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Effects of acute fresh water exposure on water flux rates and osmotic responses in Kemp's ridley sea turtles (*Lepidochelys kempi*)

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Abstract

Water flux rates and osmotic responses of Kemp's Ridley sea turtles (*Lepidochelys kempi*) acutely exposed to fresh water were quantified. Salt-water adapted turtles were exposed to fresh water for 4 d before being returned to salt water. During the initial salt water phase, absolute and relative water flux rates were 1.2 ± 0.1 l d^{-1} and 123.0 ± 6.8 ml kg^{-1} d^{-1}, respectively. When turtles were exposed to fresh water, rates increased by approximately 30%. Upon return to salt water, rates decreased to original levels. Plasma osmolality, Na^+, K^+, and Cl^- decreased during exposure to fresh water, and subsequently increased during the return to salt water. The Na^+:K^+ ratio was elevated during the fresh water phase and subsequently decreased upon return to salt water. Aldosterone and corticosterone were not altered during exposure to fresh water. Elevated water flux rates during fresh water exposure reflected an increase in water consumption, resulting in a decrease in ionic and osmotic concentrations. The lack of a change in adrenocorticoids to acute fresh water exposure suggests that adrenal responsiveness to an hypo-osmotic environment may be delayed in marine turtles when compared to marine mammals.

Keywords: Adrenal; Aldosterone; Corticosterone; Deuterium; Electrolytes; Osmoregulation; Water balance

1. Introduction

Salt water drinking has been suggested as a means for marine reptiles to obtain osmotically free water, and thus maintain water balance (Kooistra and Evans, 1976; Marshall and Cooper, 1988). Salt glands present in marine reptiles allow these animals to consume sea water by excreting excess electrolytes (Holmes and McBean, 1964; Evans, 1973; Kooistra and Evans, 1976; Nicolson and Lutz, 1989). Estimates of drinking in sea turtles by phenol red dilution (Holmes and McBean, 1964), salt gland secretion (Prange, 1985), and body mass gain (Bennett et al., 1986) have been calculated, however direct measurements of both fresh and salt water turnover rates in sea turtles have yet to be reported.

A reduction in the rate of Na^+ efflux was observed in green (*Chelonia mydas*) and logger-

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head (*Caretta caretta*) turtles within 25 h following exposure to fresh water (Evans, 1973; Kooistra and Evans, 1976) suggesting that water turnover rates in sea turtles may be decreased during exposure to fresh water. Changes in Na^+ efflux in response to fresh water exposure also suggest that ionic and osmotic homeostasis in sea turtles may be altered during this time. Green turtles injected with corticosterone increased their urinary Na^+ excretion rate approximately threefold 1 h after injection (Holmes and McBean, 1964) suggesting that regulation of electrolytes in sea turtles may be adrenocortically mediated. In another group of marine adapted reptiles, sea snakes exhibited a decrease in corticosterone in response to fresh water exposure (Duggan and Lofts, 1978). In the tortoise (*Testudo hermanni*) salt loading induced an elevation in plasma Na^+ associated with decreases in plasma K^+ and aldosterone, and a concomitant increase in corticosterone (Uva et al., 1982). The osmoregulatory role of aldosterone and corticosterone in those reptiles studied suggests that both of these steroids could be involved in electrolyte homeostasis in sea turtles. However, in sea turtles, aldosterone concentrations have yet to be reported, and corticosterone concentrations in response to stress have only recently been reported (Aguirre et al., 1995; Gregory et al., 1996; Valverde et al., 1999).

In the present study, a group of Kemp's Ridley sea turtles (*Lepidochelys kempi*) was acutely exposed to fresh water for 4 days. The objectives of this study were (1) to estimate water flux rates of captive Kemp's Ridley turtles exposed to both salt and fresh water; (2) to determine the osmotic and ionic responses of these turtles to acute fresh water exposure; and (3) to examine the effects of acute fresh water exposure on aldosterone and corticosterone concentrations. Although sea turtles are not normally found in fresh water, acute exposure to fresh water will provide insight to the responsiveness of the adrenal gland and of drinking behavior to an hypo-osmotic stimulus, and to the adaptability of these animals to an extreme environment.

2. Materials and methods

2.1. Animals

Four captive turtles (10.0 ± 1.1 kg), held at the Bioaquatics facility in the Department of Biology at Texas A&M University, College Station, TX, were available for the present study. Turtles were individually maintained in large cylindrical pools at a salinity of 32 (± 0.9) ppt. Salt water for the facility was prepared by adding one 18.93 l bucket of Fritz Super Salt concentrate (Fritz Industries, Dallas, TX) and one 34.6 kg bag of salt (United Salt Corp., Houston, TX) into a 2650 l reservoir of water before being pumped into the system. During the study all turtles were kept indoors, maintained on a 12 L:12 D light cycle, and fed a diet of squid. Water temperature was maintained at 25°C.

All blood samples were taken between 0800 and 0900 h and were consistently obtained within 3 min of handling. As previously described, 8 ml of blood was obtained from a sinus in the lateral dorsal region of the neck into a heparinized vacutainer (Owens and Ruiz, 1980; Lutz and Dunbar-Cooper, 1987). Half the blood sample for each animal was used for the determination of deuterium oxide (D_2O) and the other half was centrifuged and used for analyses of plasma parameters.

2.2. Estimations of water flux

At the beginning of the study all animals were weighed to the nearest 0.1 kg and blood sampled. Deuterium oxide (99.9%, Isotec Inc., Miamisburg, OH) was weighed to the nearest 0.1 g in 20 ml syringes. Each turtle was then dosed i.p. with 2 g D_2O kg body mass^{-1}. Empty syringes were then weighed to determine the amount of isotope dosed to the nearest 0.1 g. After dosing, subsequent blood sampling occurred at 12 h, and 2 (T2) and 4 (T4) d post-dosing. After collecting the blood sample on T4, water in the pools was switched to fresh water (1 ± 0.5 ppt) for 4 days and blood samples were taken on days 2 (T6) and 4 (T8) during exposure to fresh water. Following collection of the blood sample on T8, water in the pools was switched back to salt water and blood samples were obtained on days 2 (T10) and 7 (T15). Salinity changes were made by draining the water in the tanks and replacing it with the desired water. The design of the facility facilitated the rapid exchange of water (< 6 min), therefore animals were not left unsupported by water for any extended period. Turtles were exposed to an instantaneous change in salinity when water was replaced in their respective tanks, and not to a

gradual alteration. Exposure to a rapid change in salinity was required in order to fulfill our objective of inducing an abrupt osmoregulatory stimulus and not a gradual stimulus to which the animals may have become adapted.

For isotope measurements blood samples were lyophilized using a freeze trap method and D_2O in captured water measured by fixed filter infrared spectrometry (at 4 μ) (Ortiz et al., 1999). Instantaneous dilution of the isotope within the whole body was defined by the y-intercept (T_0) from a multiple point regression of natural log [D_2O] versus time as previously described (Ortiz et al., 1999). Isotopic dilution space (IDS, in l) was calculated as:

$$\mathrm{IDS} = \frac{D}{1.105[T_0]}$$

where D is the dose of isotope administered (mg), 1.105 is the correction for the density of D_2O versus H_2O, and [T_0] is the concentration of isotope at time zero in ml l^{-1} (Ortiz et al., 1999). In the present study, IDS was used to estimate total body water (TBW). TBW as a percentage of body mass (% TBW) was also calculated.

Rates of water flux were calculated as IDS times the slope (K) of the multiple point regression (Ortiz et al., 1999). For the experimental group, a new regression was determined for each animal during exposure to fresh water and the return to salt water phases. Rate of water flux was calculated from each new regression for each phase on the assumption that the TBW pool size did not change significantly since the body mass did not change significantly over the course of the study (Lifson and McClintock, 1966). Body mass was measured on T0, T4, and T15. The isotopic half time ($t_{1/2}$) of D_2O in the body pool was calculated as $0.693/K$.

2.3. Water budget

A water budget was constructed from water flux rate, dietary water, and estimated metabolic water production to determine the amount of water consumed. Samples of squid fed to the turtles were kept and desiccated to determine the contribution of preformed dietary water to water flux. The metabolic rate was estimated to determine the contribution of metabolic water production to water flux. Metabolic water production was estimated from O_2 consumption values reported for adult green sea turtles (Jackson and Prange, 1979) assuming the following: (1) average daily metabolic rate was three times resting metabolic rate (24 ml kg^{-1} h^{-1}) (Jackson and Prange, 1979); (2) 4.8 kcal l O_2^{-1} (Schmidt-Nielsen, 1990); and (3) 0.11 g H_2O $kcal^{-1}$ (Schmidt-Nielsen, 1990). The difference between mean water flux rate, and the sum of mean dietary and metabolic water was considered consumed water.

2.4. Plasma analyses

Effects of acute fresh water exposure on adrenal steroids, electrolytes, and osmolality were quantified using the same protocol as for the water flux study previously described. Following centrifugation of blood (15 min at 1500g), plasma was placed in cryo-vials and frozen at -20°C for subsequent analyses. Corticosterone was measured using a ^3H radioimmunoassay (RIA) validated for Kemp's Ridley plasma (Valverde et al., 1999). The extraction efficiency of radiolabeled hormone was determined to be 81.4%, and mean recovery for unlabeled corticosterone was 107.4 ± 2.4%. All samples analyzed for corticosterone were measured in the same RIA with an intra-assay coefficient of variability of 7.1%. Aldosterone was measured using a commercially available ^{125}I RIA kit (DPC, Los Angeles, CA) and validated for Kemp's Ridley plasma. Mean recovery of aldosterone from pooled turtle plasma was 94.8 ± 1.1%. All samples were run in the same RIA with an intra-assay coefficient of variability of 5.4%. For both assays, serially diluted pools were parallel to the standard curve. Glucose, Na^+, K^+, and Cl^- were measured on a clinical autoanalyzer (Roche Diagnostics, Somerville, NJ). Plasma osmolality was measured using a freezing point osmometer (Fiske 2400, Norwood, MA).

2.5. Statistics

Means were compared by analysis of variance adjusted for repeated measures over time. A Fisher's PLSD test was applied post hoc if a significant time effect was determined. Means ($\pm$ S.E.) were considered significantly different at $P < 0.05$. Statistics were performed on StatView software (SAS Institute, 1998).

3. Results

3.1. Water flux rates

Final (9.6 ± 1.0 kg) body mass of the turtles was not different from the initial body mass. TBW pool size and % TBW were 7.8 ± 0.7 l and 78.4 ± 1.3%, respectively. Absolute and relative turnover rates were increased significantly by 27 and 26%, respectively, during exposure to fresh water (Table 1). Upon return to salt water, absolute and relative rates were reduced to original levels (Table 1). The increase in water flux reflected an approximately 50% increase in water consumption (Table 2).

3.2. Plasma osmolality, electrolytes, and hormones

Significant changes over time were observed for osmolality, Na^+, K^+, Cl^-, and Na^+:K^+ ratio on T6 and T8 (fresh water phase) (Table 3). Mean plasma osmolality, Na^+, K^+, and Cl^- concentrations were significantly reduced from T0 during exposure to fresh water. Upon returning to salt water, mean plasma osmolality, Na^+, K^+, and

Table 1
Mean water turnover rates, slopes (K), and isotopic half-lifes ($t_{1/2}$) for captive Kemp's Ridley sea turtles ($n = 4$) switched among salt water, fresh water, and salt water

	Salt water	Fresh water	Return to salt water
Absolute turnover (l d^{-1})	1.2 ± 0.1	1.5 ± 0.1*	1.0 ± 0.1
Relative turnover (ml kg^{-1} d^{-1})	123.0 ± 6.8	156.5 ± 10.3*	106.5 ± 5.2
K (d^{-1})	−0.16 ± 0.01	−0.20 ± 0.01*	−0.13 ± 0.01
$t_{1/2}$ (d)	4.4 ± 0.2	3.5 ± 0.2*	5.3 ± 0.3

* Significantly different ($P < 0.05$) means (± S.E.) from salt water phases.

Table 2
Water budget for Kemp's Ridley sea turtles ($n = 4$) exposed to fresh water[a]

	Influx (ml kg^{-1} d^{-1})	Dietary (ml kg^{-1} d^{-1})	Metabolic (ml kg^{-1} d^{-1})	Unaccounted (ml kg^{-1} d^{-1})
Salt water	123 ± 7	6.0 ± 0.6	0.9	116 ± 6.2
Fresh water	157 ± 10*	5.5 ± 0.1	0.9	151 ± 9.9*
Return to salt water	107 ± 5	7.3 ± 0.7	0.9	99 ± 5.3

[a] 'Dietary' and 'metabolic' water refer to preformed water from the diet and estimated metabolic water production, respectively. 'Unaccounted' water was the difference between mean influx and the sum of mean dietary and metabolic water, and reflected water consumption. Means (± S.E.) are reported with significant differences ($P < 0.05$) denoted by an asterisk (*).

Table 3
Mean (± S.E.) plasma osmolality, electrolytes, glucose and adrenal steroids for turtles ($n = 4$) acutely exposed to fresh water for 4 days

	Salt water			Fresh water		Return to salt water	
	T0	T2	T4	T6	T8	T10	T15
Osmolality (mOsm l^{-1})	333 ± 6	331 ± 4	325 ± 3	318 ± 6*	317 ± 3*	331 ± 3	345 ± 5
Na^+ (mmol l^{-1})	153 ± 2	148 ± 3**	150 ± 1	145 ± 2*	144 ± 2*	150 ± 1	153 ± 2
K^+ (mmol l^{-1})	4.3 ± 0.4	4.0 ± 0.2	3.5 ± 0.1**	3.4 ± 0.2*	3.4 ± 0.2*	3.9 ± 0.1	4.3 ± 0.3
Na^+:K^+	37 ± 3	38 ± 2	43 ± 1**	43 ± 2*	43 ± 2*	39 ± 1	36 ± 2
Cl^- (mmol l^{-1})	123 ± 1	121 ± 1	121 ± 1	114 ± 2*	114 ± 2*	118 ± 1**	121 ± 2
Glucose (mmol l^{-1})	4.4 ± 0.3	4.4 ± 0.3	4.7 ± 0.2	4.4 ± 0.2	4.4 ± 0.3	4.7 ± 0.1	4.6 ± 0.3
Aldosterone (pg ml^{-1})	351 ± 32	362 ± 42	353 ± 43	357 ± 38	337 ± 33	368 ± 39	381 ± 51
Corticosterone (pg ml^{-1})	92 ± 22	339 ± 220	297 ± 103	307 ± 121	219 ± 61	227 ± 77	97 ± 28

* Significantly different ($P < 0.05$) from T0, and T10 and T15.
** Significantly different ($P < 0.05$) from T0.

Cl^- values were significantly increased to initial salt water phase levels. When turtles were exposed to fresh water for 4 d, mean Na^+:K^+ ratio was significantly elevated from T0 and significantly reduced upon returning to salt water (Table 3). A significant decrease in K^+, and a concomitant increase in Na^+:K^+ ratio, were observed between T0 and T4. However, means for K^+ and Na^+:K^+ ratio between T4 and, T6 and T8 (exposure to fresh water) were not different with means returning to original levels upon returning to salt water (Table 3). A significant change in mean plasma aldosterone and corticosterone concentrations was not detected when turtles were switched among the salt water, fresh water, and return to salt water phases (Table 3).

4. Discussion

The ability of salt glands to excrete excess electrolytes allows sea turtles to obtain free water by drinking salt water (Kooistra and Evans, 1976; Bennett et al., 1986; Marshall and Cooper, 1988). The present study not only supports previous suggestions that sea turtles drink salt water, but also indicates that water flux is elevated during acute fresh water exposure. This elevation in water flux during exposure to fresh water resulted in a decrease in plasma osmolality and electrolytes. However, decreases in plasma ionic and osmotic concentrations in response to acute fresh water exposure did not elicit a change in plasma adrenal steroids in the present study. The present study provides the first attempt to quantify the effects of acute fresh water exposure on water flux rates, adrenal steroids, and electrolyte concentrations in sea turtles as well as the first report of aldosterone concentrations in any sea turtle. Concentrations are comparable to those reported for lizards (Bradshaw et al., 1972), approximately fourfold greater than those in the tortoise (Uva et al., 1982), and approximately 36-fold lower than those in sea snakes (Duggan and Lofts, 1978).

Previous studies have estimated salt water drinking in sea turtles to range from 13.3 ml kg^{-1} d^{-1} in unfed juvenile green turtles (93.9 ± 2.3 g) (Holmes and McBean, 1964) to 40 ml kg^{-1} d^{-1} in hatchling loggerhead turtles (approximately 20 g) (Bennett et al., 1986). Prange (1985) calculated a drinking rate of salt water of approximately 15 ml kg^{-1} d^{-1} for a 50 kg turtle. Water flux rates previously estimated by the less conventional methods are approximately 2.5 to 7-fold lower than those estimated by deuterium dilution in the present study. Whether the discrepancies in estimated water flux rates can be attributed to differences in methodologies, species, or body mass remains to be investigated further, regardless all the estimates indicate that salt water drinking is a common behavior in marine turtles.

Exposure of sea turtles to fresh water results in an acute reduction in Na^+ efflux (Evans, 1973; Kooistra and Evans, 1976) which may be an adaptive mechanism to abate the potential for hyponatremia induced by an hypo-osmotic environment. Therefore, reduced Na^+ efflux may be expected to be associated with a decrease in water flux in order to more effectively reduce the loss of body Na^+. However, sea turtles in the present study exhibited an increase in water flux and estimated water consumption during exposure to fresh water. This apparent contradiction in mechanisms suggests that sea turtles may have a hindered physiological mechanism for differentiating between salt and fresh water drinking since through their evolutionary development these animals have not had to adapt to fresh water environments, and are not commonly found in fresh water.

The observed increase in water flux during exposure to fresh water resulted in a decrease in ionic and osmotic concentrations. The observed decrease in plasma osmolality, Na^+, K^+, and Cl^- during exposure to fresh water suggests that electrolyte homeostasis in sea turtles is dependent on the animal's habitat. Two possibilities associated with an increase in water flux that may be responsible for the decline in ionic and osmotic concentrations observed in the present study are: (1) increased efflux of solutes or (2) hemodilution. Because sea turtles have been shown to reduce Na^+ efflux while in fresh water (Evans, 1973; Kooistra and Evans, 1976), the observed decreases in ionic and osmotic concentrations were most likely the result of hemodilution.

Previous studies of sea turtles have demonstrated a reduction in plasma Na^+ in response to fresh water exposure and an elevation in plasma Na^+ following exposure to an increased salinity environment (Holmes and McBean, 1964; Kooistra

tra and Evans, 1976). However, the adrenocorticoid responses to the aforementioned manipulations were not quantified. Tortoises maintained on a high salt diet demonstrated an increase in Na^+ within 5 d which was associated with a reduction in aldosterone and an elevation in corticosterone (Uva et al., 1982). In a marine adapted mammal, West Indian manatees (*Trichechus manatus*) held in fresh water then acutely exposed to salt water exhibited an increase in plasma electrolytes and osmolality, a concomitant decrease in plasma aldosterone, and a decrease in water influx within 48 h (Ortiz et al., 1998, 1999). In the present study, acute fresh water exposure reduced plasma Na^+ and osmolality, but did not result in an increase in aldosterone and a decrease in corticosterone. Sea snakes exposed to fresh water also did not exhibit an increase in aldosterone even though Na^+ and osmolality were significantly reduced within 10 d of exposure (Duggan and Lofts, 1978) suggesting that the adrenocorticoid response to an hypo-osmotic environment may be delayed in marine reptiles when compared to terrestrial reptiles and marine mammals.

Alternatively, the mechanism of aldosterone stimulation may have been masked by conflicting stimuli. Although decreased Na^+ is a potent stimulant of aldosterone release, a reduced Na^+:K^+ ratio has been suggested as a better stimulus of aldosterone release (Morris, 1981; Funder, 1993). The reduced Na^+-induced aldosterone release mechanism may have been masked by elevated Na^+:K^+ ratio, which would inhibit aldosterone release. Therefore, the lack of a change in aldosterone despite a reduction in plasma Na^+ may not have been anomalous in view of the increased Na^+:K^+ ratio.

The direct relationship between Na^+ and corticosterone seen in the tortoise (Uva et al., 1982) has also been demonstrated in sea snakes (Duggan and Lofts, 1978) suggesting that corticosterone has a role in hydromineral balance in reptiles. However, this correlation was not apparent in sea turtles in the present study. The resulting increase in Na^+ following the transition from fresh water to salt water should have stimulated an elevation in corticosterone concentration, but this was not observed. As in the situation with a lack of a reduced Na^+-induced aldosterone release, the stimulus may not have been sufficient to elicit a corticosterone response comparable to that seen in the tortoise and sea snake.

In summary, acute fresh water exposure elicited an increase in water flux rates that resulted in a reduction in osmotic and ionic concentrations in Kemp's Ridley sea turtles. The increased water flux rates during fresh water exposure suggests that the control of drinking behavior while in fresh water is delayed, if not lost. Although reductions in plasma Na^+ and osmolality were observed during exposure to fresh water, these decreases did not elicit a significant change in aldosterone or corticosterone suggesting that a more severe reduction in plasma Na^+ may be required to stimulate the release of these adrenal steroids in sea turtles. Alternatively, the increased ratio of Na^+:K^+ may have masked the signal of reduced Na^+ to stimulate aldosterone release. Therefore, the lack of a change in aldosterone concentration during exposure to fresh water may have been the result of these conflicting signals. If so, corticosterone release in sea turtles may also be influenced by the Na^+:K^+ ratio. The lack of a change in adrenal steroids after 4 d of exposure to fresh water also suggests that adrenocorticoid mediation is delayed in sea turtles when compared to marine mammals, and may require prolonged exposure as in sea snakes. Our study supports the contention of Bennett et al. (1986) that osmoregulation in sea turtles depends on the ability to drink sea water and to excrete the ingested salt via the lachrymal gland. We add that prolonged exposure to fresh water without salt supplements will diminish their osmoregulatory capacity, although acute exposure appears to be inconsequential. In perspective, because acute exposure to fresh water did not produce any deleterious effects, estuaries, which have a large range in salinity, may be an important refuge for sea turtles during some aspects of their life history.

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