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Monitoring Oral Temperature, Heart Rate, and Respiration Rate of West Indian Manatees (*Trichechus manatus*) During Capture and Handling in the Field

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Abstract

West Indian manatees (*Trichechus manatus*) are captured, handled, and transported to facilitate conservation, research, and rehabilitation efforts. Monitoring manatee oral temperature (OT), heart rate (HR), and respiration rate (RR) during out-of-water handling can assist efforts to maintain animal well-being and improve medical response to evidence of declining health. To determine effects of capture on manatee vital signs, we monitored OT, HR, and RR continuously for a 50-min period in 38 healthy, awake, juvenile and adult Florida manatees (*T. m. latirostris*) and 48 similar Antillean manatees (*T. m. manatus*). We examined creatine kinase (CK), potassium (K^+), serum amyloid A (SAA), and lactate values for each animal to assess possible systemic inflammation and muscular trauma. OT range was 29.5 to 36.2° C, HR range was 32 to 88 beats/min, and RR range was 0 to 17 breaths/5 min. Antillean manatees had higher initial OT, HR, and RR than Florida manatees ($p < 0.001$). As monitoring time progressed, mean differences between the subspecies were no longer significant. High RR over monitoring time was associated with high lactate concentration. Antillean manatees had higher overall lactate values ([mean ± SD] 20.6 ± 7.8 mmol/L) than Florida manatees (13.7 ± 6.7 mmol/L; $p < 0.001$). We recommend monitoring manatee OT, HR, and RR during capture and handling in the field or in a captive care setting.

Key Words: West Indian manatees, *Trichechus manatus*, health, monitoring, heart rate, lactate, physiology, body temperature, respiration, manatee

Introduction

The Florida manatee (*Trichechus manatus latirostris*) and Antillean manatee (*T. m. manatus*) are endangered subspecies of the West Indian manatee (Deutsch et al., 2008; International Union for Conservation of Nature [IUCN], 2010). Given their status, multiple federal, state, private, and nonprofit organizations have been working together to protect and conserve the species from anthropogenic and natural threats to their populations.

Conservation plans include population monitoring studies, radio tracking studies, health assessment, research, rescues, and rehabilitation efforts (U.S. Fish and Wildlife Service [USFWS], 2001; Florida Fish & Wildlife Conservation Commission [FFWCC], 2007). These activities involve regular capture and handling of wild individuals.

During these interactions, qualified scientists and veterinarians assess the overall health of the manatee. An animal's general appearance, including skin condition, body condition, girth and other morphometrics, and dorsal subcutaneous fat thickness (Ward-Geiger, 1997), are assessed to provide indicators of individual health and condition. However, the evaluation of manatee health based on monitoring of physiological parameters such as oral temperature (OT) (as a proxy for core body temperature), heart rate (HR), and respiration rate (RR) is not well-defined.

A peracute stress response in wild manatees as a result of the capture and handling process has been documented by assaying for adrenocorticotropic hormone (ACTH) concentrations in plasma (Tripp et al., 2010). While capture-related deaths are rare, field biologists encounter animals during research captures and rescues with signs of bradycardia, apnea, hyperthermia, and hypothermia when compared to healthy resting individuals in a captive setting (Scholander & Irving, 1941; Gallivan et al., 1983; Irvine, 1983; Bossart, 2001; Murphy, 2003). Such clinical signs may result from an immediate stress response to capture or from a pre-existing pathologic condition such as manatee cold stress syndrome (Bossart et al., 2002). In either case, reducing the degree and duration of possible human-induced stress during manatee captures is important for the well-being of the individual.

Understanding OT, HR, RR, and blood chemistry values of field-captured manatees aids in the assessment of manatee health. The purpose of this research was (1) to determine the ranges and dynamics of manatee OT, HR, and RR over monitoring time during field captures; (2) to assess creatine kinase (CK) activity, potassium (K^+), serum amyloid A (SAA), and lactate concentrations in blood (all biochemical parameters that may indicate stress); and (3) to improve manatee triage and handling in the field.

Exercise physiology studies of the horse demonstrate an increase in HR, RR, rectal temperature, and lactate values in relation to increased exertion (Bayly et al., 1987; Rose et al., 1988; Perrone et al., 2000). However, captured dolphins demonstrate efficient body heat regulation, and they can effectively dissipate heat via a highly vascularized dorsal fin while being held at the water's surface after capture (Meagher et al., 2002). Unlike dolphins, captured manatees are too powerful to be physically restrained in the water, requiring health assessments to occur on land. During this time, the manatee's skin is kept wet. However, the change from water to air medium could potentially compromise the ability of the manatee to dissipate body heat. We hypothesized that while being held on land, captured wild manatees would experience elevated OT, HR, RR, and lactate values. We further hypothesized that RR would have a positive correlation with lactate values.

Materials and Methods

Manatees Sampled

The capture, health assessment, and radio tagging of manatees were authorized under U.S. Fish and Wildlife Service research permits MA773494 (Florida Fish and Wildlife Conservation Commission) and MA791721 (U.S. Geological Survey), Belizean Department of Forestry permit CD/60/3/05(36) (Wildlife Trust), and Puerto Rican research permit DRNA-05-EPE-002. In this study, we monitored vital signs of 38 Florida manatees and 48 Antillean manatees captured for population, health, and behavioral studies during the years 2004 through 2006 in Florida, Belize, and Puerto Rico (Tables 1 & 2). Manatees were captured at various times of the year, but in Florida most captures occurred during the winter months (December through March) as part of studies to better understand manatee use of warm-water refugia (Laist & Reynolds, 2005).

Capture of West Indian Manatees

In general, capture of manatees consisted of efforts from many people through the use of a capture boat, support boats, and aerial observation (Weigle et al., 2001). The capture boat was a motorized, modified mullet skiff with a removable transom, a raised control console for enhanced viewing, and a large seine net with surface floats and a lead weighted bottom. One to three small motorboats provided on-the-water safety support, assistance in locating animals, and additional animal handlers once the net was set on the manatee. A capture crew consisted of a minimum of ten people. To help locate manatees, an observer in a small plane or helicopter was used when possible. We made efforts to avoid harassing or capturing of cow-calf pairs. Once located, the target manatee was encircled by the capture boat in a number 30 or 56 braided seine twine net 122 to 183 m long and up to approximately 8 m deep, with a 10- to 17-cm stretch knotted nylon mesh. The net and manatee were then pulled into the boat.

In Tampa, Florida, and Belize, capture methods were adapted to enable capture of multiple animals simultaneously (Reid et al., 1995; Deutsch et al., 1998; Auil et al., 2007). In Tampa, Florida manatees were captured using a land-based net set that typically allowed for entrapment of one to four

Table 1. West Indian manatee (*Trichechus manatus*) capture locations and dates, 2004-2006

	Coordinates		Date
Capture location	Latitude	Longitude	Month Year
Florida			
1. Apollo Beach, Tampa: near TECO Big Bend power plant	27°47'34.01"N	82°25'15.87"W	December 2004, 2005 March 2005 January 2006
2. Port of the Islands, Naples: residential and marina basin, Faka Union Canal	25°57'19.74"N	81°30'34.03"W	January 2004 April 2004
3. Everglades National Park: areas of Whitewater and Coot Bay	25°18'9.77"N	80°59'9.37"W	June-July 2005 December 2005
Belize			
4. Southern Lagoon: inshore waterway	17°15'21.21"N	88°20'39.78"W	November 2004, 2005
5. Drowned Cays: offshore waters	17°28'05.25"N	88°04'23.40"W	April 2005
Puerto Rico			
6. Boqueron: Rincón Canal	18°01'12.32"N	67°11'56.15"W	June 2004
7. Guayanilla: Bahia de Guayanilla	17°59'12.49"N	66°46'15.64"W	April 2005
8. Ceiba: nearshore waters off former Roosevelt Roads Naval Air Station	18°12'46.40"N	65°37'12.71"W	May 2005

Table 2. Size classes of West Indian manatees that were monitored for oral temperature (OT), heart rate (HR), and respiration rate (RR) during field captures, 2004-2006 [a]

	Juvenile (206-265 cm)		Adult (> 265 cm)		
Location	Male	Female	Male	Female	Total
Florida	9	5	13	11	38
Belize	8	15	6	6	35
Puerto Rico	5	4	1	3	13
Total	22	24	20	20	86

[a]Size classes are based on straight length measurements (Marmontel, 1993).

animals, and in one instance up to 11 manatees, along a shoreline. Captured manatees were placed in stretchers and carried by handlers to a nearby work-up site onshore. In Belize, a two-stage capture method was implemented. An open water net set was used to surround one to three manatees in relatively shallow (1.5 to 2.0 m deep) water. Then, experienced handlers entered the water and corralled an individual manatee into a smaller net with a bag end. Once secured in the bag end of the net, the manatee was manually guided to the stern of the capture boat and manually lifted aboard for processing. Once processing was finished, the manatee was taken a short distance away from the capture site and manually lowered in a stretcher back into the water. While not all manatees were fitted with radio telemetry tags, all manatees were observed post capture for several minutes to confirm normal swimming and surfacing behavior.

Time of net set, time when manatee was secured for health assessment, and time of release were recorded. Total straight length and body weight were measured. Relative age classifications were determined for each manatee based on straight length ranges of 206 to 265 cm for juveniles (i.e., large calves and subadults) and > 265 cm for adults (Marmontel, 1993). Fourteen calves measuring < 206 cm were also captured, but they were not included in these analyses.

Oral Temperature (OT), Heart Rate (HR), and Respiratory Rate (RR)

Monitoring of manatee OT, HR, and RR began immediately after securing the animal on boat or land, and it continued every 5 min until the animal's release. A Radioshack® (RS) indoor/outdoor thermometer (model 63-1009A or 63-1035; Radio Shack, Fort Worth, TX, USA) was used to measure OT. The manufacturer's specified accuracy of the thermometers is ± 1° C at 0 to 40° C, which was verified in the laboratory. To measure OT, the thermometer probe was placed in the buccal cavity past the last molar by gently pushing it along the buccal aspect of the mandibular teeth to the back of the mouth (Wong et al., 2007). Temperature was then recorded manually every 5 min. The position of the probe was frequently checked to ensure consistency of OT measurements.

A Sonocardia® waterproof stethoscope (Magnafortis, Seattle, WA, USA) was used to auscult the heart and obtain HR values every 5 min. The stethoscope bell was inserted under the manatee's left or right axilla and placed ventrally, close to the midline beneath the sternum (Wong et al., 2007). A single heart beat was defined as an atrial and ventricular contraction. The number of heart beats were counted over 15 s and multiplied by four to determine beats/min (bpm). Additionally, a Mac 500® electrocardiogram (ECG) (GE Healthcare, Milwaukee, WI, USA) was used to assess heart electrical activity and HR. Limb leads were attached as described by Siegal-Willott et al. (2006) with two fore-limb leads secured 2 to 7 cm cranial to the left and right pectoral flippers, and two hind-limb leads secured 10 cm caudal to the left and right pectoral flippers using LLEBL Electrode Series® electrodes (Lead-Lok, Inc., Sandpoint, ID, USA). Electrocardiographs displaying artifacts such as distorted baselines or indistinguishable PQRS complexes were considered unreliable for measurement of HR and other ECG parameters and were discarded from the study (Siegal-Willott et al., 2006). HR obtained by auscultation by the authors (Wong, Siegal-Willott, & Bonde) was considered the optimal sampling method and was used to assess accuracy and reliability of the ECG recording by comparison.

Documentation of a breath was based on observation of thoracic movement, auscultation of respiratory exchange at the nares, and/or palpation of air movement at the external nares (Wong et al., 2007). Time of respiration was recorded manually. RR was defined as the number of breaths/5 min. For the safety of the manatee, when RR was less than 2 breaths/5 min, an attendant poured water onto the dorsal cranium to induce a breath (Lanyon et al., 2010; Stamper & Bonde, in press).

Blood Sampling and Biochemical Analysis

Phlebotomy from either the medial or lateral aspects of the brachial vascular bundle was performed, and blood was collected into EDTA, lithium heparin, and serum separator tubes within 20 min of capture (Medway et al., 1982; Bossart et al., 2001; Stamper & Bonde, in press). Blood samples were stored in a cooler, on top of a small foam pad on wet ice, following collection. Heparinized plasma and serum were separated by centrifugation at 6,000 × g for 10 min within 1 h of collection. Florida processed samples were transported on ice at 4 to 10° C until analysis within 48 h after collection. Belize and Puerto Rico processed samples of serum and plasma were stored at -20° C until they could be transported for analyses. The University of Florida's College of Veterinary Medicine Clinical Pathology Laboratory performed complete blood count, biochemical analysis of plasma and serum, and plasma protein gel electrophoresis as previously described (Harr et al., 2006; Harvey et al., 2007, 2009).

Statistical Analyses

For a 50-min monitoring session, OT, HR, and RR data were considered incomplete and discarded if 10 min or more of continuous monitoring time was not recorded for each manatee. A repeated measures linear mixed model was carried out using *SAS* (*Proc Mixed*; SAS Institute, Cary, NC, USA) to determine if there was a significant difference between the OT, HR, and RR of manatees based on the fixed effects of location: Puerto Rico, Belize, and Florida. No significant difference between Puerto Rico and Belize was found. Based on these results further analyses were conducted based on subspecies, with time since capture, sex, and size class as additional independent variables. Type 3 sums of squares and differences of the least square means for significant fixed effects were calculated.

Descriptive statistics were generated for capture times in Florida, Puerto Rico, and Belize. Descriptive statistics were also generated for air and water temperatures between captured Florida and Antillean manatees using *Microsoft Office Excel 2003 for Windows* (*Excel*) (Microsoft Corp., Redmond, WA, USA). To assess the difference in Florida and Antillean manatee mass size, an unpaired *t*-test was conducted comparing the relative weights of the subspecies based on their straight length in *Excel*.

Using *Sigma Stat for Windows*, Version 3.1 (Systat Software Inc., Point Richmond, CA, USA), *t*-tests were calculated at a significance level (Type I error rate) of $\alpha = 0.05$, whereby the null hypothesis was defined as the two groups lacking significant difference. Specifically, unpaired *t*-tests were conducted to examine differences among Florida and Antillean manatee CK activity, K^+, SAA, and lactate values to determine if there were any significant variations between the subspecies. Based on normal reference intervals for Florida manatees (Harr et al., 2006; Harvey et al., 2007), we examined manatees with CK activity > 482 U/L, K^+ > 6 mEq/L, SAA > 70 µg/mL, and lactate values > 25 mmol/L for any abnormalities in OT, HR, and RR.

A Pearson's product moment correlation was conducted between mean RR over the first 20 min of monitoring (20-min mean RR) and lactate values. To further assess the relationship between RR and lactate, we conducted an unpaired Student's *t*-test between lactate values associated with a 20-min mean RR ≤ 5.25 breaths/5 min (25th percentile) and a RR > 5.25 breaths/5 min using *Excel*.

Results

Capture Conditions

Capture times averaged 42.2 ± 62.6 min in Florida and 43.0 ± 27.4 min in Belize from the time of net set to when the manatee was secured for examination. In Puerto Rico, animals were secured for examination within 5 min of being netted. The time to process the animal (i.e., measurements, health assessment, tagging, etc.) averaged just under 1 h in Florida and Puerto Rico, and at 85 min in Belize (Table 3). During captures on 14 December 2004 in TECO, Tampa Bay, Florida, 11 manatees were netted at one time in a land set. Logistical constraints prevented these manatees from all being immediately processed. Five of these captured manatees (CTB046, TTB117, TTB109, TTB081, and TTB115) were included in this study. These manatees were held for extended time periods prior to examination in water that was just deep enough for them to be fully submerged. Their mean time from net set to being secured for examination was longer than normal, 189 ± 68.3 min (range: 146 to 271 min). If we omit the capture times of these animals, the adjusted mean Florida capture time from net set to being secured for examination is calculated to be 22.7 ± 24.6 min (Table 3). All the manatees in this study appeared to swim and surface normally immediately upon release.

In Florida, the predominance of captures during the winter months (Table 1) resulted in relatively colder air and water temperature exposures for 31 of the 38 sampled manatees. The mean air temperature of all 38 sampled Florida manatees was 23.8° C, and the mean water temperature was 25.9° C (Table 4). Seven of these manatees were captured and monitored in the warmer months of April, June, and July when the mean air temperature was 30.5 ± 2.8° C and the mean water temperature was 29.0 ± 4.4° C. In Belize and Puerto Rico, mean air temperature was 30.7° C and the mean water temperature was 28.9° C (Table 4).

Florida and Antillean Manatee Size

For the sample overall, manatees ranged in straight-line length from 208 to 316 cm and in body mass from 176.9 to 597.8 kg. Weights were collected for 31 of the 38 monitored Florida manatees and 16 of the 48 Antillean manatees. Although data were incomplete, in general, Florida manatees appeared larger and heavier than Antillean manatees of the same straight-line length (Figure 1). Due to limited weight data, statistical comparison of subspecies mass was limited to a straight length range of 212 to 299 cm. Within this comparable data range, Florida manatees ($n = 25$) were significantly larger than Antillean manatees ($n = 16$; $p = 0.0375$), with mean weights of 380.2 ± 108.6 kg and 312.7 ± 77.5 kg, respectively.

Oral Temperature

Oral temperature ranged from 29.5 to 36.2° C, with no difference in OT over monitoring time based on size class or sex. During the initial 5-min monitoring interval, Florida manatees' mean OT (32.6 ± 1.8° C) was significantly lower than that of Antillean manatees (34.6 ± 0.9° C; $p < 0.0001$) (Figure 2; Tables 4 & 5). At 40 min post capture, there was no statistically significant difference in OT values between Florida and Antillean manatees, with mean OT values of 34.2 ± 1.6° C and 35.2 ± 1.6° C, respectively ($p = 0.4186$) (Figure 2;

Table 3. Mean capture times (min) of West Indian manatees, 2004-2006

	Net set to secured			Secured to release			Total time		
Location	Mean (±SD)	Median	Range	Mean (±SD)	Median	Range	Mean (±SD)	Median	Range
Florida	42.2 (±62.6)	16.0	4-271	56.2 (±19.1)	52.0	26-110	99.6 (±61.9)	83.0	35-311
Florida (adjusted)[a]	22.7 (±24.6)	15.5	4-109	56.2 (±19.4)	52.0	26-110	78.4 (±25.9)	73.0	35-137
Belize	43.0 (±27.4)	44.0	1-108	85.0 (±22.8)	84.97	42-125	129.7 (±33.3)	133.0	61-177
Puerto Rico	4.9 (±0.9)	5.0	3-6	55.6 (±6.1)	57.0	43-65	60.5 (±6.1)	63.0	48-69

[a]Excludes extended capture times of five manatees from Tampa, Florida, on 14 December 2004.

Table 4. Oral temperatures of field-captured West Indian manatees with associated air and water temperatures from the capture regions, 2004-2006

	Florida			Antillean		
	Mean (±SD)	Median	Range	Mean (±SD)	Median	Range
Air	23.8(±6.0)	22.0	14.7-31.9	30.7 (±3.9)	30.4	24.2-40.8
Water	25.9(±4.2)	25.6	21.5-30.9	28.9 (±1.5)	28.9	25.1-31.5
OT initial	32.6(±1.8)	32.8	29.5-35.1	34.6 (±0.9)	34.9	32.6-36
OT final	34.8(±1.5)	35.1	30.5-35.9	35.2(±0.6)	35.3	34.0-36.1

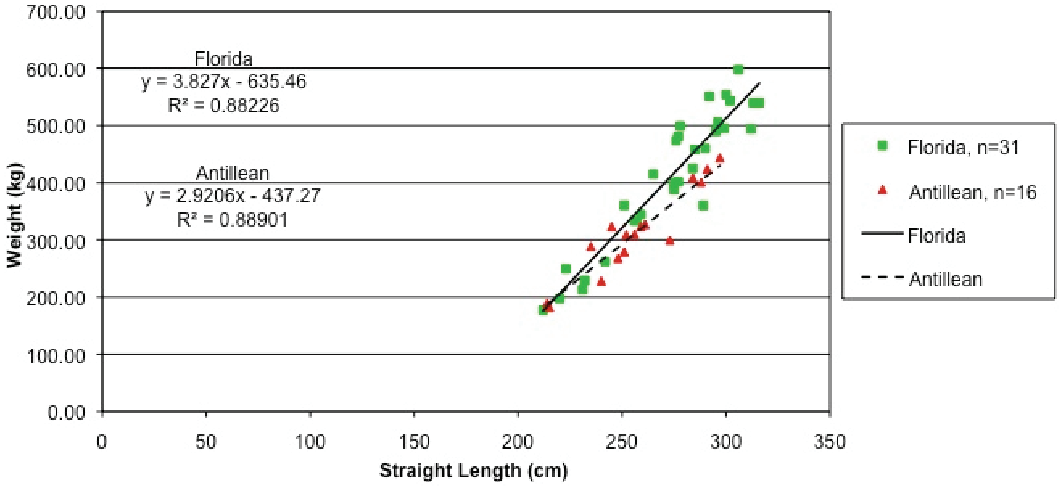

Figure 1. Length and weight relationship of wild-caught Florida manatees and Antillean manatees from Puerto Rico and Belize during the years 2004 through 2006

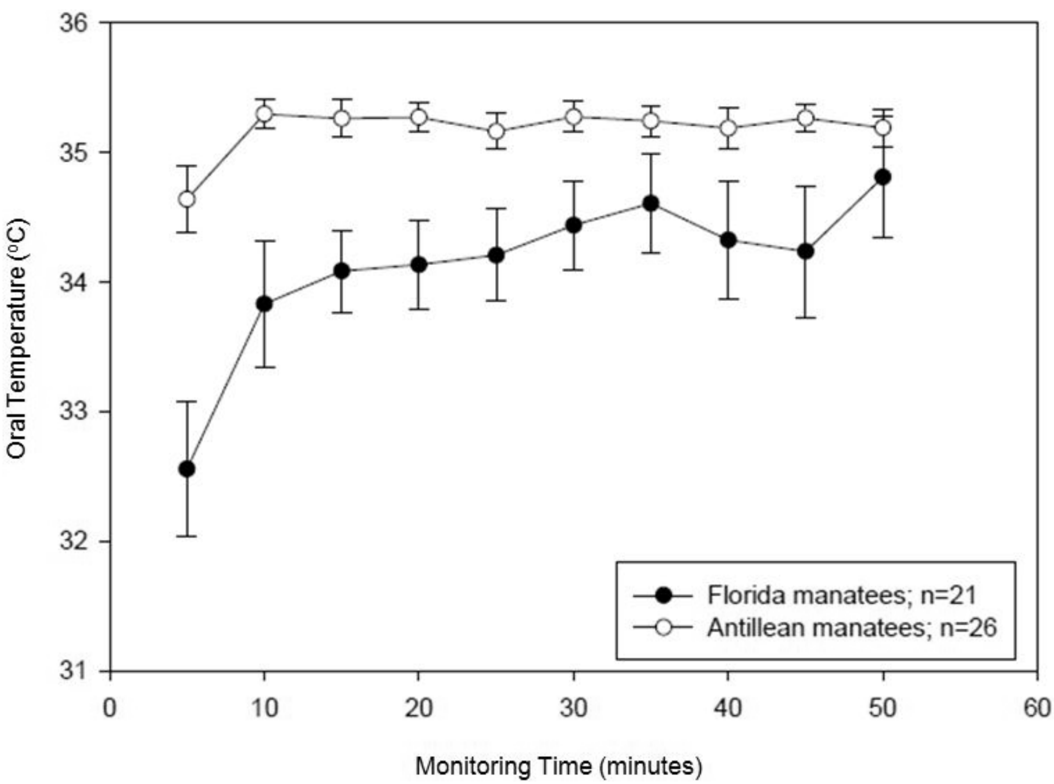

Figure 2. Mean oral temperatures (OT) over time, with standard error bars, for wild-caught juvenile and adult Florida and Antillean manatees; data were collected immediately following capture during the years 2004 through 2006.

Table 5). The seven Florida manatees captured during the warmer months displayed an OT range of 34.5 to 36.2° C.

Heart Rate

Manatee HR ranged from 32 to 88 bpm, with no difference in HR over monitoring time based on

Table 5. Descriptive statistics for OT (°C) of Florida and Antillean manatees at different time intervals during field monitoring

Time (min)	Florida *n*	Mean (±SD)	Median	Range	Std error	Antillean *n*	Mean (±SD)	Median	Range	Std error
5	21	32.6 (±1.8)	32.8	29.5-35.1	0.5	26	34.6 (±0.9)	34.9	32.6-36.0	0.3
15	21	34.1 (±1.4)	34.5	29.8-35.7	0.3	26	35.3 (±0.6)	35.3	34.1-36.2	0.1
25	21	34.2 (±1.4)	34.7	30.1-35.7	0.4	26	35.2 (±0.7)	35.3	33.6-36.2	0.1
40	21	34.3 (±1.6)	34.9	30.7-35.8	0.5	26	35.2 (±1.6)	35.3	33.3-36.2	0.2
50	21	34.8 (±1.5)	35.1	30.5-35.9	0.5	26	35.2 (±0.6)	35.25	34.0-36.1	0.1

size class or sex. The initial mean HR of Florida manatees (66.3 ± 9.7 bpm) was significantly lower than that of Antillean manatees (75.2 ± 7.7 bpm; $p = 0.0058$). At 25 min post capture there was no significant difference between Florida and Antillean manatees, with mean HR values of 60.4 ± 12.6 bpm and 62.7 ± 10.1 bpm, respectively ($p = 0.0739$) (Figure 3; Table 6).

Respiration Rate

Manatee RR ranged from 0 to 17 breaths/5 min, with no significant difference over monitoring time based on size class or sex. The initial mean RR of Florida manatees (6.4 ± 2.9 breaths/5 min) was significantly lower than that of Antillean manatees (9.4 ± 3.5 breaths/5 min; $p < 0.0001$). There was no statistically significant difference between Florida and Antillean manatee RR at the end of the 50-min monitoring period, with mean RR values of 4.3 ± 2.0 and 5.4 ± 2.3 breaths/5 min, respectively ($p = 0.0943$) (Figure 4; Table 7). Some manatees displayed periods of apnea despite efforts to stimulate respirations by pouring water over the dorsal cranium. Four Florida manatees and one Antillean

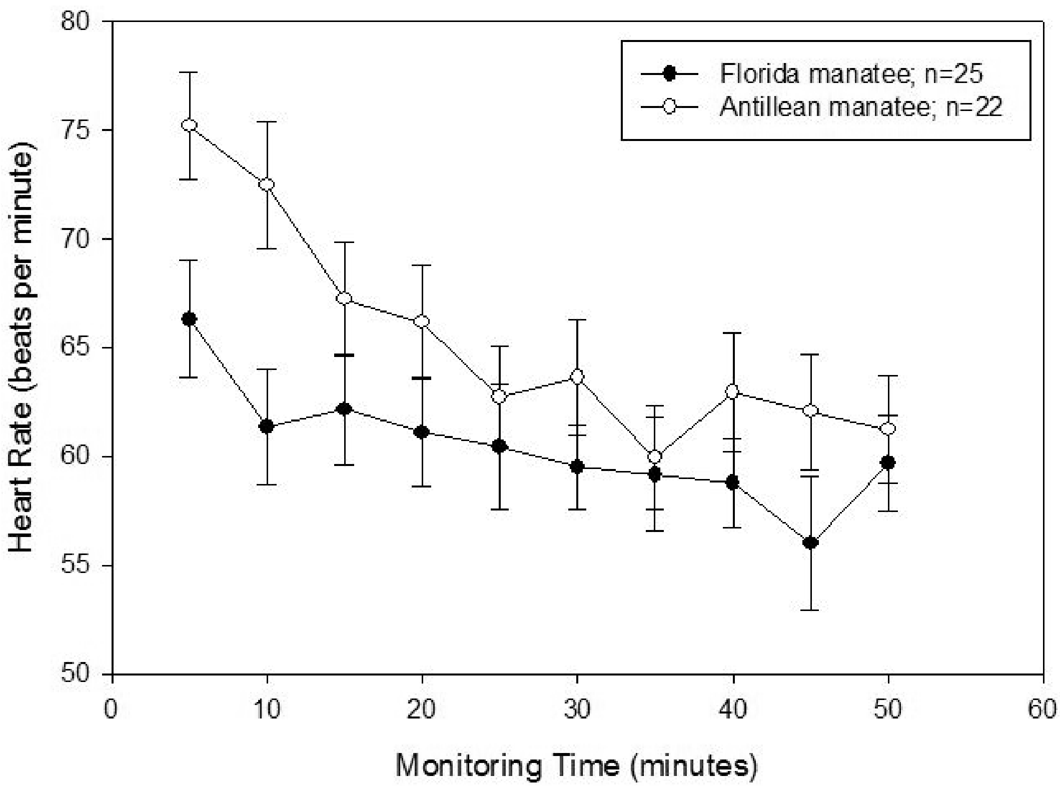

Figure 3. Mean heart rates (HR) over time, with standard error bars, for wild-caught juvenile and adult Florida and Antillean manatees; data were collected immediately following capture during the years 2004 through 2006.

Table 6. Descriptive statistics for HR of Florida and Antillean manatees at different time intervals during field monitoring

	Florida					Antillean				
Time (min)	*n*	Mean (±SD)	Median	Range	Std error	*n*	Mean (±SD)	Median	Range	Std error
5	25	66.3 (±9.7)	70	48-80	2.7	22	75.2 (±7.7)	74	64-88	2.4
15	25	62.2 (±11.1)	64	32-84	2.5	21	67.2 (±9.4)	68	52-80	2.6
25	25	60.4 (±12.6)	57	40-80	2.9	22	62.7 (±10.1)	63	44-80	2.3
40	25	58.8 (±8.7)	60	44-72	2.0	22	62.9 (±10.9)	63	44-80	2.7
50	23	59.7 (±6.9)	62	45-68	2.2	22	61.2 (±10.5)	60	48-84	2.5

manatee had RRs of 0 breaths/5 min for one 5-min monitoring interval. One Florida manatee had an RR of 0 breaths/5 min for 10 min. Hyperventilation, > 5 breaths/5-min interval occurring for a duration of three consecutive 5-min intervals, occurred in two Florida and two Antillean manatees. A total of eight Florida manatees were water stimulated to breathe. Of these Florida manatees, 14 of 432 respirations, or 3.2%, were water stimulated. A total of 11 Antillean manatees were water stimulated to breathe. Of these Antillean manatees, 119 of 1,359 respirations, or 8.8%, were water stimulated.

Lactate and Respiration Rate

Lactate—Antillean manatee mean lactate values, 20.6 ± 7.8 mmol/L, were significantly higher than Florida manatee mean lactate values, 13.7 ± 6.7 ($p < 0.001$) (Table 8). In total, 14 captured manatees presented lactate values > 25 mmol/L, with a range of 26.5 to 37.0 mmol/L. Twelve of these manatees with high lactate levels were Antillean (Harvey et al., 2007) (Table 9).

A Pearson product moment correlation coefficient of $r = 0.3685$ was calculated between 20-min mean RR and corresponding lactate values

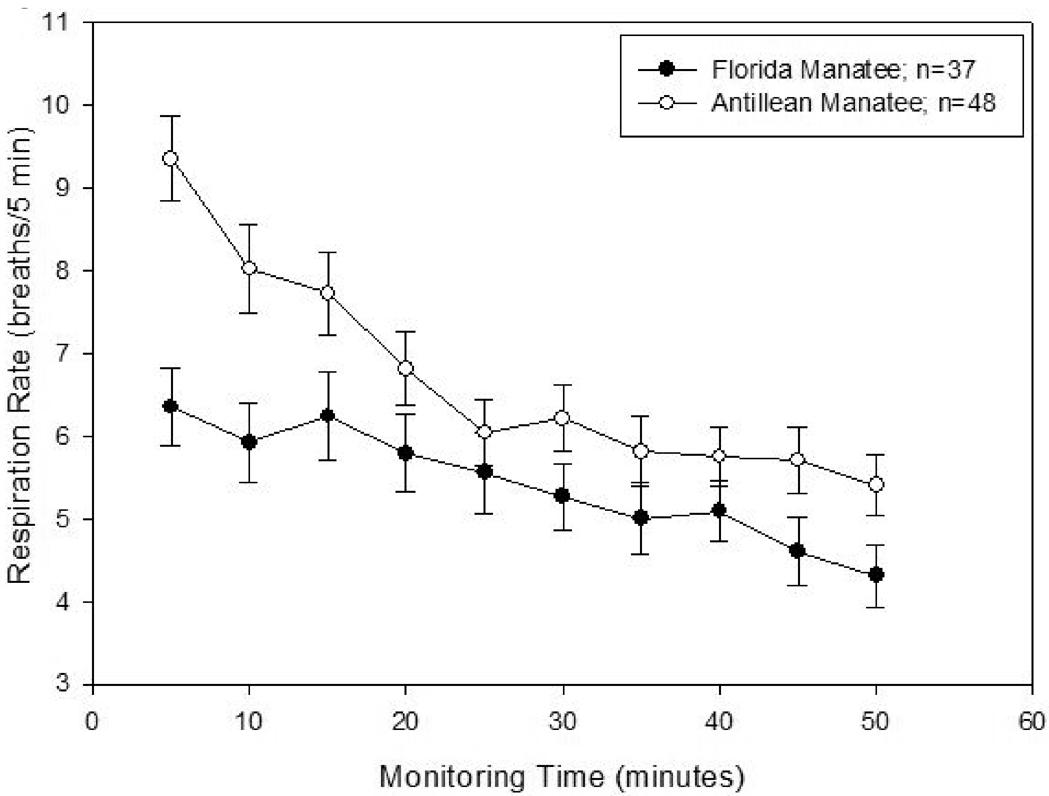

Figure 4. Mean respiration rates (RR) over time, with standard error bars, for wild-caught juvenile and adult Florida and Antillean manatees; data were collected immediately following capture during the years 2004 through 2006.

Table 7. Descriptive statistics for RR of Florida and Antillean manatees at different time intervals during field monitoring

	Florida					Antillean				
Time (min)	*n*	Mean (±SD)	Median	Range	Std error	*n*	Mean (±SD)	Median	Range	Std error
5	37	6.4 (±2.9)	6	1-13	0.5	48	9.4 (±3.5)	9	3-17	0.5
15	37	6.2 (±3.3)	6	0-17	0.5	48	7.7 (±3.5)	8	2-15	0.5
25	37	5.6 (±3.1)	5	0-17	0.5	48	6.0 (±2.7)	6	0-11	0.4
40	37	5.1 (±2.2)	5	1-10	0.4	48	5.8 (±2.5)	5.5	2-12	0.4
50	36	4.3 (±2.0)	4	0-8	0.4	48	5.4 (±2.3)	5	2-12	0.4

Table 8. Plasma clinical biochemistry descriptive statistics from 38 Florida and 48 Antillean manatees

Florida	Mean (±SD)	Median	Range
Lactate (mmol/L)	14 (±7)	13	0.5-31
K^+ (mEq/L)	5.1 (±0.6)	5.1	3.9-6.4
Na^+ (mEq/L)	150.1 (±4.2)	151	143-158
Na^+/K^+	29.7 (±3.1)	29.9	24.2-36.7
SAA (μg/mL)	97 (±235)	12	10-1,200
Antillean	**Mean (±SD)**	**Median**	**Range**
Lactate (mmol/L)[a]	21 (±8)	21	5-37
K^+ (mEq/L)	5.3 (±0.5)	5.3	4.1-7
Na^+ (mEq/L)	152 (±12.5)	153	117-204
Na^+/K^+	28.8 (±2.8)	28.1	12.2
SAA (μg/mL)	25 (±32)	10	10-190

[a] Antillean manatee lactate – *n* = 44; values not available for four manatees in Puerto Rico.

(Figure 5). Lactate values associated with a 20 min mean RR ≤ 5.25 breaths/5 min, 14.3 ± 7.6 mmol/L (*n* = 24), were significantly lower than lactate values associated with an RR > 5.25 breaths/min, 18.8 ± 7.9 mmol/L (*n* = 57; *p* = 0.020).

Other Biochemical Analysis

Creatine Kinase—Florida manatee mean plasma CK activity, 193 ± 151 U/L was not significantly different than Antillean manatee mean plasma CK activity, 195 ± 493 U/L (*p* = 0.973) (Table 8). High CK activities were detected in two Florida manatees and one Antillean manatee (Harvey et al., 2007) (Table 10).

Potassium—There was no difference between Florida manatee mean plasma K^+ concentration, 5.1 ± 0.6 mEq/L and Antillean manatee mean K^+ plasma concentration of 5.3 ± 0.5 mEq/L (*p* = 0.109) (Table 8). High K^+ concentrations were detected in three Florida manatees and three Antillean manatees (Harvey et al., 2007) (Table 11).

Serum Amyloid A—Florida manatee mean SAA concentration, 97 ± 235 μg/mL was significantly higher than Antillean manatee mean SAA concentration of 25 ± 32 μg/mL (*p* = 0.038) (Table 8). High SAA concentrations were detected in nine Florida manatees and four Antillean manatees (Harr et al., 2006) (Table 12).

Table 9. High lactate values, > 25 mmol/L (*n* = 14), identified from 38 Florida and 48 Antillean field-captured manatees; lactate reference interval from Harvey et al. (2007). The ID prefixes TTB and TEP represent Florida manatees, and the ID prefixes BZ and TPR represent Antillean manatees.

ID	Date	Lactate (mmol/L)
TTB126	04/01/06	26.5
BZ04M71	21/11/04	27
BZ97F03	21/11/04	27.5
BZ04M74	22/11/04	28
TPR27	28/04/05	28
BZ05M93	18/11/05	28.5
BZ04F68	19/11/04	29
BZ04M77	22/11/04	29.5
TPR31	30/04/05	29.5
BZ04F75	22/11/04	31
TEP04	01/07/05	31
BZ04F67	17/11/04	32
BZ04M66	17/11/04	35
BZ04F72	21/11/04	37

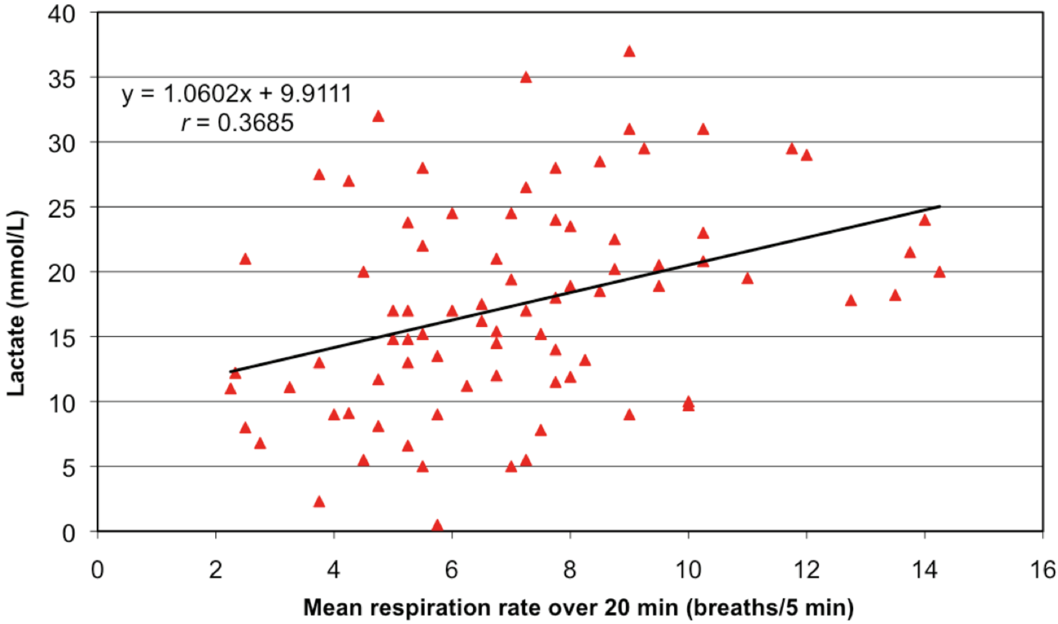

Figure 5. Correlation analyses with trend line for RR vs lactate of Florida and Antillean manatees

Table 10. High creatine kinase (CK) activity, > 482 U/L (n = 3), identified from 38 Florida and 48 Antillean field-captured manatees; CK normal reference interval from Harvey et al. (2007). The ID prefixes TNP and CNP represent Florida manatees, and the ID prefix TPR represents Antillean manatees.

ID	Date	CK (U/L)
TNP028	16/01/04	572
CNP0401	17/01/04	799
TPR20	07/06/04	3522

Table 11. High potassium (K^+) values, > 6 mEq/L (n = 6), identified from 38 Florida and 48 Antillean field-captured manatees; K^+ normal reference interval from Harvey et al. (2007). The ID prefix TTB represents Florida manatees, and the ID prefixes TPR and BZ represent Antillean manatees.

ID	Date	K^+ (mEq/L)
TPR29	29/04/05	6.1
TTB119	05/12/05	6.2
TTB132	05/01/05	6.3
TTB127	04/01/05	6.4
BZ04F72	21/11/04	6.5
BZ04M66	17/11/04	7

Significant Case Studies

A 231-cm long, juvenile male manatee (CTB051), captured in Tampa Bay on 5 January 2006, had an irregular cardiac rhythm and a mean HR of 35 bpm based on ECG recordings. Heart rate determined by auscultation with a stethoscope was recorded seven times during a 5-min period and ranged from 28 to 48 bpm (*mode* = 35 bpm). The RR during this time was 4 breaths/5 min. Water was poured on top of the head to stimulate breathing. Thirty minutes later, the HR stabilized at 60 bpm, the rhythm was regular, and RR was 6 breaths/5 min. The OT ranged from 34.1 to 34.6° C. The plasma SAA concentration

Table 12. High serum amyloid A (SAA), > 70 ug/mL (n = 13), values identified from 38 Florida and 48 Antillean field-captured manatees; SAA normal reference interval from Harr et al. (2006). The ID prefixes TEP, CTB, TTB, and TNP represent Florida manatees, and the ID prefixes BZ and TPR represent Antillean manatees.

ID	Date	SAA (µg/mL)
TEP05	01/12/05	73
BZ05F89	16/11/05	76
BZ05M87	15/11/05	79
CTB051	05/01/06	83
TPR22	08/06/04	88
TTB126	04/01/06	99
TNP032	19/04/04	117
CTB049	05/01/06	120
TPR20	07/06/04	190
TNP028	16/01/04	350
TNP027	15/01/04	360
TNP025	15/01/04	800
TNP029	18/04/04	>1,200

was mildly elevated at 83 µg/mL. The manatee had a normal lactate level of 9 mmol/L and a normal CK of 124 U/L (Harvey et al., 2007).

An adult female Florida manatee (TNP029), captured and radio-tagged in Port of the Islands, Florida, on 18 April 2004, was found dead 32 d after capture from a chronic infection due to a watercraft impact-related injury. At necropsy, a pyothorax was confirmed (FFWCC, necropsy report MSW0444). The SAA value at the time of capture of 1,200 µg/mL confirms systemic inflammation due to blunt trauma associated with a boat strike suffered prior to capture. Telemetry data on movements, activity, and diving that might have been indicative of behavioral distress warranting a rescue were not observed. Mean RR was 6.2 breaths/5 min (OT ranged from 34.3 to 34.7° C, HR ranged from 52 to 60 bpm, and RR ranged from 2 to 15 breaths/5 min).

Discussion

The monitoring of manatee OT, HR, and RR was effectively performed based on the methods described. The results of our monitoring support the hypothesis that HR and RR of wild manatees are initially elevated as a result of capture. Over the course of monitoring, manatees showed a trend toward recovery to normal HR and RR. However, OT did not exceed normal values as we had hypothesized (Murphy, 2003). Manatees appeared to effectively regulate oral temperature in both cool and warm environments. Recovery of vital signs should be closely monitored after capture as lactate levels suggest possible susceptibility to lactate acidosis as a result of struggle. Monitoring of RR may help in the assessment of a manatee's condition upon capture, given its apparent association with lactate during research capture conditions.

Capture Conditions

The method of capture and environmental conditions must be considered when interpreting OT, HR, and RR as these factors can significantly affect a manatee's physiological response to capture and handling. Manatees exerted themselves at different times during the capture process. However, capture events that prolong fleeing or struggle are more likely to support elevated HR, RR, and lactate values. Evidence of this can be seen in the longer two-stage captures which occurred in Belize (Tables 3, 6 & 7; Figures 3 & 4). During such technical capture events, an experienced crew is strongly recommended to safely secure the manatee as quickly as possible.

Captures during cool or cold weather will likely result in low OT values (< 35.5° C) as manatees attempt to conserve body heat through vasoconstriction of peripheral vasculature (Tables 1, 4 & 5; Figure 2). While we did not see elevated OT values (> 36.0° C) during this study, the increased possibility of hyperthermia occurring during hot weather should still be considered by capture crews, and appropriate measures should be taken to cool the manatee by keeping it shaded and wet.

Oral Temperature

The lower initial OT of Florida manatees compared to Antillean manatees was likely a result of the cooler water in which they were captured. The Florida manatees that were captured in relatively warmer water displayed higher OT similar to the Antillean manatees. Manatees have a limited tolerance to cold water, and they are susceptible to a potentially fatal condition known as manatee cold stress syndrome below water temperatures of 20° C (Irvine, 1983; O'Shea et al., 1985b; Bossart et al., 2002). The Florida manatee is annually exposed to winter water temperatures below 20° C and regularly seeks refuge at warm water discharges of power plants and springs during this time (Laist & Reynolds, 2005). We suspect that because of the cooler water environment in which the Florida manatees were captured, their peripheral blood vessels were greatly vasoconstricted to reduce body heat loss and maintain core body temperature, resulting in initial OT values below the normal 35.5 to 36.0° C (Murphy, 2003). Exposure to cold water may have contributed to the relatively higher occurrence of manatees in Florida with systemic inflammation and elevated SAA concentrations (Bossart et al., 2002; Harr et al., 2006; Table 12).

Both subspecies of West Indian manatee demonstrated an increase in OT over the monitoring time, which was likely a result of reduced body heat loss while being held on land as heat is 20 times less transferable through air as compared to water (Sterba, 1990). Moreover, increased muscle activity as a result of struggle may have increased body heat production. These circumstances suggest that while held on land, manatees increased blood perfusion to the surface of the skin via vasodilation to increase body heat loss and avoid hyperthermia. However, OT ranges in this study did not exceed the reported normal OT range of 35.5 to 36.0° C (Murphy, 2003). The lack of a measured OT hyperthermia, particularly in Antillean manatees given their notably warmer climate, may be due in part to a lower subcutaneous fat thickness as suggested by their leaner body composition. A high RR also may have assisted manatees in venting excess body heat. However, it is most likely that compensatory body heat loss occurs orally and via regulation of blood flow along other areas of the body such as the tail. Manatees are able to increase arterial blood flow to the skin of the tail via regulation of blood flow through deep caudal veins located collateral to the caudal vascular bundle of the chevron canal to dissipate heat (Rommel & Caplan, 2003). Measurement of tail temperature through infrared thermography may improve our understanding of manatee heat exchange and temperature regulation as it has with dolphins (Williams et al., 1999).

Heart Rate

In both subspecies of West Indian manatee, initial HR was well above the reported normal range of 40 to 60 bpm obtained from resting captive manatees, and some individuals did not return to normal HR during the monitoring period (Scholander & Irving, 1941; Murphy, 2003). The higher HR displayed by Antillean manatees compared to Florida manatees may be due to greater exertion and excitation during capture and handling. Alternatively, the differences in HR between the subspecies may be due to innate differences in physiology as a result of differences in body condition and relative size (Figure 1).

The irregular rate and bradycardia found in a juvenile manatee with systemic inflammation in this study is similar to that observed in a forced dive study on a captive manatee in Brazil (Gallivan et al., 1986) and during the rescue of RNE0501, a cold stressed Florida manatee (Wong, FFWCC, unpub. report). The observed HRs in these instances were 5 bpm and 16 to 24 bpm, respectively. Bradycardia and arrhythmia in field-captured manatees can be a sign of distress. More importantly, the observed HR during our study demonstrates the importance of monitoring manatees during capture and handling for possible stress or dive response.

In general, the combination of excitation and struggle are stressors contributing to the increased HR in these captured manatees. We recommend HR be monitored in the field to better assess overall status of the manatee's condition. The use of an ECG machine to monitor HR proved especially useful when auscultation via stethoscope was not possible due to ambient noise.

Respiration Rate

At the end of the 50-min monitoring period, over half of all the manatees captured still had RR values higher than the reported normal range of 2 to 4 breaths/5 min for captive resting manatees (Scholander & Irving, 1941; Bossart, 2001; Murphy, 2003).

Previous dive studies demonstrated breath holding of between 10 to 16 min in manatees (Scholander & Irving, 1941; Gallivan et al., 1986). Upon surfacing from bottom resting for extended periods of time, manatees have been observed with increased rhythmic respirations (Parker, 1922; Hartman, 1979). Elevated respirations in apparently healthy captured manatees may help to rapidly replenish O_2 stores while eliminating CO_2, which is typical of slow-breathing marine mammals (Kooyman, 1973; Gallivan & Best, 1980). The max RR of 17 breaths/5 min that we observed slightly exceeds the previously reported max RR of 15 breaths/5 min (Walsh & Bossart, 1999). During RR monitoring, manatees displayed varying degrees of exhalation and inhalation during a monitoring session, indicating that quality of respirations may have varied. We hypothesize that a manatee's blood O_2 stores are depleted rapidly during the capture process due to struggle; blood CO_2 levels are increased; and breath-holding ability is considerably reduced (Scholander & Irving, 1941; Gallivan et al., 1986). Water stimulation during RR monitoring proved effective in preventing severe prolonged apnea and in re-establishing a normal breathing rhythm following an apparent dive response.

Lactate and Respiration Rate

Lactate is a biochemical byproduct of the anaerobic metabolism of glucose. Increased lactate concentrations are positively associated with increased muscular exertion, which can lead to metabolic acidosis. Harvey et al. (2007) found that mean lactate in normal wild manatees is 13 mmol/L, while normal captive manatees have a mean lactate of 4 mmol/L. Consistent with their findings, the captured wild manatees in this study were found to have similarly high lactate concentrations. The high lactate concentrations are indicative of lactic acidosis as a result of muscular exertion and struggle related to the capture event (Harvey et al., 2007). Lactate concentrations are also positively correlated with cortisol and ACTH concentrations, indicators of chronic and peracute stress, respectively (Tripp et al., 2010).

Manatees in this study demonstrated a range of lactate levels similar to those exhibited by thoroughbred horses under mild to severe degrees of exertion (Bayly et al., 1983, 1987; Rose et al., 1988). We hypothesize that manatees with high lactate levels were still recovering from lactic acidosis upon release. The Antillean manatees exhibited a mean lactate level indicative of moderate struggle, whereas the mean lactate levels in Florida manatees were considered normal for wild manatees. The higher lactate concentrations exhibited by Antillean manatees compared to Florida manatees suggest a higher level of exertion during capture. The high lactate values exhibited by 14 manatees in this study suggest possible lactic acidosis, but these values were not necessarily associated with serious muscle injury (Harvey et al., 2007).

Harvey et al. (2007) showed that high lactate values in wild Florida manatees are associated with low plasma CO_2, which could be a result of a compensatory increase in RR to offset metabolic acidosis and maintain optimal blood pH (Scholander & Irving, 1941; Robergs & Roberts, 1997). Heavily exercised, conditioned thoroughbred horses have shown elevated RR, elevated lactate levels, and low arterial pH following 30 min of recovery (Butler, 1982). Our analysis of RR suggests that animals with a mean RR at 20 min of ≥ 5.25 breaths/5 min may have relatively high lactate levels, presumably as a result of struggle.

We also consider that as with HR, the differences in RR between the subspecies may be due to innate differences in physiology and body condition as suggested by their differences in size. When lactic acidosis is suspected, we recommend the use of supplemental O_2 and working quickly to release the animal to assist in post-capture recovery.

Other Biochemical Analyses

In this study, the majority of captured manatees presented normal serum biochemistry profiles and were considered healthy, wild animals based on physical examination and blood value findings. CK and K^+ were evaluated to assess muscular damage associated with exertion or injury, and SAA concentrations were evaluated to assess systemic inflammation associated with pathological disease or trauma.

Creatine kinase is an enzyme commonly found in the cytoplasm of myocytes. Its primary function is to catalyze the formation of phosphocreatine, a source of energy for muscular activity. It is typically quickly metabolized, and so increased concentrations represent acute muscular trauma. High CK activity maintained over time is generally associated with continual muscle damage from muscle disease and exertion. The reported normal range of a captive manatee's CK is 79 to 302 U/L (Walsh & Bossart, 1999; Manire et al., 2003). Elevated CK in field-captured Florida manatees is uncommon. In one previously reported instance, elevated CK levels (1,365 to 1,381 U/L) in a wild-captured manatee following a severe struggle were reported 0 to 3 d after capture (O'Shea et al., 1985a). The abnormal CK values indicating myocyte damage in three manatees in this study may have been a result of differences in struggle intensity, phlebotomy technique, individual variation, or underlying disease (Harvey et al., 2007).

The reported normal range of K^+ concentration is 3.8 to 6.3 mEq/L (Harvey et al., 2007). Extreme physical exertion and lactic acidosis can lead to rhabdomyolysis (breakdown of skeletal muscle), which can cause hyperkalemia. Clinical signs of severe hyperkalemia may include cardiac arrest and paralysis (Spraker, 1993; Montané et al., 2002). The majority of serum K^+ values reported in this study were normal despite increased lactate concentration. This result is not unusual as during organic acidosis a hydrogen ion and potassium gradient fails to occur, due to a free organic anion influx of lactate into cells (Harvey et al., 2007). The abnormally high serum K^+ values displayed by six manatees may have been influenced by hemolysis during blood collection or processing, or it may represent muscular damage.

Serum amyloid A is a characteristic acute phase response protein in manatees, which increases rapidly during acute systemic inflammation and infection, and has been established as an indicator of systemic inflammatory disease in manatees (Harr et al., 2006). Healthy manatees have a mean SAA value of 22 ± 25 µg/mL. In comparison, diseased manatees have a mean SAA value of 266 ± 398 µg/mL (Harr et al., 2006). SAA concentrations in 13 manatees from this study were indicative of systemic inflammation, which was not evident on physical examination. SAA is a more sensitive indicator of inflammation than physical examination and can be used quantitatively to assess health (Harr et al., 2006). The case of TNP029 demonstrated the limited sensitivity of OT, HR, and RR in detecting underlying disease, such as chronic inflammation due to a boat strike, when monitoring wild manatees. While RR was high, this abnormality alone could not confirm the severity of the manatee's condition. Hence, a brief (< 1 h) physical examination may miss significant underlying disease. Additionally, behavioral monitoring with telemetry did not detect any abnormality. In this situation, biochemical assessment of SAA concentration was necessary to definitively diagnose TNP029 as severely ill.

Management Implications

This marks the first study to document a protocol for the monitoring of key vital signs (OT, HR, and RR) to better assess the condition of field-captured manatees. The monitoring methods developed in this study also have been effectively implemented in the first out-of-water health assessments of field-captured dugongs (*Dugong dugon*) (Lanyon et al., 2010). Use of these monitoring methods can likely be adapted to other animal species. Creative use of common and sturdy instrumentation, such as the indoor/outdoor thermometer used in this study to measure OT, should be considered when attempting to provide reliable monitoring support in challenging field settings. Medical devices such as a stethoscope and ECG can be utilized in the field to help monitor manatee HR and overall status. Additional investigations on implementation of other medical instruments (e.g., portable blood analyzer, capnograph, and infrared thermography) to improve health assessment are greatly needed. Simple procedures such as RR monitoring can yield important data regarding a manatee's condition and its response to capture and handling. During captures of apparently healthy manatees, RR may serve as a useful indicator of the degree of struggle and peracute stress. We strongly recommend regular monitoring of manatee OT, HR, and RR during all human interventions (e.g., field research, rescue, and captive care). Based on these results, and the fact that there have been very few manatee deaths associated with capture, and those were caused by known factors (drowning), no changes in capture methodologies appear to be warranted. We believe the current methods of capture used in Florida, Puerto Rico, and Belize are appropriate for each region. We stress the importance of using experienced capture teams to help reduce capture time and to effectively handle the monitored manatee, thereby reducing struggling and out-of-water time.

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