanisms employed by manatees and to evaluate the endocrine responses of manatees to acute saltwater exposure and freshwater deprivation. Endocrine mechanisms necessary to maintain electrolyte homeostasis have not been thoroughly studied in marine mammals. Some data on the role of vasopressin, aldosterone, and plasma renin activity have been reported for dolphins, seals, and sea lions (Eichelberger et al. 1940; Malvin et al. 1971, 1978; Hong et al. 1982; Skog and Folkow 1994; Ortiz et al. 1996). The antidiuretic role of vasopressin has not been clearly established in marine mammals, but aldosterone and plasma renin activity have been shown to regulate Na^+ (Geraci 1972; Malvin et al. 1978). Plasma renin activity provides an indication of angiotensin II generation,

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which stimulates aldosterone release in response to a decrease in circulating Na^+ levels (Blair-West et al. 1979). The role of these hormones has yet to be examined in manatees.

Since published data on electrolytes, osmolality, and hormones are limited for manatees, data were initially measured in blood samples taken from captive and wild animals in various salinities. In order to determine the osmoregulatory capabilities of manatees, endocrine responses to acute saltwater exposure and freshwater deprivation were also examined. Because West Indian manatees are capable of inhabiting waters of varying salinities, they offer a rare opportunity to study the osmoregulatory capabilities of sirenians.

Material and Methods

Levels of Na^+, K^+, Cl^-, osmolality, vasopressin, and aldosterone were determined from captive and wild manatees in fresh- and saltwater, as well as from a group of wild animals captured in brackish water. An experiment manipulating water salinity and another manipulating the animal's access to freshwater were conducted separately.

Captive and Wild Manatees

Captive Animals in Freshwater. At Sea World of Florida, three calves (two males, one female, 12–18 mo old) were sampled in October 1992, and eight adults (four males, five females) in January 1993. At Lowry Park Zoo, Tampa, Florida, two adult males were sampled in October 1993. All captive animals were maintained on a romaine lettuce diet. The three calves at Sea World were also provided with a milk formula supplement. All animals were maintained in freshwater for more than a year prior to blood sampling.

Captive Animals in Saltwater. Two male Antillean manatee calves, found orphaned, were held in saltwater at the Caribbean Stranding Network's facility, Isla Magüeyez, Puerto Rico. One of the calves (18 mo old) was sampled in May 1993 and the other (12 mo old) in May 1994. The calves were held in a pool supplied with saltwater from the adjoining bay (30‰–34‰). These calves received a milk formula diet. Four Florida manatees held in saltwater at EPCOT's Living Seas, Orlando, Florida, were also sampled. One adult female and her calf (male) were sampled in February, May, August, and November 1992, and March and October 1993, and two other adult males were sampled in October 1993. The calf nursed until May 1992, at which time he began to eat romaine lettuce. Adults were maintained on a romaine lettuce diet. Water salinity of the tank was maintained at approximately 34‰.

Wild Animals in Freshwater. In Colombia, nine Antillean manatees (four males, five females) were captured in a freshwater lake during July and August 1993. These animals did not have access to saltwater. Animals ate the vegetation found in the lake (Montoya 1994).

Wild Animals in Saltwater. In May 1993, five Antillean manatees (four males, one female) were captured by net in the vicinity of Ceiba, Puerto Rico (salinity ~32‰). Because these animals were a free-ranging group, their diet could not be ascertained, but it is believed to have consisted primarily of marine vegetation (A. Mignucci, Caribbean Stranding Network, personal communication).

Wild Animals in Brackish Water. In Tampa Bay, near the Tampa Electric Company power plant effluent, blood samples were taken from 12 Florida manatees (seven males, five females) in February 1993 and from eight others (four males, four females) in January 1994. Because these animals were caught in relatively low salinity waters (<10‰; B. Weigle, Florida Department of Environmental Protection, personal communication), they were considered a brackish-water population. These animals represent a group that is known to actively move between fresh- and saltwater (B. Bonde and J. Reid, U.S. Fish and Wildlife Service, personal communication); therefore, their diet could potentially consist of both fresh- and saltwater vegetation.

Experiment 1: Effects of Acute Saltwater Exposure on Freshwater-Acclimated Manatees

Four Florida manatees (2–4 yr old) held in freshwater at Sea World of Florida were acutely exposed to saltwater for 5 d before being returned to freshwater. All animals were held in a large community pool. Throughout the experiment, animals were fed romaine lettuce. During exposure to saltwater, animals were provided access to a freshwater hose for about 1 h d^{-1}. Previous data on these animals' water turnover rates suggest that they do not drink saltwater during exposure to saltwater (Ortiz 1994). At the initiation of the experiment, while the animals were in freshwater, a blood sample from each animal was taken, and another was taken at 12 h, 2 d, and 5 d later. After the initial blood sample, manatees were orally intubated and dosed with deuterium oxide as part of a water-flux study (Ortiz 1994). Blood samples were taken after 2 d and 5 d of exposure to saltwater. Blood samples were taken again at 2 d and 5 d after the animals were returned to freshwater.

Opportunistic urine samples were obtained from three animals during this experiment while the animals were lying on their backs during blood sampling. Samples were collected into either a 20-mL Nalgene vial or 10-mL scintillation vial.

Experiment 2: Effects of Acute Freshwater Deprivation on Saltwater-Acclimated Manatees

Five manatees (three males, two females, 3–8 yr old) held at Centro Peixe-Boi in Itamaracá, Brazil, were normally fed lettuce, cabbage, beets, and carrots (88%–94% water) as well as sea grass (57% water) (R. M. Ortiz and G. A. J. Worthy, unpublished data) and had been maintained on this diet for over 2 yr. To examine the effects of acute freshwater deprivation on saltwater-acclimated manatees, these animals were maintained in saltwater (~34‰) and exclusively fed sea grasses for 19 d. Experimental protocol consisted of an initial 5-d period (predeprivation phase) in which animals had access to freshwater from a hose, followed by a 9-d freshwater-deprivation phase, and concluded with a 5-d period (postdeprivation phase) in which they again had access to freshwater. During the periods when the freshwater was offered, animals were seen taking the hose into their mouth. Previous data suggested that the animals drink freshwater but that they do not consume saltwater (R. M. Ortiz and G. A. J. Worthy, unpublished data). Blood samples were taken at the initiation of the experiment and at 10 h, 2 d, and 5 d later. After the initial blood sample, manatees were orally intubated and dosed with deuterium oxide as part of a water-flux study (Ortiz 1994). After the 5-d sample, animals were deprived of freshwater, and blood was sampled at 2, 5, and 9 d into the deprivation phase. After 9 d of deprivation, animals were again provided access to freshwater, and blood samples were collected 2 and 5 d postdeprivation. An attempt was made to obtain urine samples from each animal during each blood sampling session; however, this was not successful.

Sampling Procedures and Sample Analyses

For all captive manatees, blood samples were obtained by stranding the animals on the bottom of their tank and laying them on large foam mattresses as water was drained from the tank. Once animals were situated properly on the foam mattresses, blood samples were usually collected within 10 min. All wild animals were captured with a net and stranded on shore. These blood samples were obtained within 20 min of capture.

One 10-mL K^+-EDTA and one 10-mL Na^+-heparin Vacutainer (Becton-Dickinson, Rutherford, N.J.) were each filled with blood from a vascular bundle near the plantar surface of the pectoral limb. Blood samples were immediately placed on ice. They were then centrifuged (10 min × 2,200 rpm), and the plasma was transferred to cryovials (Nalge, Rochester, N.Y., and Vangard, Neptune, N.J.) and frozen at −70°C. At times, sample volumes were limited; therefore, not all parameters were measured for all groups. Aldosterone, cortisol, glucose, Na^+, K^+, and Cl^- levels were determined from heparinized samples. Vasopressin, plasma renin activity, and osmolality were measured with EDTA samples. Commercial radioimmunoassay (RIA) kits were used to measure levels of plasma renin activity (Rianen Angiotensin I RIA kit, Dupont, Wilmington, Del.), aldosterone (Coat-a-Count Kit, Diagnostic Products, Los Angeles), and cortisol (Coat-a-Count Kit, Diagnostic Products, Los Angeles). Vasopressin levels were determined with a double-antibody ^{125}I RIA developed at National Aeronautics and Space Administration's Ames Research Center, Moffet Field, California (Keil and Severs 1977). Aldosterone, vasopressin, and cortisol assays were validated by recovery tests from pooled manatee plasma, with cold hormone recoveries of 103%, 98%, and 97%, respectively. Serially diluted samples of these three hormones ran parallel to the standard curve. Intraassay and interassay variability for all three hormones was less than 12%, on the basis of at least four determinations per hormone. Plasma osmolality was measured with a Wescor 5500 vapor-pressure osmometer (Wescor, Logan, Utah). Plasma electrolytes and glucose were measured on a Cobas Mira autoanalyzer (Roche Diagnostic Systems, Montclair, N.J.). Urine osmolality and electrolytes were measured on the same instruments as for plasma.

Parameters for captive and wild animals were compared by Fisher's protected least significant difference ANOVA. Parameters for serially sampled animals in experiments 1 and 2 were analyzed by ANOVA corrected for repeated measures over time. If significance was determined, Fisher's protected least significant difference analysis of variance was applied post hoc. Correlations were determined for plasma renin activity-aldosterone and vasopressin-osmolality relationships by a Fisher's R to Z correlation test. Means (±SE) were considered significantly different or correlated at $P < 0.05$ for all statistical tests. All statistical tests were performed on StatView for the Macintosh software (Abacus Concepts 1992).

Results

Basal Levels for Captive and Wild Manatees

Life-history data on wild-caught animals were very limited; however, straight-length measurements provided an indication of age category. With the exception of two or three animals, measurements for wild fresh- (straight length: 198–302 cm), salt- (straight length: 209.5–272.5 cm), and brackish water (straight length: 180–305 cm) manatees indicated that they were subadults or adults based on previously determined length-age correlations (O'Shea et al. 1985). Data for captive Antillean and Florida manatees in saltwater were pooled, since means for each measured parameter were not statistically different as determined by a *t*-test.

Electrolytes and Osmolality. Captive saltwater animals had significantly lower osmolality than all other groups (Table 1). Captive freshwater manatees displayed significantly lower plasma Na^+, K^+, and Cl^- levels than wild brackish water and saltwater animals (Table 1). Wild freshwater manatees exhibited significantly higher K^+ levels than all other groups (Table 1).

Table 1: Plasma osmolality, electrolyte, and hormone levels for captive and wild manatees in fresh-, salt-, and brackish water

	Captive Freshwater	Wild Freshwater	Wild Brackish Water	Captive Saltwater	Wild Saltwater
Plasma composition:					
n	14	9	20	8	6
Osmolality (mOsmol L^{-1})	305.5 ± 1.9^{a}	306.8 ± 2.2^{a}	314.5 ± 4.7 (19)^{b}	299.3 ± 2.6^{c}	310.2 ± 2.2^{a}
Na^+ (mmol L^{-1})	143.8 ± 1.0^{a}	149.6 ± 2.1^{b}	151.2 ± 1.5^{b}	146.5 ± 1.3^{a,b}	155.0 ± 3.6^{b}
K^+ (mmmo L^{-1})	5.0 ± .2^{a}	6.4 ± .1^{b}	5.4 ± .1^{c}	5.2 ± .1^{c}	5.7 ± .1^{c}
Cl^- (mmol L^{-1})	96.8 ± 1.0^{a}	100.2 ± 1.5^{b}	102.8 ± 1.3^{b}	100.4 ± 1.2^{b}	102.6 ± 1.6^{b}
Plasma hormones:					
n	13	9		5	5
Vasopressin (pg mL^{-1})	1.1 ± .30^{a,c}	.6 ± .04^{a}	2.5 ± .78 (12)^{b}	.5 ± .01^{a}	2.1 ± .48^{b,c}
Aldosterone (pg mL^{-1})	153.6 ± 39.8^{a}	659.6 ± 103.3^{b}	68.6 ± 12.7 (16)^{c,d}	37.4 ± 5.1^{c}	95.0 ± 12.6^{a,d}
Cortisol (ng mL^{-1})	ND	ND	1.5 ± .04 (8)	ND	ND

Note. Values in parentheses next to mean (±SE) for wild brackish water animals are the sample size for that measurement and correspond to one sample per animal. Means were considered significantly different at $P < 0.05$. Common superscripts within a row indicate no significant difference. ND, Not determined.

Hormones. Wild freshwater manatees exhibited significantly higher aldosterone concentrations than all other groups (Table 1). Aldosterone concentrations in captive saltwater manatees were significantly lower than in wild saltwater and captive freshwater animals (Table 1). Wild brackish water manatees displayed significantly higher vasopressin concentrations than all other groups, except wild saltwater manatees (Table 1). Wild saltwater animals had significantly greater vasopressin concentrations than did captive saltwater and wild freshwater animals. A significant correlation ($R^2 = 0.89$; $P = 0.02$) existed between mean vasopressin and mean osmolality for captive and wild groups (Fig. 1).

Experiment 1: Effects of Acute Saltwater Exposure on Freshwater-Acclimated Manatees

Animal mass and the amount of food provided did not change over the course of the 15-d experiment, indicating that the acute saltwater exposure did not alter food consumption.

Plasma analyses. Manatees exhibited a transient increase in plasma renin activity and aldosterone for the first day following intubation. Both hormones returned to original levels by day 2. This was followed by a significant increase in osmolality, Na^+, and Cl^- during exposure to saltwater and a significant decrease upon returning to freshwater (Table 2). Aldosterone concentrations decreased significantly between the initial freshwater phase and the saltwater phase and increased significantly upon returning to freshwater (Fig. 2). Plasma renin activity also decreased significantly between the initial freshwater phase and the saltwater phase (Fig. 2) and returned to initial freshwater levels following 7 d of exposure to freshwater. Vasopressin and K^+ concentrations did not change during the course of this experiment. Plasma renin activity and aldosterone concentrations were significantly correlated ($R^2 = 0.99$; $P = 0.0001$).

Urinalyses. A total of nine urine samples were obtained from three animals during the initial freshwater and the return to freshwater phases. During blood sampling of the saltwater phase when the tank was drained and animals were on their backs, frequency of urination and the number of animals urinating decreased noticeably. Therefore, only one urine sample was collected from a single animal during this phase. Urine osmolalities collected during the three phases were significantly lower than their respective plasma osmolalities, with the mean for the return to the freshwater phase being the lowest (Table 3). Urine Na^+ from the saltwater manatee sample was 31-fold greater than that from samples of manatees in freshwater (Table 3). Corresponding plasma aldosterone concentrations from one animal in both the initial phase and return to freshwater were approximately four- and eightfold greater than the concentration for the saltwater manatee from which the sole saltwater urine sample was obtained (Table 3).

Experiment 2: Effects of Acute Freshwater Deprivation on Saltwater-Acclimated Manatees

During the course of this experiment, body mass fell between 5% and 17% of starting mass, which was constant, with a reduction in sea grass consumption throughout the 19-d experiment. During the pre- and postdeprivation phases, animals were seen drinking from a freshwater hose, which provided their sole source of exogenous freshwater (R. M. Ortiz and G. A. J. Worthy, unpublished data).

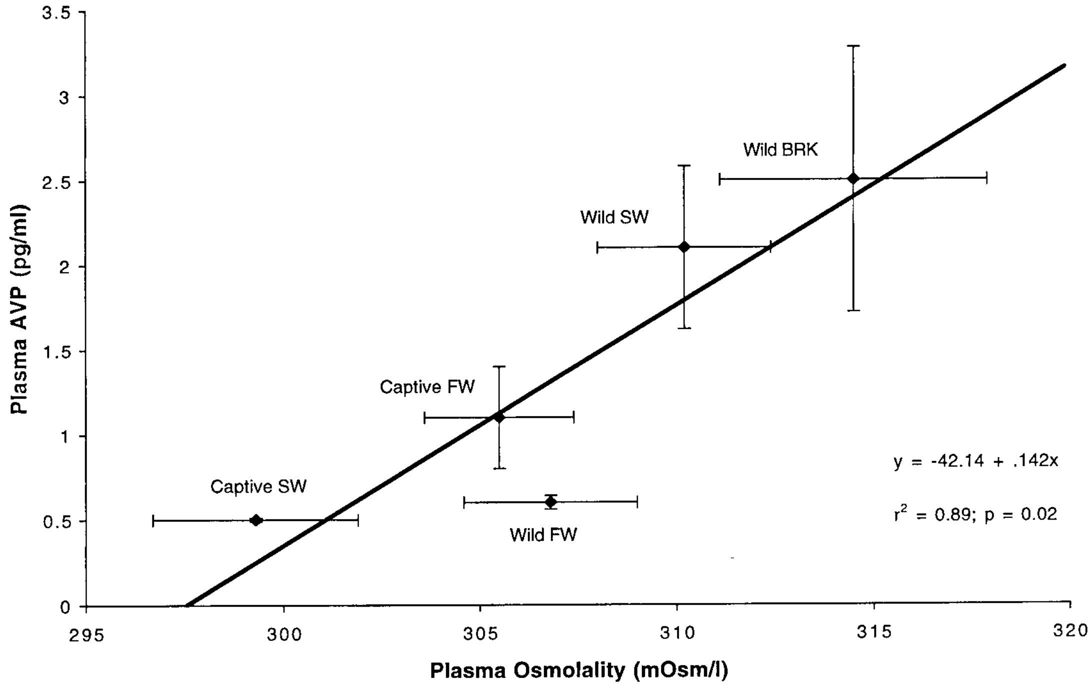

Figure 1. Plasma vasopressin (AVP)-osmolality correlation. Plasma vasopressin and osmolality were significantly correlated for wild and captive manatees held in fresh (*FW*), salt (*SW*), and brackish (*BRK*) water. Means are plotted for both vasopressin and osmolality. Error bars indicate standard error.

Over the course of the experiment, osmolality increased significantly (Table 4). Aldosterone levels decreased significantly between freshwater deprivation and postdeprivation phases; however, the increase between the predeprivation and deprivation phase was not significant (Table 4). Electrolytes, glucose, and cortisol concentrations and plasma renin activity did not change significantly over the course of this experiment (Table 4).

Discussion

Comparisons of plasma electrolyte levels previously reported for captive and wild manatees suggests that there is very little difference between captive and wild animals (White et al. 1976; Irvine et al. 1980; Medway et al. 1982). The lack of consistently different plasma electrolyte values across the different groups in the present study would support the conclusions of the previous studies. With few exceptions, plasma electrolytes and osmolalities in the present study were constant over a broad range of salinities, which suggests that West Indian manatees are good osmoregulators. Although previous studies did not indicate salinity of the water in which animals were kept or caught, electrolyte values from the present study are comparable to those previously reported (White et al. 1976; Irvine et

Table 2: Experiment 1: plasma osmolality and electrolytes

	Freshwater	Saltwater	Return to Freshwater
Osmolality (mOsmol L^{-1})	297.5 ± 2.1	314.0 ± 3.2[a]	296.3 ± 3.1
Na^+ (mmol L^{-1})	147.3 ± 1.7	159.1 ± 4.0[a]	148.7 ± .9
K^+ (mmol L^{-1})	4.8 ± .2	4.9 ± .1	5.0 ± .1
Cl^- (mmol L^{-1})	89.3 ± 1.5	101.8 ± 2.8[a]	90.3 ± 1.0

Note. Mean (±SE) plasma Na^+, Cl^-, and osmolality levels increased significantly ($P < 0.05$) when manatees were acutely exposed to saltwater for 5 d, and levels subsequently decreased significantly upon returning to freshwater.

[a] Significantly different from both freshwater and return to freshwater.

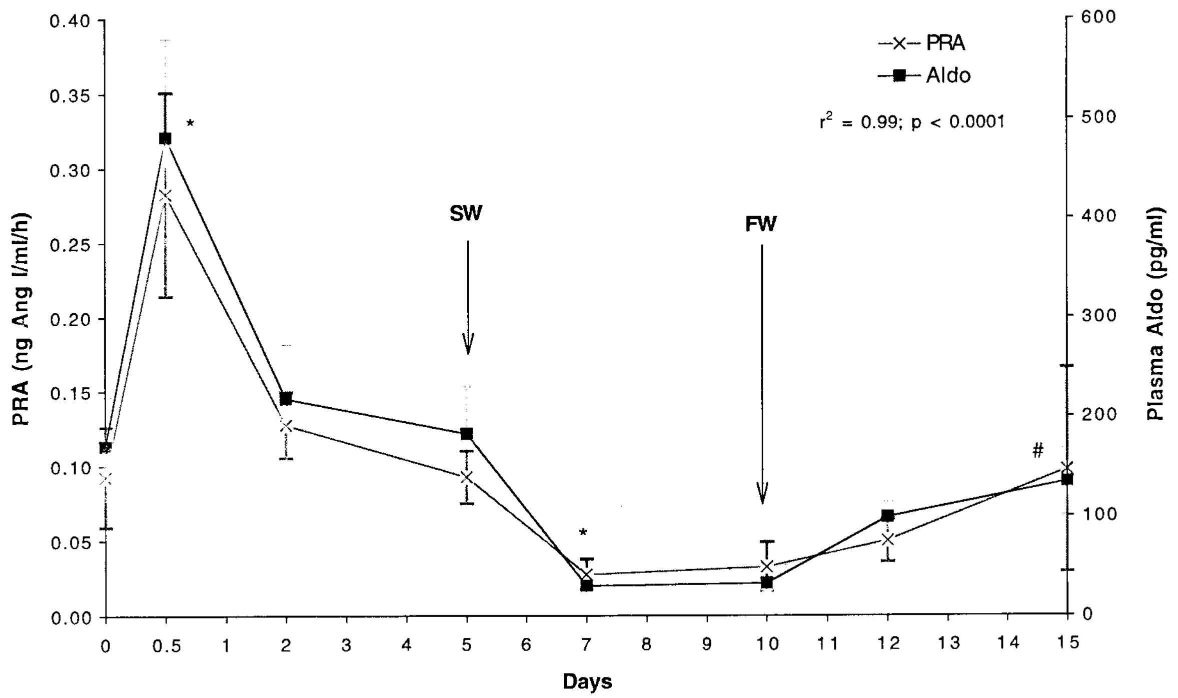

Figure 2. Experiment 1: plasma renin activity (PRA)-aldosterone (Aldo) correlation. Plasma renin activity and aldosterone concentrations decreased significantly (indicated by an asterisk) when manatees were acutely exposed to saltwater (*SW*) for 4 d. The subsequent increase in aldosterone following the return to freshwater (*FW*) was significant (indicated by a pound sign), while the increase in plasma renin activity was not. Mean plasma renin activity and aldosterone were significantly correlated. Error bars indicate one standard error.

al. 1980; Medway et al. 1982; Montoya 1994). With the exception of the captive saltwater group, plasma osmolalities were similar to previously published values (Medway et al. 1982).

Although plasma osmolalities were not significantly different in wild brackish water and wild saltwater animals, the higher levels of Na^+, K^+, and Cl^- relative to captive freshwater manatees suggests that exogenous salt sources resulted in increased plasma concentrations. The most likely sources of salt for wild animals are consumption of isosmotic sea grasses and incidental water ingestion during feeding, since mariposia appears to be absent in manatees (Ortiz 1994). Alternatively, lower levels of Na^+ exhibited by both captive groups suggests a possible deficiency of salt in captive animals. This deficiency is consistent with a lettuce diet, which has proportionally less salt and more water than does marine vegetation (Jennings 1976; Wignarajah and Baker 1981). Captive manatees appear to be potentially susceptible to hyponatremia. This suggests that manatees may require dietary salt supplements while in captivity or occasional access to marine habitats while in the wild. If occasional

Table 3: Experiment 1: urine osmolality and electrolytes with corresponding plasma aldosterone concentrations

	Freshwater	Saltwater	Return to Freshwater
n	3	1	3
Osmolality (mOsmol L^{-1})	200.8 ± 7.8	217	188.3 ± 10.4
Na^+ (mmol L^{-1})	<1.0	30.8	<1.0
K^+ (mmol L^{-1})	52.1 ± 6.9	28.0	41.1 ± 9.8
Cl^- (mmol L^{-1})	395.7 ± 3.6	406.0	388.7 ± 3.4
Plasma aldosterone (pg mL^{-1})[a]	207.7	25.8	90.8

Note. Mean (±SE) urine osmolality and electrolyte levels associated with a matching plasma aldosterone sample for animals switched between fresh- and saltwater and then returning to freshwater.

[a] Values of a single animal that had a urine sample taken at the time of blood sampling.

Table 4: Experiment 2: Plasma osmolality, electrolytes, glucose, and hormone concentrations for manatees ($n = 5$) deprived of freshwater

	Predeprivation	Deprivation	Postdeprivation
Osmolality (mOsmol L^{-1})	314.1 ± 2.2	319.5 ± 3.3	324.7 ± 2.0[a]
Na^+ (mmol L^{-1})	148.5 ± 1.1	148.2 ± 1.6	149.1 ± 1.0
K^+ (mmol L^{-1})	5.2 ± .17	4.8 ± .16	4.8 ± .10
Cl^- (mmol L^{-1})	99.5 ± 1.4	101.4 ± 1.1	102.0 ± .4
Glucose (mg dL^{-1})	88.7 ± 9.1	87.4 ± 8.6	83.0 ± 9.1
Plasma renin activity (ng Ang I mL^{-1} h^{-1})	.39 ± .09	.51 ± .04	.38 ± .06
Aldosterone (pg mL^{-1})	12.9 ± 4.6	24.7 ± 5.7	6.1 ± 2.1[b]
Cortisol (ng mL^{-1})	2.2 ± .6	2.4 ± .6	2.0 ± 0.3

Note. Animals were held in saltwater with access to freshwater for 5 d (predeprivation) before being deprived of freshwater (deprivation) for 9 d. After deprivation, animals were again provided access to freshwater (postdeprivation) for 5 d. Means (±SE) were considered significantly different at $P < 0.05$. Ang I, angiotensin I.

[a] Significantly different from predeprivation and deprivation phases.

[b] Significantly different from deprivation phase.

access to saltwater is required, observations of freshwater animals in marine areas may be explained by this need to replenish circulating Na^+ levels.

When manatees were acutely exposed to saltwater in experiment 1, Na^+, Cl^-, and osmolality increased, and they subsequently decreased to preexposure levels upon return to freshwater, indicating that an increase in exogenous salt elicits an elevation in plasma values. The lack of significant changes in electrolyte levels between the predeprivation, deprivation, and postdeprivation phases of experiment 2 demonstrates the ability of manatees to regulate and maintain electrolyte homeostasis during periods of restricted access to freshwater. The constant Na^+ level is indicative of the tight regulation of this electrolyte by animals acclimated to saltwater. Results of experiments 1 and 2 indicate that ionic and osmotic homeostasis in manatees was influenced by acute saltwater exposure and that plasma electrolyte balance was maintained in the absence of freshwater.

In mammals, an increase in plasma osmolality is the primary stimulus for vasopressin secretion during dehydration (Wade et al. 1982). Plasma osmolality and vasopressin values were correlated for all wild and captive groups, which suggests that osmolality may be a stimulating mechanism for vasopressin in West Indian manatees. This correlation may provide the first evidence that vasopressin plays a significant role in the antidiuretic activity of manatees. This is significant because the role of vasopressin in marine mammals has thus far been inconclusive (Malvin et al. 1971; Ortiz et al. 1996). The lack of a vasopressin response in experiments 1 and 2, in which an increase in osmolality was exhibited, suggests that the stimuli that induced increases in osmolality may have been inadequate to elicit a detectable increase in vasopressin.

When manatees were acutely exposed to saltwater, subsequent elevations in Na^+ and osmolality were associated with a reduction in plasma renin activity and a corresponding decrease in aldosterone concentrations. Although the increase in plasma renin activity upon returning the manatees to freshwater was not significant, values returned to pre-saltwater exposure levels. These data indicate that Na^+ homeostasis is regulated by the renin-angiotensin-aldosterone axis in manatees, as in other mammals.

West Indian manatees exhibited aldosterone levels consistent with the salinity of their environment. High levels of aldosterone in wild freshwater manatees may indicate a necessity for wild animals in freshwater to conserve Na^+, since Na^+ restriction has been shown to increase aldosterone secretion in rats (Aguilera and Catt 1983). Alternatively, the high levels may indicate a capture-stress response. In mammals, adrenocorticotropin-induced gluco- and mineralocorticoid release in response to emotional disturbance or stress has been well documented (Axelrod and Reisine 1984). However, these elevated levels did not appear to be an effect of stress, since the other two groups of wild-caught animals exhibited mean aldosterone values that were only 9.8% and 14.3% of that of the wild freshwater group. The higher aldosterone concentrations in captive freshwater animals, relative to those in captive saltwater animals, suggest that these animals have a greater need to conserve Na^+, providing further evidence of Na^+ deficiency in captive animals lacking a source of exogenous salt.

All four animals in experiment 1 responded to intubation procedures by exhibiting spikes in plasma renin activity and aldosterone concentrations within 12 h following intubation. The spike and subsequent decline in plasma renin activity were paralleled by aldosterone concentrations. The spike in plasma renin activity following intubation was likely the result of the stress of intubation. Administration of propranolol (a β-adrenergic receptor antagonist) has been shown to block plasma renin activity in cats, while renal nerve stimulation results in increased plasma renin activity (Coote et al. 1972). A similar, parallel relationship between circulating levels of plasma renin activity and aldosterone concentrations has been illustrated in dolphins and sea lions (Malvin et al. 1978). Glucocorticoid elevations and subsequent increases in glucose are other typical responses to stress (Axelrod and Reisine 1984; Tataranni et al. 1996). During the course of experiment 2, glucose and cortisol levels remained constant, which indicates that this deprivation of freshwater did not activate the hypothalamo-pituitary-adrenal axis.

Only one urine sample could be collected during the saltwater phase of experiment 1, since the frequency of micturition decreased, which suggests water conservation via a decrease in urinary water loss. Seals have been shown to conserve water by reducing urine output and increasing urine osmolality (Ortiz et al. 1978, 1996; Skog and Folkow 1994). However, none of the manatees exhibited elevated vasopressin during the saltwater phase, and the urine osmolality of the single urine sample was similar to that of urine samples obtained from animals in the freshwater phase of experiment 1. All urine osmolalities were lower than the plasma osmolality for these animals, consistent with low vasopressin levels and suggesting a lack of obligatory renal water conservation. The low urine osmolalities measured here are similar to previously reported values for captive manatees (Irvine et al. 1980). Plasma aldosterone concentrations were inversely correlated to corresponding urine Na^+ concentrations. A similar inverse correlation has been shown between plasma aldosterone and urine Na^+ in dogs (Thrasher et al. 1984). The urine data indicate that manatee kidneys are sensitive to acute saltwater exposure but respond by increasing Na^+ excretion via a reduction in plasma aldosterone.

In summary, West Indian manatees were able to maintain ionic and osmotic homeostasis in habitats of varying salinities. However, data suggest that captive animals may be susceptible to hyponatremia if an exogenous source of salt is not available. Results also indicate that manatees regulated Na^+ via the renin-angiotensin-aldosterone axis and that vasopressin release was osmotically stimulated. Furthermore, the osmolality and electrolyte data suggest that manatees acutely exposed to saltwater can tolerate incidental ingestion, incurring the subsequent salt load without dehydrating. However, these animals were fed large quantities of hydrated lettuce, which is not available to free-ranging animals. Data from saltwater animals acutely deprived of freshwater suggests that manatees may be susceptible to dehydration after an extended period if freshwater is not available. West Indian manatees can tolerate a wide range of salinities and possess renal and endocrine mechanisms similar to those employed by other mammals to maintain sodium balance and to avoid dehydration.

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