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# Body Composition and Water Turnover Rates of Bottle-Fed West Indian Manatee (*Trichechus manatus*) Calves

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## Abstract

Estimation of body composition and water turnover rates can provide important indices of an animal's health and well-being. This data becomes especially important for a highly endangered species, such as the West Indian manatee (*Trichechus manatus*). Information on body composition and water turnover rates in nursing manatee calves is unavailable; therefore, the present study describes a unique opportunity to estimate, by isotopic dilution, body composition and water flux *in vivo* in bottle-fed calves of the West Indian manatee held in captivity. A calf held in fresh water was measured at 9 mo (124.8 kg) and 12 mo (152.6 kg) to examine the effects of growth. Over this 3-mo period, absolute fat mass (FM) increased 70.4% and absolute water turnover rate increased from 4.7 to 9.7 l/d, illustrating how these parameters change as a function of the animal's growth. To examine the effects of salinity on water flux, another calf (110.0 kg), held in salt water, was restricted from fresh water. During freshwater restriction, the calf's water turnover rate was 2.9 l/d and could be accounted for solely by dietary and metabolic water. When the animal was given access to fresh water, turnover rate increased to 4.0 l/d, for which 1.3 l/d could not be accounted, suggesting that nursing calves do not drink salt water. Collectively, the results provide a unique data set for nursing manatee calves and suggest that nursing calves, similar to adults, do not engage in mariposia. In addition, nursing calves will drink when given access to fresh water; however, the contribution of drinking fresh water to the growth and development of nursing calves remains to be examined. These data should provide useful information when implementing the proper husbandry and management plans for both fresh- and saltwater habitats of such a highly endangered species as the West Indian manatee.

**Key Words:** West Indian manatee, Florida manatee, *Trichechus manatus*, endangered species, marine mammals, sirenians, fat mass, deuterium oxide, isotopic dilution, $D_2O$

## Introduction

The ability of West Indian manatees (*Trichechus manatus*) to migrate between fresh- and saltwater habitats suggests that calves may be born in either environment. Estimations of body composition and water flux rates in calves inhabiting either environment can provide useful information on the effects of their habitat on metabolism. In captivity, manatees may be held in fresh or salt water, and, thus, the occasional captive-born calf or wild-born calf held for purposes of rehabilitation may be maintained in either water type. The maintenance of captive animals in zoos and oceanaria requires the implementation of appropriate husbandry procedures. Data from captive animals, especially calves, can only augment the husbandry database of these animals, thereby increasing their probability for a successful rehabilitation and release back into their natural habitat. Reintroduction of orphaned or rehabilitated animals is paramount for a highly endangered species such as the West Indian manatee; however, to date, the lack of access to nursing manatee calves has made it extremely difficult to obtain any information on their body composition or water flux rates.

Because increased body fat in marine mammals appears to be an adaptive advantage (Young, 1976; Ortiz et al., 1978; Whittow, 1987), body composition may provide a useful index for determining the degree of rehabilitation and recovery of an orphaned or injured animal. For example, the lower body fat mass (FM) estimated previously in non-nursing manatee calves suggests that calves may be more susceptible to cold water than adults (Ortiz & Worthy, 2004). In addition, metabolically produced water from the oxidation of fat may provide a significant source of water during periods of reduced food consumption (Ortiz et al., 1978; Ortiz et al., 1999). The utilization of fat oxidation for the production of water may be of particular importance for manatees in marine habitats since it has been previously reported that adult manatees do not drink salt water (Ortiz et al., 1999). Previous studies have demonstrated that manatees have the anatomical (Hill & Reynolds, 1989; Maluf, 1989) and physiological (Ortiz et al., 1998) mechanisms to properly osmoregulate should these animals engage in mariposia; however, the potential contribution of drinking to water flux rates has not been examined in nursing manatee calves.

Data on the body composition of captive manatee calves may provide important information on their metabolism, which may be essential for their successful rearing. Therefore, body composition was used as an index to compare manatee calves maintained on a milk formula diet but held in either fresh or salt water. Water turnover rates also were estimated to determine if nursing calves drink salt water since it was reported previously that adult manatees do not engage in this behavior (Ortiz et al., 1999). The present study represents a rare opportunity to obtain data on body composition, total body water (TBW) pool size, and water turnover rates, estimated by deuterium oxide ($D_2O$) dilution (Ortiz et al., 1999), from nursing calves of the West Indian manatee.

## Materials and Methods

$D_2O$ dilution trials were conducted on captive calves that were bottle-fed a milk formula and held in either fresh or salt water. Because manatees in the present study were still bottle-fed a milk formula and were between 9 and 12 mo of age, they were considered calves. The endangered status and extremely limited number of manatees in captivity, regardless of age, resulted in the availability of only a single calf for study in each treatment.

*Animals*

Two turnover trials (October 1992 and January 1993) were conducted on one male calf (9 and 12 mo; 124.8 and 152.6 kg, respectively) held in a freshwater tank at SeaWorld of Florida in Orlando, to examine the effects of growth on body composition and water flux in a nursing calf. The calf was bottle-fed approximately 3.0 and 3.2 l, respectively, of milk formula per day during his two trials.

A male calf (12 mo, 110.0 kg), held in a saltwater tank at the Caribbean Stranding Network (CSN) facility on Isla Magüeyez, Puerto Rico (May 1993), was used to examine the effects of freshwater deprivation on rates of water turnover and to determine if calves drink salt water. Although the calf had been provided with fresh water from a hose on a daily basis prior to the study, turnover trials for this calf consisted of a 15-d freshwater deprivation period followed by 5-d with access to fresh water from a hose. Throughout the trial, the calf was bottle-fed approximately 3 l of milk formula per day.

*Isotopic Dilution*

Animal handling and experimental protocols were similar to those previously described for manatees (Ortiz et al., 1998, 1999; Ortiz & Worthy, 2004). Briefly, manatees were weighed to 0.1 kg in a sling using a hanging load cell at the beginning and end of each trial. Blood samples were taken from the venous plexus in the flipper. An initial blood sample was taken prior to administration of the isotope to determine background levels of $D_2O$. For the freshwater calf, subsequent blood samples were obtained at 12 h and at 2 and 5 d after administration of the isotope for both trials. For the saltwater calf on day 10 of the deprivation phase, a pre-dose blood sample was taken and the isotope was administered. Subsequent blood samples were collected at 12 h and at 2 and 5 d. Following the blood sample on day 5, the calf was given access to fresh water for 2 h/d. Subsequent blood samples were collected on days 2 and 5 following access to fresh water. Animals received a dose of 1 g $D_2O$/kg ($D_2O$, 99.9 atom %, Isotec, Inc., Miamisburg, OH). Blood samples were placed into 10 mL scintillation vials (Kimble Glass, Vineland, NJ), and frozen at -70° C for later analyses of $D_2O$. Water was distilled from whole blood using a freeze trap method, and $D_2O$ was measured using an infrared analyzer (Model 5651, Wilks Scientific, Foxboro/Wilks, Inc., South Norwalk, CT) (Byers, 1979).

Instantaneous dilution space of the isotope was defined as the y-intercept ($T_0$) from a 3-point regression of ln [$D_2O$] versus time (days) (Figure 1) (Ortiz et al., 1999; Ortiz & Worthy, 2004). Isotopic dilution space (IDS) was calculated by

$$\text{IDS (l)} = \frac{\text{DOSE}}{1.105*[T_0]}$$

where, DOSE is the administered dose of $D_2O$ (mg), 1.105 is the correction for the density of $D_2O$ vs $H_2O$, and [$T_0$] is the concentration of $D_2O$ at time zero in ml/l (Ortiz et al., 1999). Because hydrogen isotope dilution has been reported to overestimate TBW by approximately 3 to 4% in many mammals (Nagy & Costa, 1980; Costa, 1987), IDS was corrected by 4% to provide a conservative estimate of TBW. Water turnover rate (l/d) was calculated as the product of TBW and the slope of the 3-point regression. Because body

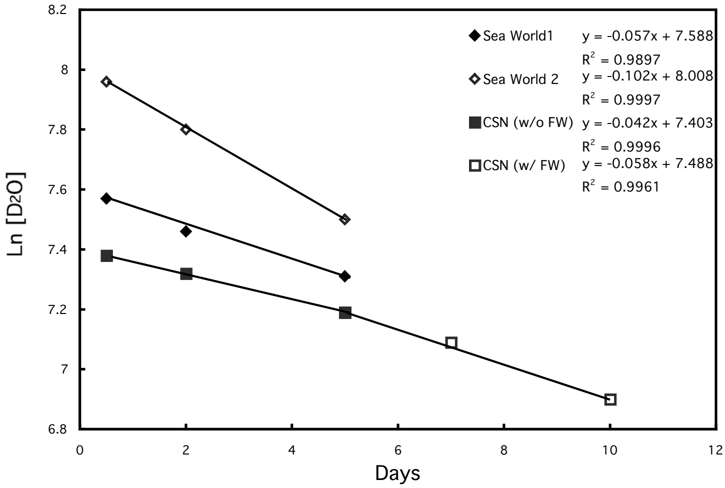


**Figure 1.** Isotopic dilution curves for each manatee's trial; see "Materials and Methods" section for calculations. "Sea World 1" represents the freshwater calf's first trial (10/92), and "Sea World 2" represents the calf's second trial (1/93). "CSN (w/o FW)" represents the saltwater calf's trial during the freshwater deprivation phase, and "CSN (w/ FW)" represents the calf's trial when fresh water was made available.

mass did not change between the beginning and end of the measurement periods, TBW pool size was assumed to stay constant.

Samples of the animal's milk formula were desiccated to estimate the contribution of dietary water to turnover rates. Water content of milk formulas at SeaWorld and CSN were 79.2% and 90.0%, respectively. Dietary water was determined as the amount of milk consumed times its hydration value. Because manatees have been reported to exhibit metabolic rates that are lower (30 to 50%) than predicted from body mass (Gallivan & Best, 1986), metabolic water was calculated from an estimated metabolic rate based on one-half of that predicted from body mass (MR = $70M_b^{0.75}$), and assumed 0.12 ml $H_2O$/kcal (Ortiz et al., 1978). Metabolic water production for all animals averaged 1.20 ± 0.04 ml/kg/d.

*Estimation of Body Composition*

Body composition of the calves was estimated from TBW, assuming that body hydration was 73% (Pace & Rathbun, 1945) and that the GI-tract accounted for 10% of body mass (Reynolds & Rommel, 1996) and was 90% hydrated (Byers & Schelling, 1986). Empty body mass (EBM) was the difference between BM and GI-tract mass. Empty body water (EBW) was the difference between TBW and GI-tract water. Lean body mass (LBM) was equal to EBW divided by 0.73, and % LBM was calculated as a function of EBM. The difference of LBM from 100 was equal to fat mass (FM). Percent FM also was calculated as a function of EBM.

## Results

During January 1993, the calf at SeaWorld possibly was seen eating small quantities of lettuce leaves fed to the other manatees in the tank; however, the absolute amount was too small to be quantified. Over the 3-mo between each of his trials, this calf increased his TBW pool size by 16.1% and his absolute FM by 70.4% (Table 1). Also, relative (37.3 and 63.6 ml/kg/d, respectively) and absolute

**Table 1.** Body composition estimates of manatee calves fed artificial milk formula

| Calves | Age (mo) | Body mass (kg) | Empty body mass (kg) | Total body water (l) | Lean body mass (kg) | Fat mass (kg) | Fat mass/ Empty body mass (%) |
|---|---|---|---|---|---|---|---|
| SeaWorld (10/92) | 9 | 124.8 | 112.3 | 82.0 | 98.1 | 14.2 | 12.6 |
| SeaWorld (1/93) | 12 | 152.6 | 137.3 | 95.2 | 113.1 | 24.2 | 17.6 |
| CSN (5/93) | 12 | 110.0 | 99.0 | 67.8 | 80.3 | 18.7 | 18.9 |

CSN = Caribbean Stranding Network calf

**Table 2.** Estimates of water turnover rates and a water budget in bottle-fed manatee calves

| Calves | Water | $H_20$ turnover (l/d) | Dietary $H_2O$ (l/d) | Metabolic $H_2O$ (l/d) | Unaccounted (l/d) |
|---|---|---|---|---|---|
| SeaWorld (10/92) | FW | 4.7 | 2.6 | 0.150 | 1.9 |
| SeaWorld (1/93) | FW | 9.7 | 2.7 | 0.184 | 6.8 |
| CSN (w/o fresh water) | SW | 2.9 | 2.8 | 0.132 | 0.0 |
| CSN (w/fresh water) | SW | 4.0 | 2.6 | 0.132 | 1.3 |

FW = freshwater habitat; SW = saltwater habitat; "Dietary $H_2O$" refers to preformed water in the milk; "Metabolic $H_2O$" was calculated assuming a metabolic rate one-half of that predicted from body mass; "Unaccounted" represents the approximate volume of water consumed

water turnover rates almost doubled between the two sampling periods (Table 2).

While the calf in salt water (CSN) was deprived of fresh water, his turnover rate could be accounted for by dietary and metabolic water. When fresh water was made available, the calf increased his turnover rate by 1.3 l/d (Table 2). When the calf had access to fresh water, his relative water turnover rate (35.9 ml/kg/d) was similar to that of the freshwater calf when their body masses were most similar. Interestingly, when his tank was drained for blood sampling, fecal samples and urination were only observed during the freshwater phase of the trial.

## Discussion

For marine mammals, percent body fat may provide a useful index for monitoring the care of captive animals, which could lead to a successful rehabilitation and release back into the wild. Body fat may be of particular importance since this component of body composition may provide a significant source of water and energy, especially during unfavorable conditions such as reduced food availability (Ortiz et al., 1978; Ortiz et al., 1999). For example, during the dry season in the Amazon, Amazonian manatees (*Trichechus inunguis*) may experience periods of reduced food availability of 6 to 8 mo (Best, 1983). Best calculated that a 300-kg manatee with a manatee's standard metabolic rate (about 36% of the normal eutherian metabolic rate) could survive 310 d of fasting before rendering its lipid reserves. Although nursing calves would continue to obtain nutrition from their mothers, and thus, place the burden on the mother, an idea of water flux rates in nursing calves can be useful in determining the amount of fat a mother should have to maintain both herself and a calf during periods of reduced food availability.

Water produced from the oxidation of body fat could be a significant source of water for saltwater manatees during periods of reduced food consumption (Best, 1983; Ortiz et al., 1999). Therefore, calves with a greater FM have the potential to produce a greater volume of water from fat oxidation, which may be significant, for instance, during the movement of a mother-calf pair between salt- and freshwater habitats. Despite the 42.6-kg discrepancy in body mass, the two calves at 12 mo of age had similar relative FM, suggesting that factors such as quality of milk formula, condition of captivity, and water salinity may have a greater influence on body composition than age alone in nursing calves. The 70% increase in absolute FM measured in the freshwater calf provides an indication of the rate of FM deposition (approximately 3.3 kg/mo) in nursing calves. Calves in the present study that were fed a milk formula diet exhibited TBW pool size and % FM values similar to those previously reported for calves on a lettuce diet (Ortiz & Worthy, 2004).

Consistent with a previous study on water turnover rates in adult manatees (Ortiz et al., 1999), the present study suggests that nursing West Indian manatee calves also do not drink salt water. The turnover rate for the bottle-fed calf in salt water could be solely accounted for by the contribution of dietary and metabolic water when the calf was deprived of fresh water. When fresh water was made available, his turnover rate increased, for which 1.3 l/d could not be accounted. This increased turnover is most likely attributed to freshwater drinking, which is supported by observations made of the calf holding his mouth open under the stream of water for periods as long as 20 min. Age also does not appear to influence water flux rates as the rates between the two calves were most similar when the freshwater calf was 9 mo and the saltwater calf (with access to fresh water) was 12 mo. Also, the mass-specific turnover rate for the calf in salt water (26.1 ml/kg/d) is similar to that reported for saltwater manatees without access to fresh water (23 ± 8 ml/kg/d) that did not drink salt water (Ortiz et al., 1999); however, calves in or given access to fresh water drank between 1.3 and 6.8 l/d. Since mariposia appears to be absent in this species, the fact that nursing calves can drink 32 to 70% of their turnover rate suggests that fresh water habitats may be a more important environment for rearing offspring. Thus, a study comparing the growth and development of manatee calves reared in fresh vs salt water would be fruitful to better understand the impacts of salinity on their metabolism.

In summary, mass-specific water turnover rates in the saltwater calf were similar to those reported for adult manatees in salt water, which also did not drink; however, calves in or given access to fresh water drank up to 70% of their turnover rate. This latter observation is important for researchers considering the application of methodologies employing isotopes of water to manatees held in fresh water. Although interpretation of these data is restricted by the limited sample size, important observations were still made on the calves of an extremely endangered species and in an extremely rare opportunity to make such physiological observations. Because the nursing calf in fresh water or given access to fresh water drank relatively large amounts of water, freshwater habitats may prove to be important for the proper development and growth of nursing calves in the wild. To this end, a study addressing the hypothesis that naturally nursing freshwater calves drink more than their saltwater counterparts, and thus, have greater growth curves may be fruitful to a better understanding of the importance of the different habitats to the development of manatee calves. These results may provide a significant contribution to the implementation of husbandry procedures during the rehabilitation of orphaned or injured manatee calves, and to the management policies for free-ranging West Indian manatees.

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