MONITORING ORAL TEMPERATURE HEART RATE, AND RESPIRATION RATE OF FIELD-CAPTURED FLORIDA AND ANTILLEAN MANATEES (*Trichechus manatus latirostris* and *T. m. manatus*)

By

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To my parents: Bill and Alice. Thank you for everything.

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Abstract of Thesis Presented to the Graduate School
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(*Trichechus manatus latirostris* and *T. m. manatus*)

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Knowledge gained from the capture of manatees for health assessment and telemetry based studies is required for the conservation of the species. Understanding how free-ranging manatees respond to capture and how to effectively monitor the awake individual animal is of the utmost concern for researchers charged with their care in the field. The main objectives of this study were to: 1) determine practical field methods to monitor oral temperature (OT), heart rate (HR), and respiration rate (RR) of captured manatees; 2) establish normal OT, HR, RR parameters with correlations to blood chemistry; 3) provide an easy to reference OT, HR, RR monitoring field guide for manatee researchers. Three digital thermometer types and a human bedside electrocardiogram (ECG) were tested in the field. Oral temperature measurements between three digital thermometer types showed no statistical significant difference (p=0.125). However, a thermocouple type thermometer did present several clinically significant incorrect measurements. The ECG unit was 84% successful in acquiring a heart rate during field application. Electrocardiogram HR measurements were not significantly different from those obtained by stethoscope (p=0.313). Thirty-eight Florida manatees (*Trichechus manatus latirostris*) were captured in Florida. Thirty-five Antillean manatees (*T. m. manatus*) were

captured in Belize and thirteen Antillean manatees were captured from Puerto Rico. Manatees were monitored for OT, HR, and RR following capture and during handling. Blood was concurrently sampled from monitored individuals. Creatine kinase activity (CK), potassium (K^+), serum amyloid A (SAA), and lactate levels were measured. Animals were removed from mean water temperatures of 25.9 (±4.2) °C in Florida; and 28.9 (±1.5) °C in Belize and Puerto Rico. Mean OT of Florida (n=21) and Antillean (n=26) manatees increased during capture from 32.6 (±1.8) °C and 34.6 (±0.9) °C; to 34.8 (±1.5) °C and 35.2 (±0.7) °C, respectively. There was no statistically significant difference of OT between Antillean and Florida manatees after 40 minutes (p=0.4186). Mean HR of Florida (n=25) and Antillean (n=22) manatees decreased from 66 (±9.7) beats per minute (bpm) and 75 (±7.7) bpm, respectively, to 60 (±6.9) bpm and 61 (±10.5) bpm, respectively. There was no statistically significant difference of HR between Florida and Antillean manatees after 25 minutes (p=0.0739). Mean RR of Florida (n=37) and Antillean (n=48) manatees decreased from 6 (±2.9) breaths/5min and 9 (±3.5) breaths/5min, to 4 (±2.0) breaths/5min and 5 (±2.3) breaths/5min, respectively. There was no statistically significant difference in RR after 50 minutes between Florida and Antillean manatees (p=0.0943). Higher respiratory rate over time was associated with higher lactate values. Antillean manatees had higher overall lactate values than Florida manatees (p=<0.001). Further study regarding manatee physiology is suggested to better explain the differences observed between subspecies in this study. The effect of capture on manatee OT, HR, and RR in the field shows biological significance. Monitoring of these parameters can help to improve the ability to obtain individual health assessments of manatees in the field.

CHAPTER 1
INTRODUCTION

The Florida manatee, *Trichechus manatus latirostris*, is classified as an endangered species in the State of Florida, protected by the U.S. Endangered Species Act, and by the Marine Mammal Protection Act, listed in the Convention on International Trade of Endangered Species of Wild Flora and Fauna under Appendix I, and defined as vulnerable by the International Union for Conservation of Nature and Natural Resources (U.S. Fish and Wildlife Service 2004, Convention on International Trade of Endangered Species of Wild Flora and Fauna 2004, Haubold *et al*. 2006, International Union for Conservation of Nature and Natural Resources 2007). Given its status, multiple federal, state, private, and non-profit organizations have been working together to protect and conserve the species from anthropogenic and natural threats to its population. Long term tracking studies, population studies, rescues, and rehabilitation of manatees, all involve regular capture and handling of free-ranging individuals.

During these interactions, scientists attempt to assess the overall health of the manatee. An animal's overall appearance including girth, skin condition, and semi-quantitative dorsal subcutaneous fat thickness is examined (Ward-Geiger 1997). This assessment is a superficial method to assess external integument and body condition of an individual manatee, and is performed regularly by biologists. However, the evaluation of the health of a manatee is typically performed by select veterinarians or more experienced biologists. In this assessment, respiration, heart rate, body temperature, and blood chemistry are often recorded. Protocols for monitoring these vital signs are well defined in humans and in many species of domestic and wild animals, but protocols do not exist for manatees. Methods used to evaluate temperature, heart rate, and respiratory rate vary between rehabilitation facilities. While literature exists on manatee blood chemistry (White *et al*. 1976, Medway *et al*. 1982, Walsh & Bossart 1999, Bossart *et a*l. 2001, Manire *et al*. 2003, Murphy 2003), study methods are changing due to advances in medical technologies. Furthermore, correlations in body temperature, heart rate, and respiration with manatee blood chemistry and the overall health of the animal have not been studied in a field setting.

Since the late 1970s, only six manatees have died in over 1000 field research captures (U.S. Geological Survey, Florida Fish and Wildlife Conservation Commission, Wildlife Trust, unpublished data). However, monitoring of oral temperature (OT), heart rate (HR), and respiration rate (RR) of captured manatees can provide additional information that may be useful to improve the care of individuals and increase our understanding of manatee physiology.

This thesis: 1) Determines practical field methods that can be performed by researchers in order to monitor oral temperature (OT), heart rate (HR), and respiration (RR) as a basic health assessment in the manatee following capture and during handling. 2) Establishes a normal range of OT, HR, and RR in relation to blood chemistry values. 3) Provides a manatee monitoring field guide to aid researchers during health assessments. To help accomplish the aforementioned objectives, several aims were defined, and associated hypotheses were proposed to be tested:

- **Specific Aim 1:** Establish temperature, heart rate, and respiration rate parameters in captured manatees.
- **Hypothesis 1a**: Oral temperature will show a time dependent increase when ambient air temperature is above 26°C.
- **Hypothesis 1b**: A healthy captured manatee's heart rate is higher than 40 beats/minute.
- **Hypothesis 1c**: Healthy captured manatees will show a normal respiration rate of 3-4 breaths/5 minute cycle (Bossart 2001).
- **Specific Aim 2:** Determine possible correlations of OT, HR, RR with blood chemistry.
- **Hypothesis 2a**: Oral temperature will show a time dependent increase in value after capture; heart rate and respiration rate will stabilize over time.

- **Hypothesis 2b**: A positive correlation exists between heart rate, potassium, and lactate concentration amongst healthy captured manatees.

This information is novel and important as the existing literature reflects information on manatees under captive care and not free-ranging manatees during capture. Additionally, there are no published studies on simultaneous OT, HR, and RR in field captured manatees.

This thesis is organized with the intent to publish the information in peer reviewed journals as a contribution to the scientific community. The second chapter describes assessment and quality control of techniques chosen to measure OT, HR, and RR. The third chapter is an article presenting and discussing the physiological results of the study. The fourth chapter references a practical monitoring field guide located in Appendix F, which is intended for manatee researchers, and presented in book publication format. The fifth and final chapter provides an overall thesis conclusion.

CHAPTER 2

METHODS DEVELOPMENT FOR FIELD MONITORING OF MANATEE (*Trichechus manatus latirostris, T. m. manatus*) ORAL TEMPERATURE AND HEART RATE

Abstract

Prior to this study, continuous monitoring of oral temperature and heart rate was not regularly performed during health assessments of free-ranging manatees (*Trichechus manatus latirostris, T. m. manatus*). Three digital thermometers, an electrocardiogram (ECG), and stethoscope were tested for efficacy in manatee field health assessments. While there was no statistically significant difference between thermometer types in measuring oral temperature (p=0.125), the thermocouple thermometer still presented several skewed oral temperature readings in the field which were clinically significant, falsely indicating hyperthermia, in a normothermic manatee. No statistically significant difference was found between ECG and stethoscope heart rate measurements (p=0.313). The ECG was 84% successful in measuring heart rate and provided continuous cardiac monitoring in a field capture setting. The most common challenge was water and sand fouling electrode connections. The ECG, stethoscope, and basic digital thermometer are recommended as tools to monitor the heart rate, and oral temperature of manatees in the field.

Introduction

It is standard practice in many medical fields to monitor temperature, heart and respiration rate as part of regular health assessment (George 1965, Osofsky 1997). While respiration rate (RR) can be assessed using human senses alone, monitoring oral temperature (OT) and heart rate (HR) requires instrumentation. Electronic monitoring equipment is generally developed for use in clinical settings where environmental factors, such as extreme temperature, humidity, and dirt exposure are limited. These cannot always be controlled in a field setting and can render equipment inoperable. Furthermore, many devices are designed for human or domestic animal use, and may not be useful in manatees due to anatomic and physiologic variability, e.g. large size and thick dermal layers.

Reliable monitoring equipment and implementation is necessary to ensure proper care of manatees during field captures. In order to accomplish this goal, this study assessed the efficacy of three digital thermometers, and an ECG for monitoring oral temperature and heart rate in the Florida manatee (*Trichechus manatus latirostris*), and Antillean manatee (*T. m. manatus*), without chemical immobilization and under limited human restraint in the field.

Materials and Methods

Manatees Sampled

Captures were performed under U.S. Fish and Wildlife Service permits MA773494, MA791721-4, and the Belizean Department of Forestry permit CD/60/3/05(36) for tracking-based population studies, conducted by the Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute, the U.S. Geological Survey Sirenia Project, and Wildlife Trust, respectively. Manatees in this study were concurrently monitored for oral temperature (OT), heart rate (HR), respiration rate (RR) and sampled for research purposes as well as for complete health assessment.

Oral Temperature Method and Thermometer Validation

A Digisense® (DS) thermocouple thermometer (Cole-Parmer, Vernon Hills, IL) equipped with type T, copper-constantin thermocouple probes, coated in flexible blue plastic (Physitemp Instruments Inc., Clifton, NJ), a Radioshack® (RS) thermometer model 63-1009A, and a RS thermometer model 63-1035 (Radio Shack, Fort Worth, TX) were used to measure oral temperature. The RS 63-1035 continuously displayed ambient air and oral temperatures simultaneously. The manufacturer's specified accuracy of the DS thermocouple thermometer is +/- 0.4°C for temperatures greater than -150°C. A "useful range" is specified by the manufacturer at 0 to 40°C. The manufacturer's specified accuracy of the RS thermometer model 63-1009A and model 63-1035 is +/- 1°C, at 0 to 40°C.

To test manufacturer claims of accuracy and precision, each thermometer was subjected to ten rounds of a series of different temperature water baths in a controlled lab setting. The temperature probes were randomLy placed in water temperatures of 0°C (ice bath with distilled water), 15°C (Fisher Scientific Isotemp Refrigerated Circulator Model No. 9100 Pittsburgh, PA), 30°C (Dubnoff Metabolic Shaking Incubator), 45°C (Fisher Scientific 20 Liter Water Bath, Pittsburgh, PA), and 60°C (Fisher Scientific 20 Liter Water Bath, Pittsburgh, PA). Additionally, only the DS thermocouple thermometer probe was tested at 75°C (Sybrone Corp., Thermolyne Model No.SP-A1025B Dubuque, IA). The thermometers were allowed to adjust for a minimum of one minute at each temperature before a reading was recorded. Accurate temperatures in the baths were maintained using calibrated mercury thermometers, considered to be the gold standard.

For field use, the RS 63-1035 and DS thermocouple thermometer were each placed in a modified dry bag (West Marine, Watsonville, CA) or Ziploc® bag to prevent water damage. To determine field accuracy, probe tips from the two thermometer types were taped together to ensure simultaneous measurements of OT from the same location. OT probes were placed once the manatee was secured on land or on a boat deck. The soft wire temperature probe was placed by hand, towards the back of the mouth, adjacent to the inferior dental mandibular cavity, located buccally past the molars. The temperature probes were removed just prior to release.

Heart Rate Monitoring and ECG Validation

Heart rate was ausculted with a Sonocardia water proof stethoscope (Magnafortis, Seattle, WA). The stethoscope bell was inserted underneath the manatee's axilla and placed ventrally close to midline. The number of heart beats, defined as atrial and ventricular contractions, were counted for 15 seconds and multiplied by four to determine beats per minute (bpm). The resulting stethoscope measurements were considered the gold standard when compared to ECG measurements.

Prior to use in the field a Mac 500 ECG (GE Healthcare, Milwaukee, WI) was tested using a four electrode placement technique on a 280 cm straight-line length, 540 kg, healthy, sexually mature, male, captive manatee at the Parker Manatee Aquarium in Bradenton, Florida. Four commercially available adhesive LLEBL Electrode Series electrodes (Lead-Lok, Inc., Sandpoint, ID) were placed on the manatee. Two anterior leads were placed dorsally on the right and left side, approximately 2-7 cm cranial to the pectoral flipper, anterior to the scapula. Two caudal leads were placed laterally approximately 10 cm caudal to the pectoral flippers at the level of the manatee's shoulder (Siegal-Willott *et al*. 2006). This lead placement was the standard used throughout this study.

In the field, algae or dirt on the skin at the lead placement sites were first cleaned. The skin area was wiped with an alcohol pad, and dried with a clean paper towel to ensure proper adherence of sticky electrode pads. Occasionally, a small amount of tissue glue was used directly on the cleaned site to improve electrode pad adherence. A drop of Aquasonic®) 100 ultra sound transmission gel (Parker laboratories, Fairfield, NJ) was placed at the connection point of the lead clip and the electrode pad to improve electrical conductivity.

Following field exposure, lead connection points were wiped down with alcohol gauze pads to prevent oxidation. Lead clips were taken apart, soaked in a small jar of alcohol, and carefully brushed down with a small piece of sturdy paper towel, to rid them of sand, rust, and salt.

Electrocardiogram HR was compared with simultaneous measurements obtained by stethoscope to ensure its accuracy. Printed electrocardiograms were examined to ensure validity of the unit's displayed HR and to assess heart function (Siegal-Willott *et al*. 2006). Electrocardiograms that showed artifacts such as distorted baselines, or unclear PQRS complexes were considered as evidence for an inaccurate HR reading. Data from inaccurate tracings were not used and these were considered equipment failures.

Based on its smaller size and simpler interface, the Mac 500 ECG unit was selected for field use. The unit was placed in a clear dry bag (West Marine, Watsonville, CA) to prevent water damage. To determine if ECG and stethoscope readings were significantly different, a paired t-test was performed comparing 56 randomLy sampled HRs, simultaneously measured by the two instruments, from 20 manatees selected at random. To determine the success rate of the ECG while being used in the field, successful and failed attempts to collect HRs were documented during HR monitoring of 25 manatees selected at random. A successful ECG HR measurement was determined by a steady HR accompanied by a readable electrocardiogram. ECG HR was checked by stethoscope for clinical accuracy. A percentage of success and error was then calculated.

Statistics

Basic statistical analyses were conducted using Microsoft Office Excel 2003 for Windows (Microsoft Corp., Redmond, WA), and Sigma Stat for Windows, Version 3.1 (Systat Software Inc., Point Richmond, CA). T-tests were conducted 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. To evaluate differences in field OT measurements between RS 63-1035 and DS thermocouple thermometers, a paired t-test was performed comparing 109 randomLy sampled OTs, simultaneously measured by the two instruments, from 11 wild caught manatees.

Results

Oral Temperature Method andThermometer Validation

Under laboratory testing, one DS thermocouple thermometer with two probes, one RS 1009A, and four RS 1035 thermometers performed within their respective manufacturer's specified levels of accuracy in the 0 and 30ºC water baths (Table 2-1, 2-3). However, at the other temperature water baths, the digital thermometers showed several readings that were less accurate, with the DS thermocouple thermometer showing the highest range of variability outside of the 0 to 40°C "useful range" (Table 2-2,Table 2-4 through Table 2-6). This was most evident when the DS thermometer presented a reading of 33.5ºC after a minute in the 45ºC water bath. In the 15°C and 60°C water baths the RS 1009A and DS thermocouple showed several inaccurate readings, but mean temperature readings were not significantly affected.

A grand total of 109 randomLy selected simultaneous oral temperature readings were taken from 9 Florida manatees, 2 Belize manatees using the RS and DS thermometers. The RS 1035 mean temperature was 34.5 (±1.2) ºC and the DS thermocouple mean temperature was 34.3 (±1.1) ºC. A paired t-test of the grand total resulted in no statistically significant difference between the oral temperature readings of the two thermometer types (p=0.125). However, a paired t-test of 12 consecutive temperatures gathered from one individual resulted in a statistically significant difference between the measurements of the two thermometers (p<0.001). In this instance, the RS 1035 mean temperature was 35.7 (±0.2) ºC and the DS thermocouple mean temperature was 34.0 (±0.9) ºC. The biggest measurement discrepancy from this data sampling session was an RS 1035 measurement of 35.6ºC and a DS thermocouple measurement of 32.5ºC.

Heart Rate Monitoring and ECG Validation

A paired t-test of 56 randomLy selected simultaneous ECG and stethoscope measurements from 10 Florida manatees and 13 Antillean manatees resulted in no significant difference between the two instrument's values ($p = 0.313$). Mean HR via ECG was 58 bpm (±11) and mean HR via stethoscope was 60 bpm (±12). However, several clinically significant differences between ECG HR and stethoscope HR were apparent from three Florida manatees. Whereby, combined ECG HR values were 56, 53, 38, 46, 60 bpm; and simultaneous stethoscope HR values were 32, 44, 48, 61, and 80 bpm respectively.

A total of 116 ECG readings were attempted on 11 Florida manatees and 14 Antillean manatees (Table 2-7). The ECG was 83.6% successful in obtaining HRs and paper tracings in the field; 9.5% of the unsuccessful tracings were associated with poor lead setup such as wet or dirty electrode clips and 6.9% were associated with printer problems.

Discussion

This study provides a minimally invasive and effective OT and HR monitoring protocol for manatees using commercially available instrumentation. Oral temperature is effectively measured using a basic indoor/outdoor thermometer. The ECG monitoring as described by Siegal-Willott *et al*. (2006) produces reliable results that can effectively be repeated by field researchers. Furthermore, the resulting information from this study provides useful detailed information as to the challenges faced when attempting to continuously monitor OT and HR of free ranging manatees.

Oral Temperature Method and Thermometer Validation

Oral temperature measurement has been performed in a captive setting on manatees and its reported normal range of 35.5°C to 36°C is reflective of the reported normal core body temperature range of 35.6°C to 36.4°C (Gallivan *et al*. 1983, Irvine 1983, Murphy 2003). The placement of a soft wire temperature probe orally is considerably easier than attempting to place a probe rectally to obtain core body temperature, as has been performed on many large animal species such as dolphins, elephants, and moose (Sweeney & Ridgway 1975, Osofsky 1997, Franzmann *et al*. 1984). Rectal temperature probe placement is not practiced on manatees as they are most safely secured in a ventrally recumbent position thereby blocking rectal access. Should rectal access even be obtained, the reported range of 27°C to 32°C is significantly less than core body temperature, and may be clinically less significant (Irvine 1983). Furthermore, fecal build up in the manatee colon may affect temperature readings, as has been demonstrated with elephants (Buss & Wallner 1965). Typically for free ranging ungulates, rectal temperature is only taken on chemically immobilized individuals. For free-ranging dolphins, human restraint is generally adequate for rectal access. However, physical restraint of large, powerful, and awake manatee is dangerous.

The DS thermocouple thermometer under laboratory and field testing showed the highest variability in measurements. While the DS thermocouple thermometer can function in a wide range of temperatures, this is not necessary for the narrow biological range of manatee body temperature. The DS thermocouple thermometer has two connection points to allow for simultaneous measurements with many different probe types. This function adds to the versatility of the unit, but may also add to environmental exposure of electronic elements, which could affect measurement accuracy. The outlier measurements seen in the field are possibly due to comparably less ideal environmental conditions such as salt water, humidity, sand, and heat. In contrast the RS 63-1035 and 63-1009A are much simpler units, with no option to calibrate, and no probe connection points. With fewer parts, these thermometers may have less of a chance for malfunction and error. Assessing accuracy of thermometers in a laboratory setting provides an insight to performance potential in the field. Under such ideal conditions individual measurements outside the predicted scope of accuracy should be given careful consideration as dirt, water, and salt spray can seriously damage electrical equipment. Regular temperature bath testing of electronic thermometers should be performed in order to ensure accuracy over time, using a mercury thermometer as the gold standard.

Pushing the thermometer probe by hand into the manatee's mouth was performed with minimal effort. Proper placement was ensured by feeling for the most distal molar. Occasionally an animal would move their head slowly up and away from the direction of the probe. This behavior occurred with a few animals, but was never enough to require assistance in animal restraint, and all animals quickly relaxed following probe placement. The DS thermometer's soft wire probe tip was chewed open by one animal, exposing the underlying wires. The RS thermometer's hard plastic probe tip was more resilient and did not break. Both probe types were relatively easy to maneuver by hand, as the loose skin around the manatee's mouth provided adequate room along the jaw line. While we did not rigidly test for differences in temperature at the front of the mouth versus the back of the mouth, temperatures measured toward the back of the mouth were observed to be up to 0.5°C warmer than when the thermometer probe was displaced several centimeters away, toward the front of the mouth.

Heart Rate Monitoring and ECG Validation

The stethoscope is traditionally the simplest method to monitor HR. However, monitoring HR in exotic species has been accomplished with a variety of other instruments. Trained dolphins can be ECG monitored using a 2 lead setup. Suction cup electrodes can be placed along the ventral midline and the right scapula; or cross chest electrodes can be held in place with a nylon strap (Noren *et al*. 2004, Williams *et al*. 1999). In elephants, a pulse oximeter clipped to the ear has proven effective (Osofsky 1997). Wireless wrist-watch style monitors have been used to monitor HR of deer (Montané *et al.* 2002). For the manatee, suction cup electrodes do not properly adhere to their irregular skin surface. Ventral placement of cross chest electrodes or small wireless monitors are a challenge to place on large manatees, requiring a team of handlers to properly position an animal, and risking the crushing of equipment. A viable site for pulse oximetry has not yet been identified for the manatee.

Monitoring HR via auscultation was the riskiest procedure as it placed the examiner's head near the body of the manatee in a sometimes semi-prone position. Injury was prevented by sampling HR only when the manatee was fully secured, and not during an invasive sampling procedure such as venipuncture or tissue sampling. Manatees showed little to no irritation to repeated stethoscope placement. The only evasive behavior observed was a short slow turning of the body away from the direction of the stethoscope by an individual.

Failure of HR measurement via auscultation occurred. When the stethoscope bell was submerged in water, the gasket around the diaphragm of the "waterproof" stethoscope did not eliminate water flooding the bell. Position of the bell was important. Some animals required direct bell placement on the ventral midline, while placement proximal to the axilla was sufficient for others. Adequate pressure on the bell as well as placement of the bell was necessary for accurate auscultation. In this study weak sounding heart beats were often a result of poor bell placement rather than a sign of cardiac disease. Additionally, some individuals appear to be unable to auscult the low frequency and/or decibel level of the manatee heart beat. Over the duration of this study, repeated placement of the stethoscope underneath manatees caused the researchers knuckles to be rubbed raw, reddened, or slightly scarred.

The performance of the ECG on manatees in the field, with lead set up as described by Siegal-Willott *et al.* (2006) was generally accurate and reliable, with no permanent malfunctions or repairs needed. Manatees tolerated ECG monitoring quite well. Cleaning of the sites for electrode pad placement elicited no reaction. The occasional outliers in ECG HR measurements were likely due to salt and sand contacting the electrodes and thereby affecting electrode pad contact to the skin, as well as contact of electrode pads to their lead wires. This can be corrected by cleaning, applying ultrasound gel, and using tissue glue. When proper lead connection is attained, HR by ECG and stethoscope are statistically and clinically similar. However, there are key issues which would favor one versus the other. A stethoscope should be used in conjunction with the ECG to ensure proper function. Once it has been determined through stethoscope that the ECG is measuring correctly, the ECG can effectively be used to continue to monitor HR.

The benefits of ECG are its ability to provide a HR during transport by boat or car, where engine noise and vibration inhibit auscultation. Collected ECG tracings can serve as documentation of monitoring and enhance knowledge of heart function. The ECG has been used during transport of sick or injured manatees successfully, allowing for HR to be monitored under noisy conditions, while limiting the manipulation of the injured animal.

ECG failure did occur approximately 15% of the time, generally due to water or sand contamination of the lead clips or electrodes. The beach environment with sand and salt spray was more challenging than workup sites on a boat or paved ground. Warmer weather may allow for the adhesive electrode pads to better adhere to the manatee’s skin. Tissue glue on the electrode pads showed no peeling of the manatee skin when electrode pads were removed. Extra lead clips were kept on hand in the field to readily replace lead clips that were affected by excess water or debris. In general, the lead connection points tolerated the occasional fine mist of water spray and debris. Crossing of lead wires did not seem to affect tracing printouts.

There are some additional points that need attention during use of the ECG. Coordination of lead setup was necessary during processing and sampling, as leads would occasionally detach due to manipulation of the manatee for various procedures. Shading of the ECG unit is recommended, as direct sunlight can wash out the ECG display. Paper printouts needed to be carefully prepared and stored in plastic bags, to prevent them from becoming degraded by moisture and excessive handling. Analyses of paper printouts needed to be performed manually. It is recommended that the researcher have a minimum of 30 cm in front of them for setting up the particular unit tested. The ECG can be extended 3-4 feet away from the animal. The ECG should be checked annually with an ECG simulator device to ensure proper electronic function.

Future Considerations: Oral Temperature and Heart Rate Monitoring

Oral temperature and heart rate monitoring in manatees can effectively be performed with a basic digital thermometer and ECG in conjunction with a stethoscope. The ECG can be used while on a powered boat or in a moving transport truck, where noise and vibration can make it difficult to auscult via stethoscope. The ECG should be used as a method to alert the researcher if HR falls out of a normal range. In such a case, immediate assessment should follow with a stethoscope as the gold standard to confirm ECG measurements. A wireless thermometer transceiver unit with a hand held display console could improve versatility. A wireless ECG lead transceiver unit with a computer lap top display and storage is recommended for future field use.

Table 2-1. Results of 0ºC water bath measurements by Radioshack® (RS) and Digisense® thermocouple (DS) thermometers

Thermometer	Mean Temperature (± SD); n=10	Range
RS 63-1009A	-0.2 (± 0.0)	----*
RS 63-1035 (1)	0.5 (± <0.1)	0.4-0.5
RS 63-1035 (2)	0.2 (± <0.1)	0.2-0.3
RS 63-1035 (3)	0.2 (± 0.0)	----*
RS 63-1035 (4)	0.5 (± 0.0)	----*
DS (probe 1)	0.2 (± 0.1)	0.1-0.2
DS (probe 2)	0.1 (± 0.1)	0.0-0.3

The useful range for both thermometer types was 0 to 40ºC. RS and DS thermometers had a manufacturer's specified accuracy of ±1ºC and ±0.4ºC respectively.* No range was determined as all temperature displays were identical.

Table 2-2. Results of 15ºC water bath measurements by Radioshack® (RS) and Digisense® thermocouple (DS) thermometers

Thermometer	Mean Temperature (± SD); n=10	Range
RS 63-1009A	14.3 (± 0.4)	13.4-14.5**
RS 63-1035 (1)	15.0 (± 0.2)	14.5-15.2
RS 63-1035 (2)	14.7 (± 0.1)	14.5-14.9
RS 63-1035 (3)	14.7 (± 0.3)	14.0-14.9
RS 63-1035 (4)	15.1 (± 0.1)	14.9-15.3
DS (probe 1)	14.7 (± 0.9)	12.2-15.1**
DS (probe 2)	14.8 (± 0.4)	13.6-15.0**

The useful range for both thermometer types was 0 to 40ºC. RS and DS thermometers had a manufacturer's specified accuracy of ±1ºC and ±0.4ºC respectively. ** Highlights values that were obtained outside of the thermometer's respective accuracy.

Table 2-3. Results of 30ºC water bath measurements by Radioshack® (RS) and Digisense® thermocouple (DS) thermometers

Thermometer	Mean Temperature (± SD); n=10	Range
RS 63-1009A	29.5 (± 0.2)	29.3-29.9
RS 63-1035 (1)	30.3 (± 0.2)	30.0-30.6
RS 63-1035 (2)	29.8 (± 0.2)	29.5-30.2
RS 63-1035 (3)	29.8 (± 0.2)	29.5-30.2
RS 63-1035 (4)	30.2 (± 0.2)	30.0-30.6
JTEK (probe 1)	30.0 (± 0.2)	29.7-30.3
JTEK (probe 2)	30.0 (± 0.2)	29.7-30.4

The useful range for both thermometer types was 0 to 40ºC. RS and DS thermometers had a manufacturer's specified accuracy of ±1ºC and ±0.4ºC respectively.

Table 2-4. Results of 45ºC water bath measurements by Radioshack® (RS) and Digisense® thermocouple (DS) thermometers

Thermometer	Mean Temperature (± SD); n=10	Range
RS 63-1009A	44.0 (± 0.1)	43.8-44.1
RS 63-1035 (1)	44.6 (± 0.2)	44.4-44.9
RS 63-1035 (2)	44.1 (± 0.1)	44.0-44.2
RS 63-1035 (3)	44.1 (± 0.1)	43.9-44.2
RS 63-1035 (4)	44.6 (± 0.1)	44.4-44.8
DS (probe 1)	43.3 (± 3.5)	33.5-44.6**
DS (probe 2)	44.3 (± 0.1)	44.2-44.7**

The useful range for both thermometer types was 0 to 40ºC. RS and DS thermometers had a manufacturer's specified accuracy of ±1ºC and ±0.4ºC respectively. ** Highlights values that were obtained outside of the thermometer's respective accuracy.

Table 2-5. Results of 60ºC water bath measurements by Radioshack® (RS) and Digisense® thermocouple (DS) thermometers

Thermometer	Mean Temperature (± SD); n=10	Range
RS 63-1009A	59.05 (± 0.5)	58.4-59.9**
RS 63-1035 (1)	59.74 (± 0.3)	59.4-60.2
RS 63-1035 (2)	59.16 (± 0.2)	58.8-59.5
RS 63-1035 (3)	59.27 (± 0.3)	58.9-59.9
RS 63-1035 (4)	59.76 (± .0.2)	59.4-60.0
DS (probe 1)	59.60 (± 0.4)	59.3-60.6**
DS (probe 2)	59.42 (± 0.3)	59.1-60.0**

The useful range for both thermometer types was 0 to 40ºC. RS and DS thermometers had a manufacturer's specified accuracy of ±1ºC and ±0.4ºC respectively. ** Highlights values that were obtained outside of the thermometer's respective accuracy.

Table 2-6. Results of 75ºC water bath measurements by Digisense® thermocouple (DS) thermometer

Thermometer	Mean Temperature (± SD); n=10	Range
DS (probe 1)	74.5 (± 0.8)	73.4-75.8**
DS (probe 2)	74.7 (± 0.9)	73.3-76.1**

The useful range for the Digisense® thermocouple (DS) thermometer was 0 to 40ºC. The DS thermometer had a manufacturer's specified accuracy of ±0.4ºC respectively. ** Highlights values that were obtained outside of the thermometer's respective accuracy.

Table 2-7. Evaluation of ECG performance on 25 manatees

Animal ID	Straight length (cm)	Weight (kg)	Sex	Date	Successful reading	Printer jam	Lead malfunction	Comments
TTB119	251	361	F	05 Dec 2005	2		1	Not reading, loose connection with plug, replaced electrode clip.
TTB120	259	345	M	05 Dec 2005	2			
TTB122	276	474	F	06 Dec 2005	5	1		
TTB123	292	551	F	06 Dec 2005	3	2		
CTB051	231	470	M	05 Jan 2006	6			
TTB125	277	401	M	04 Jan 2006	5	1		
TTB101	290	460	M	05 Jan 2006	1		1	Tracings 2 and 3 not showing for augmented print.
TTB128	296	506	M	04 Jan 2006	11			
TTB132	302	816-907*	F	05 Jan 2006	2	3	1	Electrode clips replaced
TTB127	313	540	M	04 Jan 2006	1			
TTB006	316	540	F	05 Jan 2006	5		1	ECG searching for signal. Added gel to electrode terminals.
BZ05F78	191	132	F	14 Nov 2005	2			
BZ05F92	204	166	F	17 Nov 2005	1			
BZ03F28	210	190	F	15 Nov 2005	2			

n=11 Florida manatees (TTB and CTB prefix IDs) and n=14 Antillean manatees (BZ prefix IDs). * A range of weight is given because lifting chains broke while lifting animal in stretcher for weight.

Table 2-7. Continued

Animal ID	Straight-line length (cm)	Weight (kg)	Sex	Date	Successful Reading	Printer Jam	Lead Malfunction	Comments
BZ05F89	215	182	F	16 Nov 2005	7			
BZ98M05	235	289	M	14 Nov 2005	1			
BZ01M15	240	228	M	16 Nov 2005	2			
BZ05M85	245	323	M	12 Nov 2005	5	1	3	Checked leads. Salt in electrode clips-replaced
BZ05F86	248	268	F	13 Nov 2005	2		1	ECG searching for signal, clips were wet, had to replace
BZ05F91	251	279	F	17 Nov 2005	7		1	Electrode clips wet-replaced
BZ97F03	252	309	F	14 Nov 2005	2		1	ECG searching for signal. Electrode clip came off-replaced
BZ05M93	261	327	M	18 Nov 2005	2			
BZ05M88	259	323	M	16 Nov 2005	2		1	Electrode clip came off-replaced
BZ05M90	284	409	M	16 Nov 2005	6			
BZ05M87	291	424	M	15 Nov 2005	13			
TOTAL					97	8	11	
Percentage					83.6	6.9	9.5	

CHAPTER 3
ORAL TEMPERATURE, HEART RATE, AND RESPIRATION RATE OF MANATEES (T*richechus manatus latirostris, T. m. manatus*) EXPOSED TO CAPTURE AND HANDLING IN THE FIELD

Abstract

Many manatee research studies require the capture and handling of animals to collect data. Understanding how manatee oral temperature (OT), heart rate (HR), and respiration rate (RR) can change during a capture event is important for researchers who want to ensure an animal's well-being. To determine the effects of capture on healthy, awake, juvenile/adult manatee vital signs: a total of 38 Florida manatees (*Trichechus manatus latirostris*) and 48 Antillean manatees (*T. m. manatus*) were continuously monitored for OT, HR, and RR during field capture events. Creatine kinase (CK), potassium (K^+), serum amyloid A (SAA), and lactate values were examined for each animal to assess possible systemic inflammation and muscular trauma. Manatee OT, HR, and RR all changed over a 50 minute period, with Antillean manatees having higher parameters than Florida manatees. Mean (±SD) OT of Florida and Antillean manatees increased during capture from 32.6 (±1.8) ºC and 34.6 (±0.9) ºC; to 34.8 (±1.5) ºC and 35.2 (±0.7) ºC respectively. Animals were removed from water temperatures of 25.9 (±4.2) ºC in Florida; and 28.9 (±1.5) ºC in Belize and Puerto Rico. There was no statistically significant difference of OT between Antillean and Florida manatees after 40 minutes (p=0.4186). Mean (±SD) HR of Florida and Antillean manatees decreased from 66 (±9.7) beats per minute (bpm) and 75 (±7.7) bpm to 60 (±6.9) bpm and 61 (±10.5) bpm, respectively. There was no statistically significant difference of HR between Florida and Antillean manatees after 25 minutes (p=0.0739). Mean (±SD) RR of Florida and Antillean manatees decreased from 6 (±2.9) breaths/5min and 9 (±3.5) breaths/5min to 4 (±2.0) breaths/5min and 5 (±2.3) breaths/5min, respectively. There was no significant difference in RR after 50 minutes between Florida and

Antillean manatees (p=0.0014). Higher respiratory rate over time was associated with higher lactate values. Antillean manatees had higher overall lactate values than Florida manatees (p<0.001). Monitoring of manatee OT, HR, and RR in the field is recommended as standard protocol for researchers, to better assess the condition of an animal.

Introduction

The Florida manatee, *Trichechus manatus latirostris*, has a minimum population size calculated to be 3,300 animals (Haubold *et al.* 2006). It is classified as an endangered species in the State of Florida, protected by the U.S. Endangered Species Act, and by the Marine Mammal Protection Act, listed in the Convention on International Trade of Endangered Species of Wild Flora and Fauna under Appendix I, and defined as vulnerable by the International Union for Conservation of Nature and Natural Resources (U.S. Fish and Wildlife Service 2004, Convention on International Trade of Endangered Species of Wild Flora and Fauna 2004, Haubold *et al.* 2006, International Union for Conservation of Nature and Natural Resources 2007).

Since the early 1970s multiple federal, state, private, and non-profit organizations have been working together to protect and conserve the species from anthropogenic and natural threats to its population. As part of the conservation effort, healthy free-ranging manatees are routinely captured for population studies and research purposes. Between the years 1975 and 1983, O'Shea *et al.* (1985) reviewed net captures of 92 manatees in Florida. Based on blood chemistry parameters including aminotransferase activity, creatine kinase, potassium, and lactate, they determined that both healthy and distressed manatees were generally not susceptible to severe capture stress. Since the late 1970s, only six manatees have died in over 1000 capture events (U.S. Geological Survey, Florida Fish and Wildlife Conservation Commission, Wildlife Trust, unpublished data). Upon necropsy,of the carcasses, symptoms of capture myopathy as defined by Spraker (1993) were not found.

While capture related deaths are low, field researchers encounter animals during research captures and rescues with symptoms such as bradycardia, apnea, hyperthermia, and hypothermia. Recognizing these physiological abnormalities is important to biologists and veterinarians who want to improve manatee triage and husbandry in the field. Understanding oral temperature (OT), heart rate (HR), respiration rate (RR) and blood chemistry parameters of manatees under various field circumstances may aid with the interpretation of physiologic and pathologic findings. The purpose of this research was to determine normal ranges and dynamics of manatee OT, HR, and RR over time during field captures and determine their relationships with creatine kinase (CK), potassium (K^+), serum amyloid A (SAA), and lactate.

Materials and Methods

During the years 2004-2006, free ranging manatees were captured at the following locations:

Florida: Apollo Beach, in Tampa Bay, near the TECO Big Bend power plant (27°47'34.01"N, 82°25'15.87"W); Port of the Islands' residential and marina basin, in Naples, Faka Union Canal (25°57'19.74" N, 81°30'34.03"W) ; Everglades National Park, Whitewater and Coot bays (25°18'9.77"N, 80°59'9.37"W).

Belize: Inshore waterway of Southern Lagoon (17°15'21.21"N, 88°20'39.78"W); and offshore isles of the Drowned Cays (17°28'05.25"N, 88°04'23.40"W).

Puerto Rico: Boqueron, Rincón Canal (18°01'12.32"N, 67°11'56.15"W); Guayanilla, Bahia de Guayanilla (17°59'12.49"N, 66°46'15.64"W); Ceiba, near-shore waters off former Naval Station Roosevelt Roads (18°12'46.40"N, 65°37'12.71"W).

Captures were performed under United States Fish and Wildlife Service research permits MA773494 (Florida Fish and Wildlife Conservation Commission), M791721/4 (U.S. Geological Survey), MA791721/4 (U.S. Geological Survey), and the Belizean Department of Forestry permit CD/60/3/05(36) (Wildlife Trust). Manatees in this study were concurrently monitored for OT, HR, and RR, sampled for research purposes and given complete health assessments. A total of 86 apparently healthy, free-ranging, juvenile and adult manatees were monitored for OT, HR, and RR in this study, 38 were Florida manatees and 48 were Antillean manatees (Table 3-1). Capture of manatees consisted of efforts from many people through the use of a capture boat, support boats, and aerial observation. 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 motor boats 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 from the air, an observer in a small plane or helicopter was used when possible. Manatees were selected visually based on individual size and number of grouped individuals. Preference was given to larger animals and efforts were made to avoid harassing or capture of mother-calf pairs. Once located, the target manatee was encircled by the capture boat, in a number 30 or 56 braided seine twine net approximately 122-183 meters long, approximately 8 meters deep, with an approximately 10-17 cm stretch knotted nylon mesh. The net and manatee were then pulled into the boat. Slight modifications to the basic capture method occurred in Florida and Belize. In Florida, manatees were usually captured using land based net sets, simultaneously entrapping one to four animals, and in one instance up to 12 manatees along a shoreline. In Belize, one to three manatees were typically caught in large open water net sets at one time. The manatees were corralled into a smaller net with a bag end, guided to the stern of the boat, and pulled aboard. If manatees needed to be moved to a work-up site, they were placed in a stretcher and carried by handlers. Capture times varied.

Monitoring of OT, HR, and RR began immediately upon safely securing the manatee on the deck of the capture boat or on land. OT, HR, and RR were monitored continuously during the holding period until the manatee was released.

A Radioshack® (RS) thermometer model 63-1009A, and RS thermometer model 63-1035 were used to measure oral temperature. Thermometers were lab tested to ensure accuracy prior to field deployment (CHAPTER 2). OT probes were placed once the manatee was secured on land or on a boat deck. The soft wire temperature probe was placed by hand, along the jaw line, buccally past the rear mandibular molars (CHAPTER 4). OT values were recorded every 5 minutes simultaneously with HR and RR. The temperature probes were removed just prior to release.

A Sonocardia® waterproof stethoscope (Magnafortis, Seattle, WA) was used to auscult the heart. The stethoscope bell was inserted under the manatee's axilla and placed ventrally, close to midline. The number of heart beats, defined as atrial and ventricular contractions, were counted for 15 seconds and multiplied by four to determine beats per minute (bpm). HR was recorded every 5 minutes, in conjunction with OT and continuous RR monitoring.

A Mac 500 electrocardiogram (ECG) (GE Healthcare, Milwaukee, WI**)** was used to assess heart function and HR. Skin with dirt or slime was scraped, wiped with an alcohol pad, and dried extensively with an absorbent sponge, heavy duty paper towels, or beach towel, to ensure proper adherence of sticky electrode pads. Ultra sound gel was used at the connection point of the lead clip and the electrode pad to improve electrical conductivity. A small amount of tissue glue was applied to the cleaned site. ECG HR was compared to stethoscope to ensure its accuracy. Printed ECG tracings were examined to ensure validity of the unit's displayed HR and to assess heart function (Siegal-Willott *et al*. 2006). ECG tracings that showed artifacts such as distorted baselines, or unclear PQRS complexes were considered as evidence for an inaccurate HR reading. Data from inaccurate tracings were not used and these were considered equipment failures.

Monitoring of respiration began when a manatee was secured on a boat or land, and ended upon release back into the water. Time and quality of breaths were recorded. Evidence of respiration needed to be seen, heard, or felt. Opening of the nares alone did not constitute a breath. Visible respiratory exchange was determined by thoracic excursion. Sounds of respiratory exchange were also considered as evidence of breathing. A hand was placed in front of a manatee's nares to feel for an exhalation or inhalation. Respiratory abnormalities such as air leaking out of closed nares were described and recorded. Lungs were ausculted dorsally to assess respiratory health. The following defined terms were commonly used to help describe observed quality of breaths.

- **Shallow breath:** A relatively short respiratory exchange time is observed, heard, or felt. There is little rising or lowering of the manatee's thoracic region.
- **Deep breath**: A relatively long respiratory exchange time is observed, heard, or felt. The rising and lowering of the manatee's thoracic region is quite obvious and great.
- **Stuttering breath**: Obvious, short, rapid cessations of inhalation occur during inhalation. No exhalation during the cessations of inhalation.

Blood Sampling and Biochemical Analysis

Blood was sampled from the brachial vascular bundle, located between the radius and ulna (Medway *et al.*1982; Bossart *et al.* 2001). The medial or lateral, mid-pectoral region of the manatee was manually restrained and sterilely prepared by alternating Betadine, a 10% povidone iodine solution (Purdue Frederick Company, Norwalk, CT), and 70% isopropyl alchohol three times in concentric circles radiating outwards. Vacutainer equipment was used with a 21 gauge, 1.5 in needle with a syringe or extension set. Blood samples were generally collected within 15 to 45 minutes of capture. Total time of blood collection varied from 5 minutes to 30 minutes, depending on the flow of blood into the tubes, the additional number of tubes being filled for other studies, and the activity of the animal. Ethyoenediamine tetra-acetic acid (EDTA) tubes and lithium heparin tubes were gently inverted for 2 minutes after collection. Tubes with anti-coagulants were placed in a cooler, on top of a small foam pad on ice, as soon as possible after collection. Plasma and serum were separated within 1 hour of collection. Samples were transported on ice at 4 to 10°C until laboratory analysis. Belize serum and plasma samples were stored at -20°C prior to analysis.

Complete blood count, biochemical analysis, and protein gel electrophoresis were performed at the University of Florida-College of Veterinary Medicine Clinical Pathology Laboratory. Creatine kinase, potassium (K^+), serum amyloid A (SAA), and lactate values were determined as described by Harr *et al*. (2006) and Harvey *et al*. (2007).

Statistical Analyses

Statistical analyses were conducted using Microsoft Office Excel 2003 for Windows (Microsoft Corp., Redmond, WA), and Sigma Stat for Windows, Version 3.1 (Systat Software Inc., Point Richmond, CA). T-tests were conducted 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. Correlation and regression analyses were conducted whereby an $r^2 = 0.5$ or greater was considered a significant relationship.

Only complete OT, HR, and RR data were used in the statistical analysis. Complete OT, HR, and RR was defined as: monitoring where no more than 10 minutes of continuous time, or two consecutive 5 minute time intervals, were missed during a 50 minute monitoring session. While some manatees were monitored for over 50 minutes, the sample size supported statistical analysis up to 50 minutes. Antillean manatees captured from Puerto Rico and Belize were grouped together for statistical comparison with Florida manatees. A repeated measures linear mixed model (Proc Mixed; SAS Institute, Cary, NC) was used to determine if there was a significant difference between the OT, HR, and RR of manatees based on the fixed effects of time, location, sex, and size class. Type 3 sums of squares and differences of the least square means were calculated for significant fixed effects. Correlation analyses of HR versus RR, for Florida and Antillean manatees were conducted.

Unpaired t-tests were conducted between respective Florida and Antillean: CK activity, K^+, SAA, and lactate values.

Based on normal Florida reference intervals (Harvey *et al*. 2007), manatees with CK activity > 482 U/L, K^+ > 6 mEq/L, SAA> 70 ug/mL, and lactate values >25 mmol/L were examined for abnormal OT, HR, and RR. Regression analyses were performed between OT, HR, and RR versus blood chemistry. Unpaired t-tests were used to compare the means of OT, HR, and RR values in groups of animals with normal and abnormal biochemical values.

Average HR and RR over 20 minutes and 40 minutes were grouped into those with six breaths or higher versus those with five breaths or fewer and at 70 bpm or higher versus 69 bpm or less, respectively. An unpaired t-test was then used to compare the mean lactate concentration within these groups.

Results

Capture

Climate conditions for captures were distinctly different for Florida and Antillean manatees. In Florida, mean air temperature was 23.8 (±6.0) °C, and ranged from 14.7°C to 31.9°C; mean water temperature was 25.9 (±4.2) °C, and ranged from 21.5°C to 30.9°C. In Belize and Puerto Rico combined, mean air temperature was 30.7 (±3.9) °C, and ranged from

24.2°C to 40.8°C; mean water temperature was 28.9 (±1.5) °C, and ranged from 25.1°C to 31.5°C.

Capture times averaged 43 minutes from the time of net set to when the manatee was secured for examination, for Florida and Belize. This was due to the logistical complexity that occurred when multiple manatees were netted. In Puerto Rico, where single individuals were captured at a time, animals were secured within 5 minutes of being netted. In TECO Tampa Bay, on 14 December 2007, 12 manatees were netted at one time in a land set. Six individuals from this group were monitored for this study. For approximately 4.5 hours, several individuals were maintained in water that was just deep enough to be fully submerged, prior to being moved for examination. Similarly, in Belize several animals swam around in the large net for a little over 2.5 hours before being secured for examination (Table 3-7).

Oral Temperature

No significant difference in OT over time was found between adult and juvenile size classes, or between sexes. Initial OT of the Florida manatees averaged 32.6 (±1.8) °C, with a range of 29.5°C to 35.1°C. The final 50 minute interval OT of the Florida manatees averaged 34.8 (±1.5) °C, with a range of 30.5°C to 35.9°C. Initial OT of the Antillean manatees averaged 34.6 (±0.9) °C, with a range of 32.6°C to 36.0 °C. The final 50 minute interval OT of the Antillean manatees averaged 35.2 (±0.7) °C, with a range of 34.0°C to 36.1°C. Initially, OTs between the two subspecies were significantly different ($p<0.0001$). By the 40 minute time interval, there was no statistically significant difference in OTs between Florida and Antillean manatees, with average OTs of 34.2 (±1.6) °C and 35.2 (±1.6) °C, respectively ($p=0.4186$) (Figure 3-2, Table 3-3). A total range of 29.5 °C to 36.2 °C was observed from Antillean and Florida manatees.

Heart Rate

No significant difference in HR over time was found between adult and juvenile size classes, or between sexes. Initial HR of the Florida manatees averaged 66 (±9.7) bpm, with a range of 49 bpm to 80 bpm. The final 50 minute interval HR of the Florida manatees averaged 60 (±6.9) bpm, with a range of 45 bpm to 68 bpm. Initial HR of the Antillean manatees averaged 75 (±7.7) bpm, with a range of 64 bpm to 88 bpm. The final 50 minute interval HR of the Antillean manatees averaged 61 (±10.5) bpm, with a range of 48 bpm to 84 bpm. Initially, there was a significant difference between the HRs of the two subspecies ($p=0.0058$). However, by 25 minutes there was no statistically significant difference between Florida and Antillean manatees, with average HRs of 60 (±12.6) bpm and 63 (±10.1) bpm, respectively ($p=0.0739$) (Figure 3-3, Table 3-4). A total range of 32 bpm to 88 bpm was observed from Antillean and Florida manatees in the field.

Respiration Rate

No significant difference in RR over time was found between adult and juvenile size classes, or between sexes. Initial RR of the Florida manatees averaged 6 (±2.9) breaths/5 minutes, with a range of 1 breath/5 minutes to 13 breaths/5 minutes. The final 50 minute interval RR of the Florida manatees averaged 4 (±2.0) breaths/5 minutes, with a range of 0 breaths/5 minutes to 8 breaths/5 minutes. Initial RR of the Antillean manatees averaged 9 (±3.5) breaths/5 minutes, with a range of 3 breaths/5 minutes to 17 breaths/5 minutes. The final 50 minute interval RR of the Antillean manatees averaged 5 (±2.3) breaths/5 minutes, with a range of 2 breaths/5 minutes to 12 breaths/5 minutes. Initially, respiration rates between the two subspecies were significantly different ($p<0.0001$). Over time the respiration rates of Antillean and Florida manatees became progressively lower. However, by 50 minutes there was no statistically significant difference between Florida and Antillean manatees with average RRs of 4 (±2.0) breaths/5 minutes and 5 (±2.3) breaths/5 minutes, respectively (p=0.0943) (Figure 3-4, Table 3-5). A total range of 0 breaths/5 minutes to 17 breaths/5 minutes was observed from Antillean and Florida manatees.

A positive correlation existed between heart rate and respiration rate in manatees. Florida manatees had an r^2=0.732 and Antillean manatees had an r^2=0.8598 (Figure 3-5).

Blood Chemistry: Creatine Kinase, Potassium, Serum Amyloid A, and Lactate

There was no statistically significant difference between Florida and Antillean measured CK activity. Mean CK activity of 38 Florida animals was 193 (±151) U/L with a range of 51-799 U/L. Mean CK activity of 48 Antillean manatees was 195 (±493) U/L with a range of 38 U/L to 3522 U/L. Within theses sampled groups, two Florida manatees and one Antillean manatee had abnormal values with a combined range of 572 U/L to 3522 U/L.

There was no statistically significant difference between Florida and Antillean potassium values. Mean K^+ of 38 Florida manatees was 5.1 (±0.6) mEq/L with a range of 3.9 mEq/L to 6.4 mEq/L. Mean K^+ of Antillean manatees was 5.3 (±0.5) mEq/L with a range of 4.1 mEq/L to 7 mEq/L. Within these sampled groups three Florida manatees and three Antillean manatees had abnormal values with a combined range of 6.1 mEq/L to 7 mEq/L.

There was no statistically significant difference between Florida and Antillean SAA values. Mean SAA for Florida manatees was 97 (± 235) ug/ul with a range of 10 to 1200.ug/mL). Mean serum amyloid A for Antillean manatees was 25 (±32) ug/mL with a range of 10 ug/mL to 190 ug/mL. Within these two sampled groups nine Florida manatees and four Antillean manatees had abnormal values with a combined range of 73 ug/mL to >1200 ug/mL.

A statistically significant difference existed between mean lactate values of Florida (n=37) and Antillean (n=44) manatees, of 13.7 (±6.7) mmol/L and 20.6 (±7.8) mmol/L, respectively (p<0.001). Ranges of lactate values were 0.5 mmol/L to 31 mmol/L and 5 mmol/Lto 37 mmol/L for Florida and Antillean manatees, respectively. Fourteen manatees presented lactate levels greater than 25 mmol/L, with a total range of 26.5 mmol/L to 32 mmol/L). Twelve of the 14 manatees were Antillean.

Respiration Rate and Lactate

A student's t-test revealed a statistically significant difference between lactate values of all manatees based on mean RR at 20 minutes, of ≤5 breaths (n=24) versus ≥6 breaths (n=57) (p=0.020). Whereby, mean lactate (±SD) was 14.3 (±7.6) mmol/L and 18.8 (±7.9) mmol/L, respectively. A statistically significant difference was also observed when conducting a student's t-test comparing lactate values of all manatees based on mean respiration rates in 40 minutes, of ≤5 breaths (n=32) versus ≥6 breaths (n=49) (p=*0*.018). Whereby, mean lactate (±SD) was 14.9 (±7.8) and mmol/L 19.1 (±7.8) mmol/L, respectively. Sample size was not sufficient enough to compare lactate values based on respiration rates within the two subspecies.

No significant correlation was observed between lactate versus HR and RR. However, a female Antillean manatee, BZ97F03, captured on 3 separate occasions showed a positive correlation between initial HR, initial RR, and lactate values (Table 3-6).

Other Significant Findings

A 231 cm long male (CTB051), captured in Tampa Bay on 05 January 2006, demonstrated a pronounced and persistent bradycardia, with irregular rhythm, an instantaneous HR of 42 bpm, and a mean of 48 bpm on the ECG. Heart rate was taken by stethoscope seven times during a 5 minute period and ranged between 28 bpm and 48 bpm. RR during this time was 4 breaths/5 minutes. Thirty minutes later, the HR stabilized at 60 bpm, RR was 6 breaths/ 5 minutes. Oral temperature ranged between 34.1 °C to 34.6 °C. The SAA was 83 ug/mL.

A 289 cm long female (TNP029), captured in Port of the Islands on 18 April 2004 was found dead 32 days later from a chronic infection due to a watercraft impact related injury. No obvious signs of illness were apparent upon capture. However, the SAA value was >1200 ug/mL. At necropsy, a pyothorax was confirmed (Florida Fish and Wildlife Conservation Commission, unpublished data). The manatee's OT was 34.7 °C, HR ranged from 52 bpm to 60 bpm, and RR ranged from 2 breaths/5 minutes to 15 breaths/5 minutes.

Discussion

Capture

The grouping of Antillean manatees captured by corralling into a bag net in Belize, and simpler open water net sets in Puerto Rico, was supported by the lack of a significant differences between OT, HR, and RR of the two groups. Considering how distinctly different these two capture methods were, the results suggest that the differences in OT, HR, and RR between Florida and Antillean manatees may also be due to other capture related factors such as pursuit time by boat prior to net set, struggle duration and intensity, or individual degrees of excitability. While pursuing manatees for capture, focal follow of individuals underwater was challenged by poor water visibility, and assessment of struggle duration and intensity was considered too subjective. Furthermore, the instances when there was a preference to pick more robust animals and avoid mother-calf pairs, did not create a sampling bias as the resulting animals sampled were relatively well distributed in size and sex.

Oral Temperature

Oral temperature monitoring is an effective technique to ascertain thermal homeostasis in a manatee, but should not necessarily be considered an exceptionally sensitive method of measuring body heat build up. Oral temperature ranges in this study did not exceed the reported normal OT range of 35.5°C to 36.0°C (Murphy 2003). The results from this study suggest that captured manatees are effectively managing near, or within the reported core body temperature of 35.6°C to 36.4°C (Gallivan *et al*. 1983; Irvine 1983), and/or that OT may be physiologically limited in terms of the maximum level of heat that is distributed to the buccal area in exercised, healthy individuals.

Measurement of fluke temperature in conjunction with oral temperature may prove useful to better assess thermal insult in field captured manatees. When forced to dissipate body heat, manatees are able to increase arterial blood flow to the skin of the fluke, via regulation of blood flow through deep caudal veins located collateral to the caudal vascular bundle of the chevron canal (Rommel & Caplan 2003).

Heart Rate

Manatee mean HR following 50 minutes post capture monitoring returned to near the normal resting max HR of 60 bpm reported for captive manatees in the water and on land (Scholander & Irving 1941, Murphy 2003). These results suggest that manatees are not reacting with a tachycardia in terms of a fright response, or a bradycardia in terms of a dive response.

In this study, initial individual HR measurements were as high as 88 bpm. Previous captive manatee HR studies demonstrated a HR of 70 bpm upon surfacing from a 5 bpm dive bradycardia, and 75 bpm while being monitored with an ECG in a drained pool (Tenney & Hanover 1958, Gallivan *et al.* 1986). The elevated initial HR of captured manatees is likely a physiological response in order to supply the increased demand for oxygen and energy by active muscles. Additionally, increased blood flow to the fluke and other extremities in response to increased body heat, may also have contributed to an increase in HR. In general, this study did not see profound bradycardic dive responses or fright responses as suggested by Butler (1982), and demonstrated by Gallivan *et al*. (1986) on manatees in water. These results suggest that manatees may recognize when they are on land and in the water, and have different responses for the two different environments.

Respiration Rate

Initial RR of captured manatees was significantly higher than the normal 2 breaths/5 minutes to 4 breaths/5 minutes (Scholander & Irving 1941, Murphy 2003, Bossart 2001). This study's RR range of 0 breaths/5 minutes to17 breaths/5 minutes exceeded the RR observed by Walsh & Bossart (1999) of 3 breaths/5 minutes to15 breaths/5 minutes, of manatees being assessed on land. It is likely that the 10 minute to 16 minute periods of breath holding recorded in previous dive studies is significantly reduced following intense struggle in the field captured manatee (Scholander & Irving 1941, Gallivan *et al*. 1986).

Increased rhythmic respirations have been observed in bottom resting manatees submerged for extended periods of time (Hartman 1979, Parker 1922). It is likely that the respirations observed in captured manatees were stronger and deeper than those of the bottom resting manatee. The increase in minute ventilation is likely a hypercapnic response in the captured manatee, whereby high CO_2 and not low O_2 was the triggering factor (Gallivan & Best 1980). Hyperventilation in manatees rapidly replenishes oxygen stores while eliminating carbon dioxide and is typical of slow breathing marine mammals (Kooyman 1973). In this study, RR was generally elevated above reported normal values. Also, many animals did not return to the reported normal RR during monitoring. The persistent elevated RR overtime suggests that full recovery from struggle can take over 50 minutes. Captured manatees released within an hour may still be in a recovery stage upon release.

Difficulty in a breathing response following a forced submergence of a 12 minute to15 minute duration was observed in a 330 kg individual (Scholander & Irving 1941). Following this extreme dive duration, respirations increased to two or three breaths per minute. Apnea and decreased respirations were occasionally observed in captured manatees, but not in association with a bradycardia of ≤40 bpm. This observation however, does not preclude the possibility of a dive response in manatees. Manatees with an RR below 2 breaths/5 minutes were stimulated to breathe by pouring water over the back of the head. The active induction of respirations is an animal care technique used by field researchers to ensure that a captured manatee is adequately ventilating, and may have prevented a dive response from occurring during this study.

Blood Chemistry: Creatine Kinase, Potassium, Serum Amyloid A, and Lactate

Standard manatee hematological and biochemical references are relatively well established (Medway *et al*. 1982, Walsh & Bossart 1999, Murphy 2003, Harvey *et al*. 2007). Furthermore, research has been conducted on specific blood parameters to support a more diagnostic approach to manatee health in the field. The evaluation of CK, K^+, SAA, and lactate are used to assess muscle damage and systemic inflammation associated with exertional or pathological distress.

CK is an enzyme commonly found in the cytoplasm of muscle cells. Its primary function is to catalyze the formation of phosphocreatine, a source of energy for muscle activity. CK has a relatively short half life in many mammals, but specific duration has not been determined in manatees. High levels of CK activity maintained overtime are generally associated with continual muscle damage from muscle disease and exertion. The reported normal range of manatee creatine kinase (CK) activity is 79 U/L to 302 U/L (Walsh & Bossart 1999). Current reported normal mean CK values for two captive adult manatees are reported by Manire *et al*. (2003) as 223.8 U/L and 233.8 U/L. CK in a free-ranging captured manatee (8247B) following severe struggle was observed as high as 1365 U/L sampled the same day, and 1381 U/L 3 days later while in captivity (O'Shea *et al*. 1985). One hundred days later, the same individual's CK activity was 227 U/L. This result was common amongst other similar individuals in the study. While it has been suggested that manatees are likely not susceptible to capture myopathy, such elevated levels of CK activity are indicative of muscle damage (Harvey *et al*. 2007). Chronically injured manatees may show mildly increased CK activities. For example, a rescued manatee with an embedded line entanglement to its pectoral flipper (SWF-TM-613B) showed a CK value of 520 U/L (Beusse *et al*. unpublished data).

Potassium is an electrolyte that helps to maintain cellular osmotic balance, and is mostly found in muscle cells. High levels of serum K^+ can be associated with acidemia, as intracellular K^+ are exchanged for H^+ in the blood to maintain cellular osmotic balance. The reported normal range of K^+ is 4.2 mEq/L to 6.6 mEq/L (Walsh & Bossart 1999). White *et al*. (1976) reports normal mean (±SD) for Na^+ as 143.8(±1.20) mEq/L and for K^+ as 5.0 (±0.14) mEq/L. Similarly, Medway *et al*. (1982) show respective values of 151 (±4.3) mEq/L and 5.2(±0.7) mEq/L. Harvey *et al*. (2007) show similar results for both free ranging and captive manatees.

Serum amyloid A is an acute phase response protein which increases during acute inflammation and infection. Harr *et al*. (2006) established use of the acute phase response protein SAA as a general indicator of tissue damage in manatees. In their research, healthy manatees were determined to have a mean (±SD) SAA of 22 (±25) µg/mL. In comparison, diseased manatees showed a mean SAA value of 266 (±398) µg/mL.

Lactate is a biochemical byproduct of the anaerobic metabolism of glucose. Increased levels of lactate are positively associated with increased levels of muscular exertion, which can contribute to metabolic acidosis. In their manatee diving experiment, Scholander and Irving (1941) observed lactate levels significantly increasing from 1.11 mmol/L pre dive to a range of 11.1 mmol/L to 16.65 mmol/L post dive. Increases in lactate occurred a few minutes after the dive, but not during the dives. Scholander and Irving (1941) explain that such phenomena is indicative of peri-vascular constriction of circulation within the muscle tissue during the dive, isolating lactic acid build up from the general circulation. Inverse to blood O_2, CO_2 increased during dives and decreased during dive recovery. Harvey *et al*. (2007) found that mean lactate in normal free ranging manatees was 13 mmol/L, and 4 mmol/L in normal captive manatees. One should consider that the increased lactate concentration in free ranging manatees may be associated with struggle due to capture.

In this study, the majority of captured manatees presented normal blood chemistry values and were considered healthy animals. Animals with abnormal CK, K^+, and SAA did not have associated abnormal OT, HR, or RR. However, significant associations were made between lactate and RR.

Respiration rate and lactate

The underlying cause of metabolic acidosis is an over abundance of H^+ protons resulting from glycolysis and ATP hydrolysis, due to anaerobic metabolism (Robergs & Roberts 1997). Clinically, metabolic acidosis has been traditionally and indirectly assessed by the associated symptomatic increase in levels of lactate. Lactate values from captured manatees show a range resembling that of mild, moderate, and extreme levels of exertion for thoroughbred horses (Bayly *et al*.1983, Bayly *et al*. 1987, Rose *et al*. 1988). Harvey *et al*. (2007) show that high lactate values in free-ranging Florida manatees are associated with low plasma CO_2, which would be attributed to increased respiration rate to blow off HCO_3^- and maintain optimal blood pH (Robergs & Roberts 1997). Similarly, Scholander and Irving (1941) observed in manatee recovery from forced dives a return to predive blood O_2 levels, and a decrease in blood CO_2 with an associated increase in lactic acid.

RR levels show potential to serve as predictors of lactate levels in captured manatees. BZ97F03, captured on three separate occasions, showed lactate levels of 27.5 U/L, 20 U/L, and 9.7 U/L. Concurrently, initial HR and RR values were 92 bpm and 16 breaths/5 minutes; 84 bpm and 8 breaths/5 minutes; 60 bpm and 3 breaths/5 minutes respectively. Individuals with elevated

RR levels that do not decrease over a 50 minute time period should be clinically assessed by a veterinarian. Such individuals are not properly adjusting and returning them to the water is recommended.

Florida and Antillean Manatees: Differences in Oral Temperature; Heart Rate, Respiration Rate, and Lactate

Oral Temperature

The initial oral temperature differences between the Florida and Antillean manatees may in part be due to differences in subcutaneous fat thickness, body condition, and water temperature. Gallivan *et al*. (1983) notes an individual manatee's core temperature at 33.0°C and falling in a water temperature of 23.0°C. At the same length and after gaining 26 kg in body weight, the same animal maintained a core temperature of 34.0 °C in a water temperature of 20.0°C. In respect to these findings, Antillean manatees may be leaner than Florida manatees as part of an adaptation to their warmer environment, and possibly have a higher minimal water temperature limit than the 20.0°C reported for Florida manatees (Irvine 1983, Bossart 2001). However, there is currently not a large enough data set regarding length, weight, and subcutaneous fat measurements of Antillean manatees to compare them with Florida manatees.

Heart Rate, Respiration Rate, and Lactate

Lower HR, RR, and lactate in Florida manatees compared to Antillean manatees may be attributed to better endurance conditioning in Florida animals. Florida manatees can have long distance seasonal migrations generally between <50km to 830 km, with a unique individual traveling greater than 2,300 km (Deutsch *et al*. 2003). Populations in Puerto Rico and Belize generally remain within a 50 km home range (O'Shea & Salisbury 1991, Lefebvre *et al*. 2001). Endurance conditioning increases mitochondria and provides more aerobic supplied energy, reducing lactate production (Holloszy & Coyle 1984, Honig *et al*. 1992). Endurance conditioned muscles also have a higher lactate capacity and increased membrane transport, further reducing blood lactate levels during recovery (Gladden 2000). Florida manatees may be more physically fit than the Antillean subspecies.

Another possible explanation for the observed differences between the manatee subspecies may be due to differences in follow time prior to capture. Upon examining lactate levels of several Everglades captured manatees, Tripp *et al*. (2007) show an association between follow time and lactate. An individual followed for 15 minutes showed a lactate level of 17 mmol/L whereas an individual followed for 50 minutes showed a lactate level of 23 mmol/L. It is possible that Antillean manatees in Belize and Puerto Rico were followed longer than animals in Florida.

Abnormal Individuals

During this study we observed two abnormal individuals, CTB051 and TNP029. The result of monitoring the OT, HR, and RR of these individuals depicts the benefits and limitations of the field monitoring protocol.

A capture on 05 January 2006 of CTB051, a 231 cm long male, captured in Tampa Bay, demonstrated a pronounced persistent bradycardia, with irregular rhythm, an instantaneous HR of 42 bpm, and a mean of 48 bpm by the ECG. HR was taken by stethoscope seven times during a 5 minute period and ranged between 28 bpm to 48 bpm. RR during this time was 4 breaths/5 minutes. Thirty minutes later, the HR stabilized at 60 bpm, RR was 6 breaths/5 minutes. Oral temperature ranged from 34.1°C to 34.6°C. The SAA was 83 ug/mL. Additional care was taken in the field, once a bradycardia was recognized. This included alerting of symptoms to all involved field staff, more care in handling, induction of respirations, and expediency in the overall health assessment. It is possible that these additional measures of care, helped to prevent the manatee from becoming further compromised.

In the case of TNP029, an animal with >1200 ug/mL, no obvious signs of illness were apparent upon capture. However, the animal was found dead 32 days later from a chronic infection due to a watercraft impact related injury. At necropsy, pyothorax was confirmed (Florida Fish and Wildlife Conservation Commission unpublished data). The manatee's OT was 34.7°C; HR ranged from 52 bpm to 60 bpm; and RR ranged from 2 breaths/5 minutes to 15 breaths/5 minutes. Not all illnesses affect OT, HR, and RR to noticeable, abnormal values. This case demonstrates the limitations of OT, HR, and RR when monitoring the manatee.

Future Monitoring Considerations

For researchers capturing and handling manatees in the field, understanding the dynamics of manatee vital signs specific to their circumstances, and recognizing abnormal trends in values overtime are essential for proper care of individuals. The effects of struggle and follow time should be studied further to improve manatee safety during capture procedures. Study of heat loss from the manatee fluke or other areas of the body, via thermography, may prove useful in further assessing thermal regulation by manatees in the field. HR of manatees should be monitored by stethoscope to better assess potential arrhythmias that might put an animal at increased risk. Manatees being captured may have significantly reduced breath holding ability, and should be allowed to frequently surface for air to prevent drowning. Quality of respirations should be studied with a capnograph, to enhance our understanding of manatee capture recovery. Field use of a portable blood analyzer may help to further assist in assessing the blood gas status of these animals. This study supports current post release behavioral and RR monitoring of field captured manatees, as physical recovery may still be occurring following a capture event. Establishing an historical record of individual manatee's OT, HR, and RR during long term studies is recommended to support health assessments. Further study of the Antillean manatee is recommended in order to better understand physiological differences compared to Florida manatees. The OT, HR, and RR values and dynamics established in this study should serve as a point of reference for those individuals tasked with monitoring vital signs. For the safety of the animal, interpretation of results should be performed by an experienced manatee veterinarian or biologist.

Table 3-1. Apparently healthy, free-ranging manatees monitored during captures from 2004 to 2006

Location	Juvenile 206-265 cm		Adult >265 cm		Total
	Male	Female	Male	Female	
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

14 calves (<206 cm) captured during this time were not included as part of this study. Size classification is based on modification of a working document by the USGS Sirenia Project, 30 September 1994. Size classes are based on straight length measurements.

Table 3-2. Air and water temperatures (˚C) associated with manatees sampled for oral temperature, heart rate, and respiration rate

	Belize and Puerto Rico			Florida		
	Mean (±SD))	Median	Range	Mean (±SD)	Median	Range
Air	30.7 (±3.9)	30.4	24.2-40.8	23.8(±6.0)	22.0	14.7-31.9
Water	28.9 (±1.5)	28.9	25.1-31.5	25.9(±4.2)	25.6	21.5-30.9

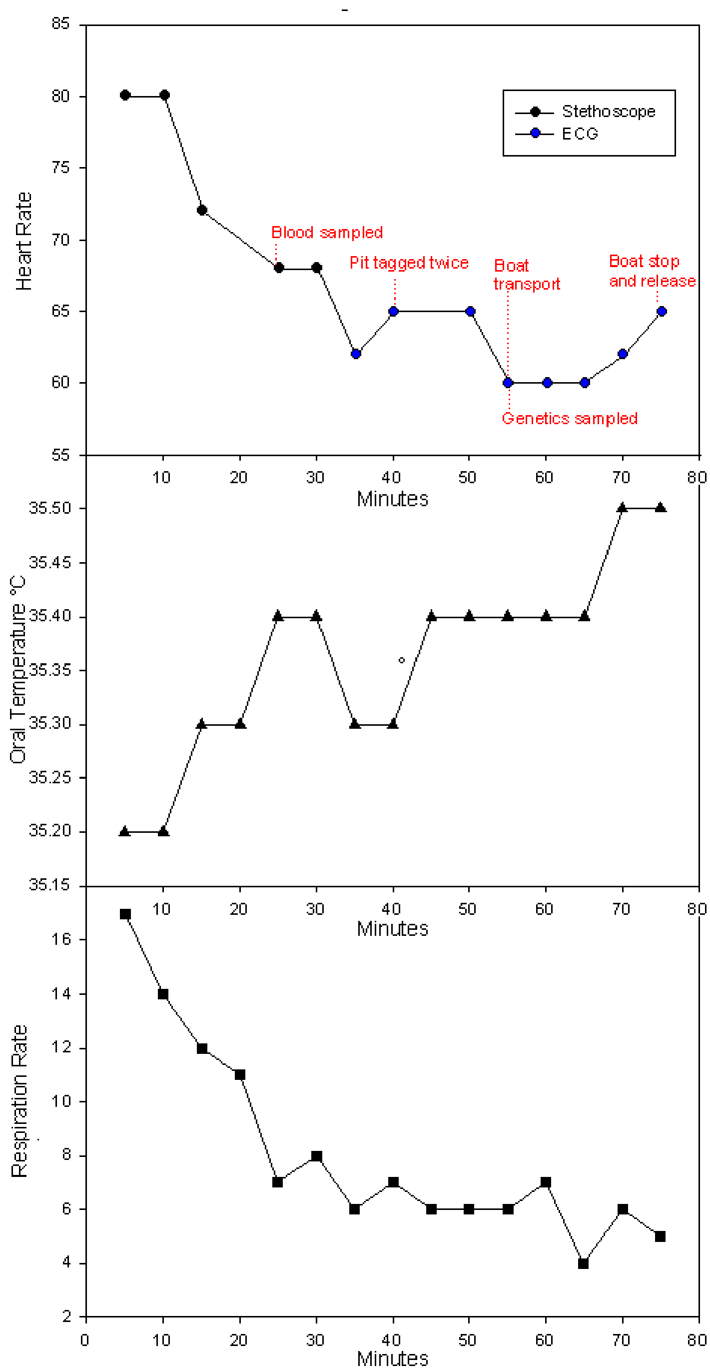

Figure 3-1. OT, HR, and RR values in relation to invasive procedures that occurred to BZ05M79, a 225 cm male, on April 17, 2005. Manatees in this study generally did not show extreme reactions in OT, HR, and RR as a direct result of a specific invasive procedure.

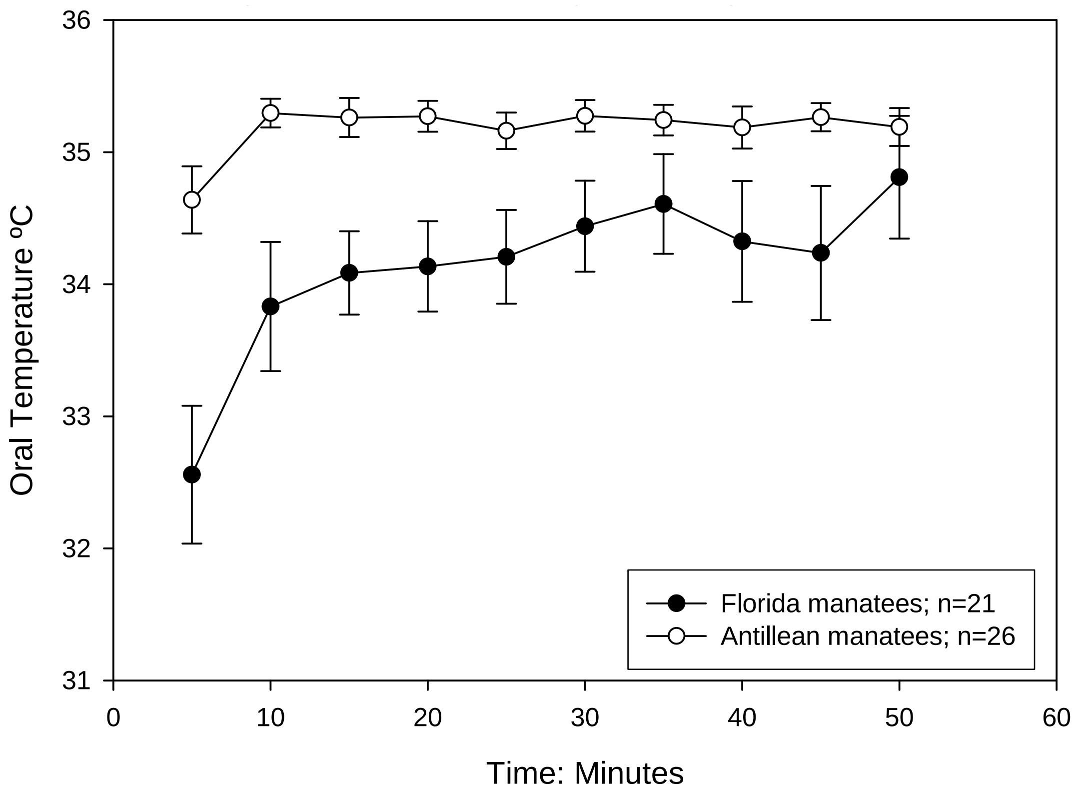

Figure 3-2. Mean oral temperatures over time, with standard error bars, for healthy juvenile and adult manatees, collected immediately following capture

Table 3-3. Descriptive statistics for oral temperatures of Florida and Antillean manatees at different time intervals during field monitoring

	Florida Manatees					Antillean Manatees				
Time (min)	n	Mean (±SD)	Median	Range	Std. Error	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

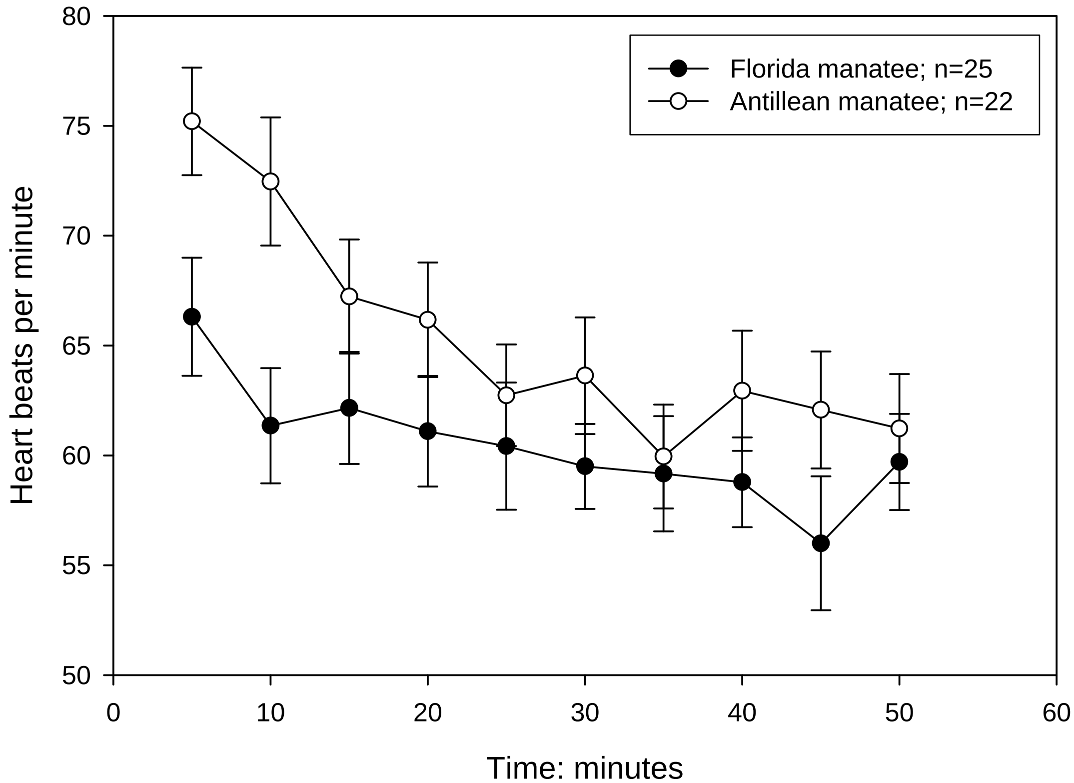

Figure 3-3. Mean heart rates over time, with standard error bars, for healthy juvenile and adult manatees, immediately following capture.

Table 3-4. Descriptive statistics for heart rates of Florida and Antillean manatees at different time intervals during field monitoring

		Florida Manatees					Antillean Manatees			
Time (min)	n	Mean (±SD)	Median	Range	Std.Error	n	Mean (±SD)	Median	Range	Std.Error
5	25	66 (±9.7)	70	48-80	2.7	22	75 (±7.7)	74	64-88	2.4
15	25	62 (±11.1)	64	32-84	2.5	21	67 (±9.4)	68	52-80	2.6
25	25	60 (±12.6)	57	40-80	2.9	22	63 (±10.1)	63	44-80	2.3
40	25	59 (±8.7)	60	44-72	2.0	22	63 (±10.9)	63	44-80	2.7
50	23	60 (±6.9)	62	45-68	2.2	22	61 (±10.5)	60	48-84	2.5

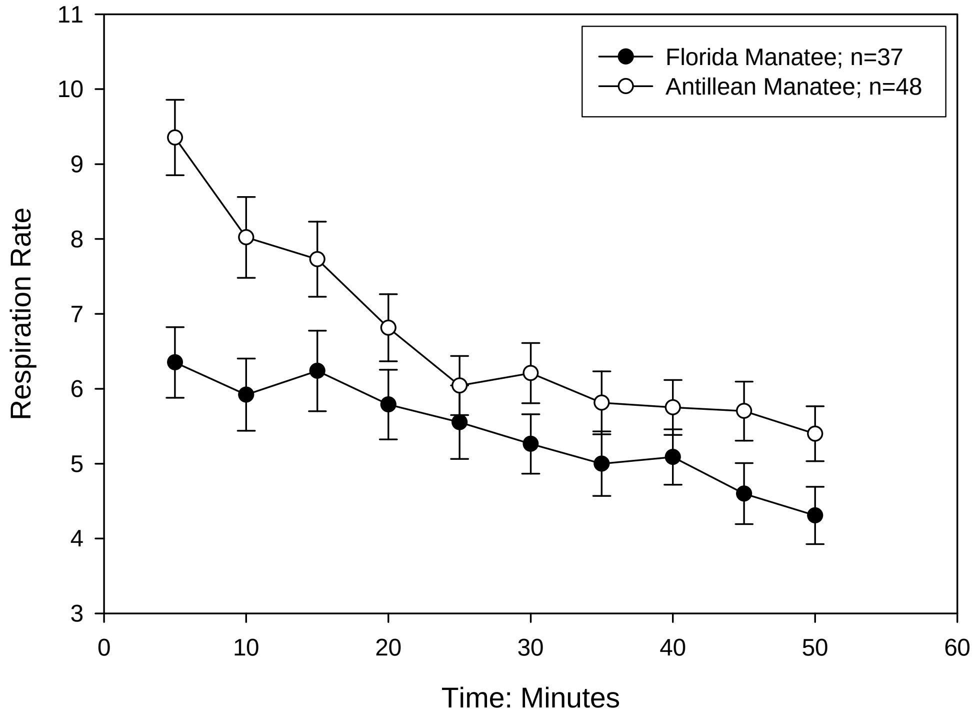

Figure 3-4. Mean respiration rates over time, with standard error bars, for healthy juvenile and adult manatees, immediately following capture.

Table 3-5. Descriptive statistics for respiration rates of Florida and Antillean manatees at different time intervals during field monitoring

Florida Manatees						Antillean Manatees					
Time (min)	n	Mean (±SD)	Median	Range	Std.Error	Time (min)	n	Mean (±SD)	Median	Range	Std.Error
5	37	6 (±2.9)	6	1-13	0.5	5	48	9 (±3.5)	9	3-17	0.5
15	37	6 (±3.3)	6	0-17	0.5	15	48	7 (±3.5)	8	2-15	0.5
25	37	6 (±3.1)	5	0-17	0.5	25	48	6 (±2.7)	6	0-11	0.4
40	37	5 (±2.2)	5	1-10	0.4	40	48	6 (±2.5)	5.5	2-12	0.4
50	36	4 (±2.0)	4	0-8	0.4	50	48	5 (±2.3)	5	2-12	0.4

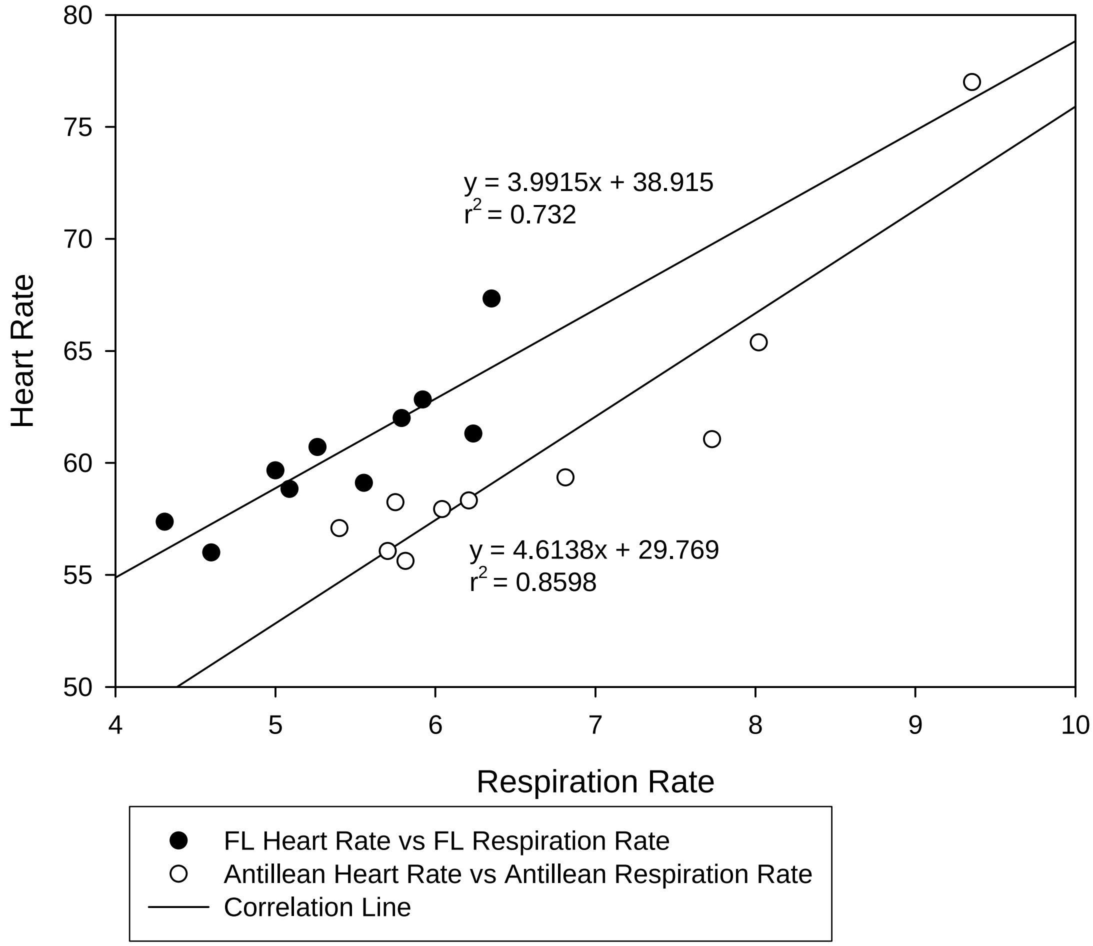

Figure 3-5. Correlation analyses of average heart rate versus average respiration for Florida and Antillean manatees. Pearson product moment correlation coefficient r^2=0.856, *p*=0.00159; and r^2=0.927, *p*=0.000112, respectively.

Table 3-6. Initial heart rate respiration rate and lactate of a female Antillean manatee BZ97F03, captured on 3 separate occasions during this study.

Date captured	Heart rate (beats per minute)	Respiration rate (breaths/ 5 minutes)	Lactate (mmol/L)
21 November 2004	92	16	27.5
16 April 2005	84	8	20
14 November 2005	60	3	9.7

Initial=within the first 10 minutes of being secured upon capture

Table 3-7. Mean times in minutes during capture and handling of manatees

Location	Net Set to Secured		Secured to Release		Total time	
	Mean (± SD)	Range	Mean (± SD)	Range	Mean (± SD)	Range
Florida	43.3 (±62.3)	4-271	57.4 (±19.3)	26-110	101.5 (±61.6)	35-311
Belize	43.0 (±27.4)	1-108	85.0 (±22.8)	42-125	129.7 (±33.3)	61-177
Puerto Rico	4.9 (±0.9)	3-6	55.6 (±6.1)	43-65	60.5 (±6.1)	48-69

CHAPTER 4
MANATEE TEMPERATURE, HEART, AND RESPIRATION MONITORING FIELD GUIDE

The field guide in Appendix F describes monitoring methods of oral temperature, heart rate, and respiration rate in manatees, that can be used during field rescues and research captures. The field guide was developed to serve as a useful point of reference for the newly introduced manatee biologist while monitoring vital signs of manatees in the field. The information collected from monitoring is intended to assist the attending field veterinarian or senior biologist with decisions regarding individual animal care. Familiarization with medical terminology is suggested in order to enhance communication between veterinarians and biologists. Training in monitoring techniques from an experienced veterinarian or manatee biologist is recommended to ensure proper implementation.

CHAPTER 5
CONCLUSION

This thesis presents a normal range of OT, HR, and RR parameters of captured free-ranging Florida (*T. m. latirostris*) and Antillean (*T. m. manatus*) manatees (Chapter 3). In so doing, it provides a successful methodology for monitoring HR and OT using electronic instrumentation (Chapter 2). The practical culmination of this research is a condensed OT, HR, and RR monitoring field guide for the field researcher (Chapter 4).

Hypotheses Addressed

● **Specific Aim 1:** Establish temperature, heart rate, and respiration rate parameters in captured manatees.

● **Hypothesis 1a**: Oral temperature will show a time dependent increase when ambient air temperature is above 26°C.

Yes, but the assumption that an increase in air temperature will cause more of a change in oral temperature is incorrect. We saw significant increases in oral temperature at ambient air values several degrees below 26°C. Lower water temperature is likely more of a contributing initial factor affecting oral temperature in manatees. In the water, blood flow is reduced to the extremities by perivascular constriction, thereby reducing the loss of heat. When placed on land manatees will compensate by returning blood flow to their periphery as a thermoregulatory response. While the medium of air is 25 times less efficient at transferring heat than water, we saw no signs of overheating in captured manatees in a tropical climate. However, keeping animals shaded and wet when examined on land is still recommended as a precaution during hot sunny days.

● **Hypothesis 1b**: A healthy captured manatee's heart rate is higher than 40 bpm.

Yes, captured manatees generally initially presented heart rates well above the reported normal minimum of 40 bpm. A normal sustained HR for field captured manatees will be closer to 60 bpm. A downward trend in HR will generally occur over time, as the manatee recovers from exertion.

● **Hypothesis 1c**: Healthy captured manatees will show a normal respiration rate of 3-4 breaths/5 minute cycle (Bossart 2001).

Yes, over time a healthy captured manatee will show a downward trend of respirations within the normal range. However, initial respiration rates will be significantly higher than the reported normal, and it is not unusual for a rescued manatee to have sustained respirations above the normal range prior to release. The level of exertion from capture and handling on land is a significant factor for this phenomenon. Also, observed respirations on land are likely not of the same quality as those respirations in the water, given the strain of weight on the animal.

● **Specific Aim 2:** Determine possible correlations of oral temperature, heart rate, and respiration rate with blood chemistry.

● **Hypothesis 2a**: Oral temperature will show a time dependent increase in value after capture; heart rate and respiration rate will stabilize over time.

No. Oral temperature stabilized quickly during the monitoring period. However, heart rate and respiration rate did not always stabilize over time. Some animals showed elevated values upon release, which was likely due to recovery time following capture struggle.

● **Hypothesis 2b**: A positive correlation exists between heart rate, potassium, and lactate concentration amongst healthy captured manatees.

No. We did not establish a true correlation for any monitoring parameter with a blood parameter. However, we established a significant positive association between lactate and respiration rate. Elevated respiration rates can be a general indicator of elevated lactate levels.

The Captured Manatee and Future Monitoring Considerations

If we consider the manatee bradycardic response while diving (Scholander & Irving 1941, Gallivan *et al.* 1986), then a classic dive response is a very possible behavior for a distressed diving manatee. However, the common occurrence and degree of a manatee dive response during free-ranging activity is debatable, given their considerably low metabolism and shallow water habitat (Scholander & Irving 1941, Hartman 1979, Butler 1982). The prevention of prolonged apnea by pouring water on the back of the manatee's head to induce breathing, may also have prevented the occurrence of a dive response in this study.

The manatee may also be more behaviorally adapted to tolerate handling more than other animals. Their natural habit of becoming tidally stranded while foraging or being chased in a mating herd (Hartman 1979) has conditioned many populations with a behavioral and physiological response that is unique to the shallow water habitats that they tend to inhabit. Manatee researchers have witnessed tidally stranded individuals behaving in a sometimes playful manner, with normal HR, OT, and RR (Wong personal observations). It is likely that manatees are fully aware of the change in their surroundings when they are pulled completely out of the water and placed on land. The inquisitive manatee generally does not demonstrate an exceptional fight or flight response as hunted ungulates do, lacking the alarm communication that is characteristic of herd animals (Hartman 1979; Bateson & Bradshaw 1997). Many individual manatees, especially those in populous Florida, demonstrate not only a tolerance for people, but will often interact with swimmers (Reep & Bonde 2006). However, even given the manatee's calm disposition, elevated heart and respiration rates were undoubtedly a consequence of the chase and struggle endured during capture.

Differences in OT, HR, and RR between subspecies were possibly a result of differences in environment, exertion, and physical conditioning. The stable mean oral temperature of 35°C shows adequate thermal regulation, possibly due to excess heat loss occurring through the fluke (Rommel & Caplan 2003). The additional progression of HR and RR values toward reported normal values, between the subspecies of manatees, suggests a physiological similarity.

Monitoring of manatee OT, HR, and RR enables the field researcher, with a measurable framework, to further assess the immediate condition of this unique marine mammal. For common terrestrial animals vital signs monitoring has long been standard practice and commercial veterinary equipment has evolved to enhance a practitioner's monitoring ability (Bright 1997). Because manatee health assessment is not common practice, an effort should be made by the researchers to validate the use of instrumentation for this species. With some ingenuity many existing medical items developed for general human and veterinary medicine can be adapted for manatees.

While we were unable to determine illness in TNP029 while in the field, abnormal OT, HR, and RR values can be apparent in critically injured manatees. For example, a critically injured boat strike adult manatee RNE0602, that died within 18 hrs from time of capture, had THR values during transport to Sea World which remained high and steady. OT was 36.6°C, HR via ECG was generally at 73 bpm, and RR was between 5 breaths/5 minutes to 7 breaths/5 minutes (Florida Fish and Wildlife Conservation Commission unpublished data). For two cold stress manatees, OT was initially 26.6°C and increased only to 27.8°C, and initially 28.3°C and increased only to 30.6°C (Florida Fish and Wildlife Commission unpublished data). Heart rates of these two cold stressed manatees ranged between 40 bpm to 54 bpm, with varying qualities of breaths, and inconsistent RR ranging between 2 breaths/5minutes to 5 breaths/5 minutes.

Additional improvements in current technologies applied in this study are recommended. A wireless thermometer transmitting to a hand held temperature display or lap top computer, would improve versatility. Assessing temperature regulation on other areas of the manatee, such as examining the body with a thermograph, would increase our understanding of thermoregulation. Heart rate monitoring could be improved with the use of a waterproof wireless ECG unit, transmitting to a lap top computer. This would allow for digital data storage and increase field versatility. This study did not adequately address quality of manatee respirations. A methodology to adequately measure tidal volume and use of a capnograph, in conjunction with OT, HR, and RR monitoring, would likely yield useful results for the clinician and researcher. A portable blood analyzer should also be considered as an additional tool when assessing the condition of an animal, as we have seen that OT, HR, and RR monitoring is limited in its ability to serve as a predictor of changes in blood chemistry.

APPENDIX A
CAPTURE LOCATIONS

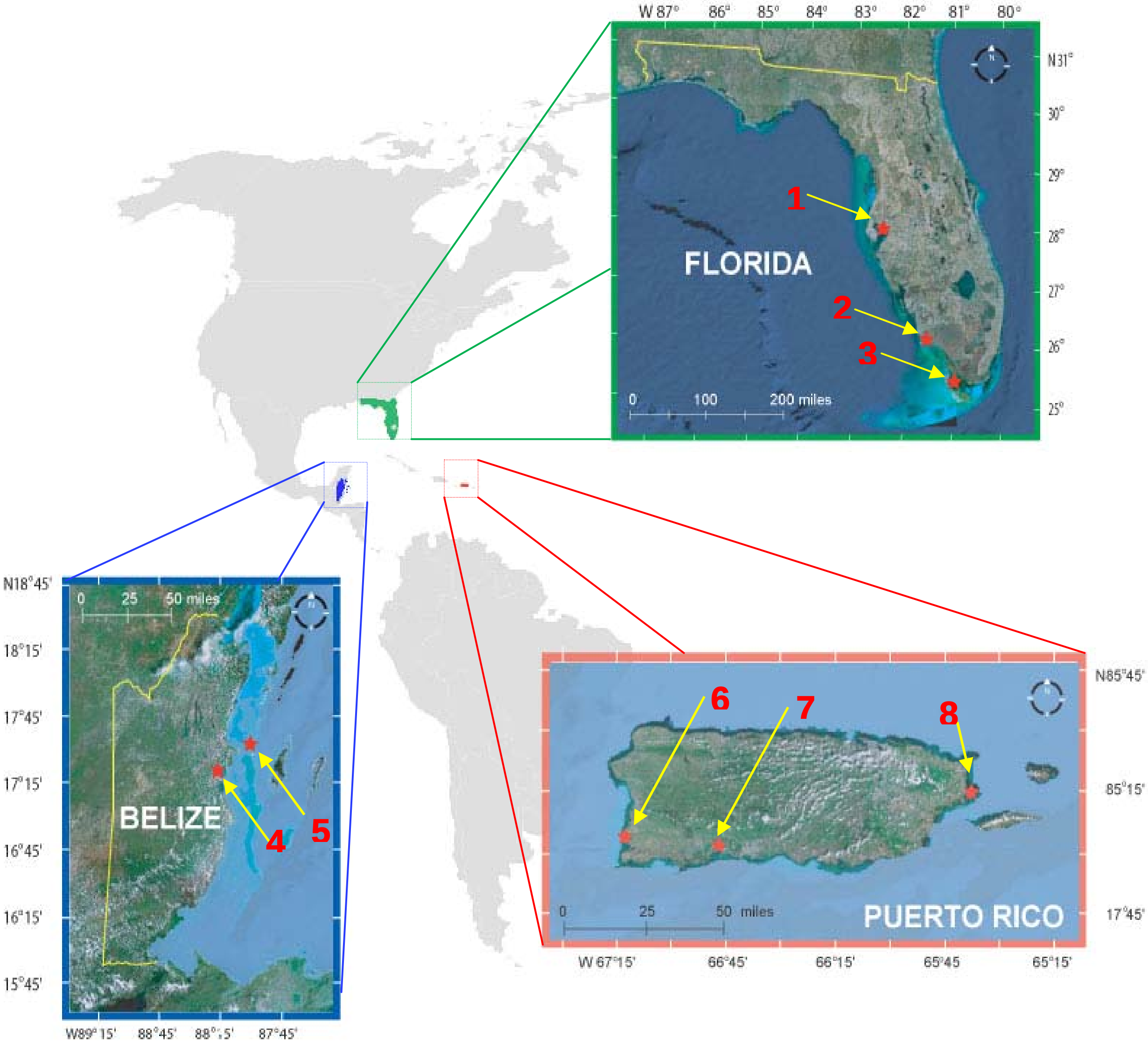

Figure A-1. Map of manatee capture locations

Table A-1. Associated location descriptions and coordinates for Figure A-1

Location description		Coordinates	
	Florida	Latitude	Longitude
1.	Apollo Beach, Tampa: near TECO Big Bend power plant	27°47’34.01”N	82°25’15.87”W
2.	Port of the Islands, Naples: residential and marina basin, Faka Union Canal	25°57’19.74”N	81°30’34.03”W
3.	Everglades National Park: areas of Whitewater and Coot Bay	25°18’9.77”N	80°59’9.37”W
	Belize	Latitude	Longitude
4.	Southern Lagoon: inshore waterway	17°15’21.21”N	88°20’39.78”W
5.	Drowned Cays: off shore waters	17°28’05.25”N	88°04’23.40”W
	Puerto Rico	Latitude	Longitude
6.	Boqueron: Rincón Canal	18°01’12.32”N	67°11’56.15”W
7.	Guayanilla: Bahia de Guayanilla	17°59’12.49”N	66°46’15.64”W
8.	Ceiba: near shore waters off former naval station Roosevelt Roads	18°12’46.40”N	65°37’12.71”W

APPENDIX B
BASIC MANATEE CAPTURE METHOD PHOTOS

The basic method of capturing a manatee using a seine net (Figures B-1 through B-3) was used in Puerto Rico, and was modified slightly to suit varying conditions in Florida and Belize.

Figure B-1. Large seine net deployed from a capture boat (Photo Credit: Robert K. Bonde 2004)

Figure B-2. Large seine net enclosed on a manatee (Photo Credit: Robert K. Bonde 2004)

Figure B-3. Manatee lifted onto a capture boat (Photo Credit: Robert K. Bonde 2004)

Figure B-4. Land set near Florida's Teco Power Plant. In this manner, multiple manatees would be captured (Photo Credit: Chip J. Deutsch 2004)

Figure B-5. A manatee capture in Belize. The manatee is contained within a small net and is being guided toward the stern of the boat by animal handlers. A surrounding large net is identified by the many buoys located in the background and the several buoys in the immediate foreground. (Photo Credit: Yvonne Dartsch, 2005)

APPENDIX C
MANATEE LENGTH VERSUS WEIGHT

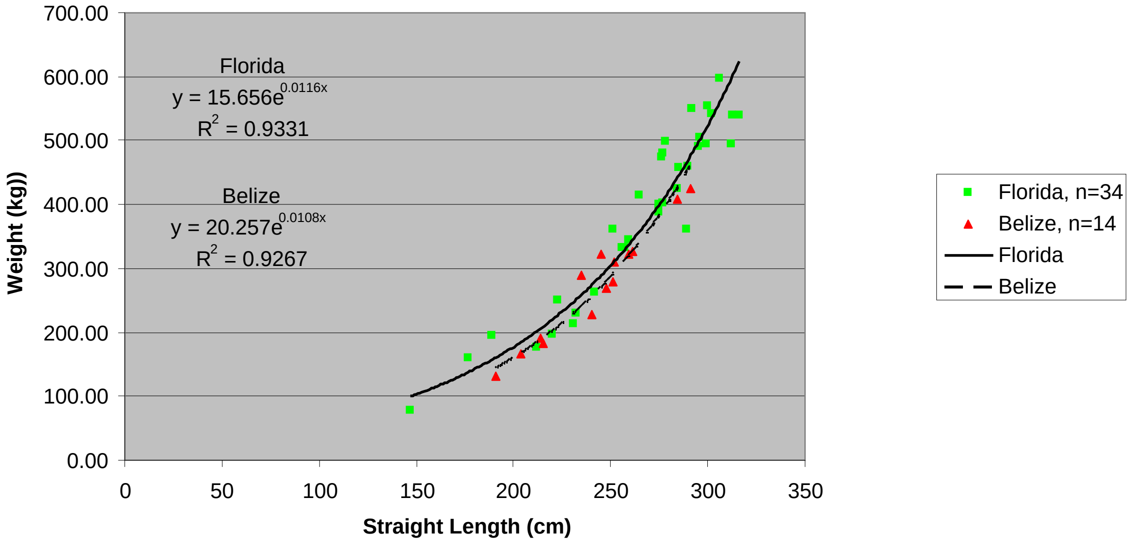

Figure C-1. Length weight relationship of manatees captured in this study, from Florida and Belize. Not all animals were weighed, as weighing equipment was not available at every capture. Manatees from Puerto Rico are not included in the graph as the n=4 of available weights, is too low for analysis.

APPENDIX D
BASIC ECG RESULTS

Table D-1. Results of ECG printed QRS tracing analyses for 25 of 47 heart rate monitored manatees

Animal Id	Date	Sex	Straight length (cm)	Instantaneous heart rate	Rhythm
BZ04F67	11/17/2004	F	219	79	Regular
TTB115	12/14/2004	F	302	50	Regular
TTB117	12/14/2004	F	277	51	Regular
BZ97F03	4/16/2005	F	250	73	Regular
BZ05F80	4/17/2005	F	234	51.4	Regular
BZ05M79	4/17/2005	M	225	65.2	Regular
BZ04M66	4/21/2005	M	294	49.1	Regular
TPR28	4/29/2005	M	273	49.9	Regular
TEP03	6/30/2005	F	246	69.8	Regular
TEP01	6/30/2005	M	275	70.4	Regular
BZ05M85	11/12/2005	M	245	67	Regular
BZ05M85	11/12/2005	M	245	63.8	Regular
BZ98M05	11/14/2005	M	235	54.5	Regular
BZ05M87	11/15/2005	M	291	57.7	Regular
BZ03F28	11/15/2005	F	214	77.1	Regular
BZ05M93	11/18/2005	M	261	44.5	Regular
TTB120	12/5/2005	M	259	57.5	Regular
TTB119	12/5/2005	F	251	41	Regular
TTB122	12/6/2005	F	276	68.5	Regular
TTB123	12/6/2005	F	292	52.7	Regular
TTB125	1/4/2006	M	277	64.7	Regular
TTB127	1/4/2006	M	313	58.8	Regular
TTB128	1/4/2006	M	296	71.4	Regular
TTB128	1/4/2006	M	296	63.5	Regular
TTB132	1/5/2006	F	302	53.6	Regular
TTB101	1/5/2006	M	290	60.7	Regular
CTB051	1/5/2006	M	231	42.7	Irregular

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APPENDIX E
PLASMA CLINICAL BIOCHEMISTRY AND PROGESTERONE 4 VALUES

Table E-1. Individual plasma clinical biochemistry of 38 Florida (*Trichechus manatus latirostrus*) and 48 Antillean (*T. m. manatus*) manatees concurrently monitored for oral temperature, heart rate, and respiration rate

Id	Date	Location	Length (cm)	Class	Sex	CK (U/L)	Lactate (mmol/L)	SAA (ug/mL)	Na (mEq/L)	K (mEq/L)	Na/K
CEP0501	06/28/05	Florida	220	2	F	82	12	30	150	5.3	28.30189
CNP0401	01/17/04	Florida	245	2	M	799	2.3	50	148	5	29.6
CTB046	12/14/04	Florida	212	2	F	332	13	31	157	5.6	28.03571
CTB048	01/04/06	Florida	232	2	M	141	17.5	<10	150	4.5	33.33333
CTB049	01/05/06	Florida	223	2	M	192	15.4	120	152	5.2	29.23077
CTB051	01/05/06	Florida	231	2	M	124	9	83	153	5.7	26.84211
TEP01	06/30/05	Florida	275	3	M	76	18.9	<10	152	5.9	25.76271
TEP02	06/30/05	Florida	284	3	M	81	18.9	<10	151	4.7	32.12766
TEP03	06/30/05	Florida	246	2	F	51	11.7	18	158	4.8	32.91667
TEP04	07/01/05	Florida	311	3	F	130	31	<10	150	5.3	28.30189
TEP05	12/01/05	Florida	275	3	M	296	17	73	152	5.9	25.76271
TNP025	01/15/04	Florida	242	2	M	323	11.9	800	147	4.7	31.2766
TNP026	01/15/04	Florida	312	3	M	211	<0.5	43	147	5.2	28.26923
TNP027	01/15/04	Florida	267	3	M	413	20	360	149	4.3	34.65116
TNP028	01/16/04	Florida	234	2	M	572	17	350	151	4.3	35.11628
TNP029	04/18/04	Florida	289	3	F	278	13.2	>1200	145	4.7	30.85106
TNP032	04/19/04	Florida	306	3	M	131	16.2	117	146	5.3	27.54717
TTB006	01/05/06	Florida	316	3	F	161	7.8	<10	152	5.6	27.14286
TTB069	12/16/04	Florida	299	3	M	107	23.2	<10	143	4.7	30.42553
TTB069	03/24/05	Florida	305	3	M	77	11.5	12	146	4.7	31.06383
TTB081	12/14/04	Florida	300	3	F	131	5.5	12	144	4.7	30.6383
TTB101	01/05/06	Florida	290	3	M	138	19.4	<10	153	4.7	32.55319
TTB109	12/14/04	Florida	295	3	F	171	6.8	<10	144	4.8	30
TTB115	12/14/04	Florida	302	3	F	106	14.8	60	145	5.7	25.4386
TTB117	02/14/04	Florida	277	3	F	194	12.2	<10	143	5.2	27.5
TTB118	12/16/04	Florida	257	2	M	263	14	<10	143	3.9	36.66667

Table E-1. Continued

Id	Date	Location	Length (cm)	Class	Sex	CK (U/L)	Lactate (mmol/L)	SAA (ug/mL)	Na (mEq/L)	K (mEq/L)	Na/K
TTB119	12/05/05	Florida	251	2	F	183	8.1	12	152	6.2	24.51613
TTB120	12/05/05	Florida	259	2	M	342	11.1	11	152	5.2	29.23077
TTB120	12/05/05	Florida	259	2	M	342	11.1	11	152	5.2	29.23077
TTB122	12/06/05	Florida	276	3	F	193	5.5	11	153	5.8	26.37931
TTB123	12/06/05	Florida	292	3	F	101	5	<10	156	5.1	30.58824
TTB124	12/06/05	Florida	285	3	M	132	11	11	155	5.1	30.39216
TTB125	01/04/06	Florida	277	3	M	61	9.1	<10	146	4.9	29.79592
TTB126	01/04/06	Florida	256	2	M	102	26.5	99	154	4.8	32.08333
TTB127	01/04/05	Florida	313	3	M	116	23.8	<10	155	6.4	24.21875
TTB128	01/04/06	Florida	296	3	M	57	20.5	<10	153	4.8	31.875
TTB130	01/04/06	Florida	278	3	F	84	24.5	15	153	4.7	32.55319
TTB131	01/05/06	Florida	265	2	F	266	14.8	19	149	4.6	32.3913
TTB132	01/05/05	Florida	302	3	F	98	11.2	<10	154	6.3	24.44444
BZ00M13	04/16/05	Belize	297	3	M	71	17.8	56	158	4.8	32.91667
BZ01M15	11/16/05	Belize	240	2	M	113	9	<10	152	4.9	31.02041
BZ03F28	11/15/05	Belize	210	2	F	120	14.5	<10	155	5.6	27.67857
BZ03F28	04/19/05	Belize	214	2	F	114	17	59	163	5	32.6
BZ03F29	04/19/05	Belize	282	3	F	118	6.6	<10	154	6	25.66667
BZ03F31	11/20/04	Belize	278	3	F	38	8	<10	136	4.8	28.33333
BZ03F31	04/18/05	Belize	280	3	F	84	20.8	15	155	5.5	28.18182
BZ03F47	11/19/04	Belize	208	2	F	193	18.5	61	117	4.2	27.85714
BZ04F67	11/17/04	Belize	219	2	F	130	32	45	161	5.1	31.56863
BZ04F68	11/19/04	Belize	252	2	F	158	29	28	130	4.7	27.65957
BZ04F69	11/20/04	Belize	285	3	F	101	21.5	14	133	5.1	26.07843
BZ04F72	11/21/04	Belize	263	2	F	147	37	<10	158	6.5	24.30769
BZ04F73	11/22/04	Belize	286	3	F	81	24	<10	127	5.3	23.96226
BZ04F75	11/22/04	Belize	260	2	F	46	31	<10	138	5.7	24.21053
BZ04M66	11/17/04	Belize	277	3	M	93	35	<10	165	7	23.57143
BZ04M66	04/21/05	Belize	294	3	M	137	17	<10	149	5.2	28.65385

Table E-1. Continued

Id	Date	Location	Length (cm)	Class	Sex	CK (U/L)	Lactate (mmol/L)	SAA (ug/mL)	Na (mEq/L)	K (mEq/L)	Na/K
BZ04M71	11/21/04	Belize	250	2	M	100	27	<10	159	5.4	29.44444
BZ04M74	11/22/04	Belize	280	3	M	74	28	<10	147	5.8	25.34483
BZ04M74	04/16/05	Belize	283	3	M	115	10	<10	154	4.8	32.08333
BZ04M77	11/22/04	Belize	256	2	M	167	29.5	32	145	4.8	30.20833
BZ05F80	04/17/05	Belize	234	2	F	149	9	23	149	4.9	30.40816
BZ05F81	04/17/05	Belize	212	2	F	182	21	23	151	5.4	27.96296
BZ05F86	11/13/05	Belize	248	2	F	118	13	24	157	5	31.4
BZ05F89	11/16/05	Belize	215	2	F	233	24.5	76	157	5	31.4
BZ05F91	11/17/05	Belize	251	2	F	126	22	34	156	5.3	29.43396
BZ05M79	04/17/05	Belize	225	2	M	85	18.2	10	154	5.6	27.5
BZ05M85	11/12/05	Belize	245	2	M	156	19.5	<10	159	5.9	26.94915
BZ05M87	11/15/05	Belize	291	3	M	168	22.5	79	131	4.1	31.95122
BZ05M88	11/16/05	Belize	279	3	M	97	23.5	<10	153	5.6	27.32143
BZ05M90	11/16/05	Belize	284	3	M	89	13.5	<10	153	4.7	32.55319
BZ05M93	11/18/05	Belize	261	2	M	89	28.5	<10	156	5	31.2
BZ97F03	11/21/04	Belize	246	2	F	69	27.5	<10	149	5.4	27.59259
BZ97F03	04/16/05	Belize	250	2	F	183	20	<10	158	5.3	29.81132
BZ97F03	11/14/05	Belize	252	2	F	93	9.7	<10	151	5.5	27.45455
BZ98M05	11/14/05	Belize	235	2	M	177	23	13	155	4.6	33.69565
CPR0501	04/27/05	PuertoRico	222	2	M	223	15.2	15	151	5.5	27.45455
TPR07	05/01/05	PuertoRico	297	2	M	61	15.2	<10	151	5.7	26.49123
TPR20	06/07/04	PuertoRico	256	3	F	3522	N/A	190	153	4.7	32.55319
TPR21	06/07/04	PuertoRico	261	3	F	62	N/A	<10	151	5.2	29.03846
TPR22	06/08/04	PuertoRico	273	2	M	242	N/A	88	146	5.2	28.07692
TPR23	06/10/04	PuertoRico	225	2	F	166	N/A	<10	149	5.6	26.60714
TPR27	04/28/05	PuertoRico	273	2	F	123	28	14	153	5.5	27.81818
TPR28	04/29/05	PuertoRico	250	3	M	82	20.2	<10	150	4.9	30.61224
TPR29	04/29/05	PuertoRico	264	2	F	95	21	19	158	6.1	25.90164
TPR30	04/29/05	PuertoRico	270	2	M	126	24	<10	158	5.9	26.77966

Table E-1. Continued

Id	Date	Location	Length (cm)	Class	Sex	CK (U/L)	Lactate (mmol/L)	SAA (ug/mL)	Na (mEq/L)	K (mEq/L)	Na/K
TPR31	04/30/05	PuertoRico	268	3	F	277	29.5	16	204	5.7	35.78947
TPR32	05/01/05	PuertoRico	265	2	F	88	18	16	158	5.8	27.24138
TPR33	05/02/05	PuertoRico	288	3	M	93	5	<10	163	5.6	29.10714

Table E-2: Plasma clinical biochemistry descriptive statistics from 38 Florida (*Trichechus manatus latirostrus*) and 48 Antillean (*T. m. manatus*) field- captured manatees

Florida	Mean(±SD)	Median	Range
CK (U/L)	193(±151)	135	51-799
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 (ug/mL)	97(±235)	12	10-1200
Lactate (mmol/L)	14(±7)	13	0.5-31
Antillean	**Mean(±SD)**	**Median**	**Range**
CK (U/L)	195(±493)	117	38-3522
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 (ug/mL)	25(±32)	10	10-190
Lactate (mmol/L)	21(±8)	21	5-37

Table E-3. Abnormal creatine kinase (CK) activity identified from 38 Florida (*Trichechus manatus latirostrus*) and 48 Antillean (*T. m. manatus*) field- captured manatees

Id	Date	CK(U/L)
TNP028	1/16/04	572
CNP0401	1/17/04	799
TPR20	06/07/04	3522

Table E-4. Abnormal lactate values identified from 38 Florida (*Trichechus manatus latirostrus*) and 48 Antillean (*T. m. manatus*) field- captured manatees

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

Table E-5. Abnormal serum amyloid A (SAA) values identified from 38 Florida (*Trichechus manatus latirostrus*) and 48 Antillean (*T. m. manatus*) field- captured manatees

Id	Date	SAA (ug/mL)
TEP05	12/01/05	73
BZ05F89	11/16/05	76
BZ05M87	11/15/05	79
CTB051	01/05/06	83
TPR22	06/08/04	88
TTB126	01/04/06	99
TNP032	04/19/04	117
CTB049	01/05/06	120
TPR20	06/07/04	190
TNP028	01/16/04	350
TNP027	01/15/04	360
TNP025	01/15/04	800
TNP029	04/18/04	>1200

Table E-6. Abnormal potassium (K^+) values identified from 38 Florida (*Trichechus manatus latirostrus*) and 48 Antillean (*T. m. manatus*) field- captured manatees

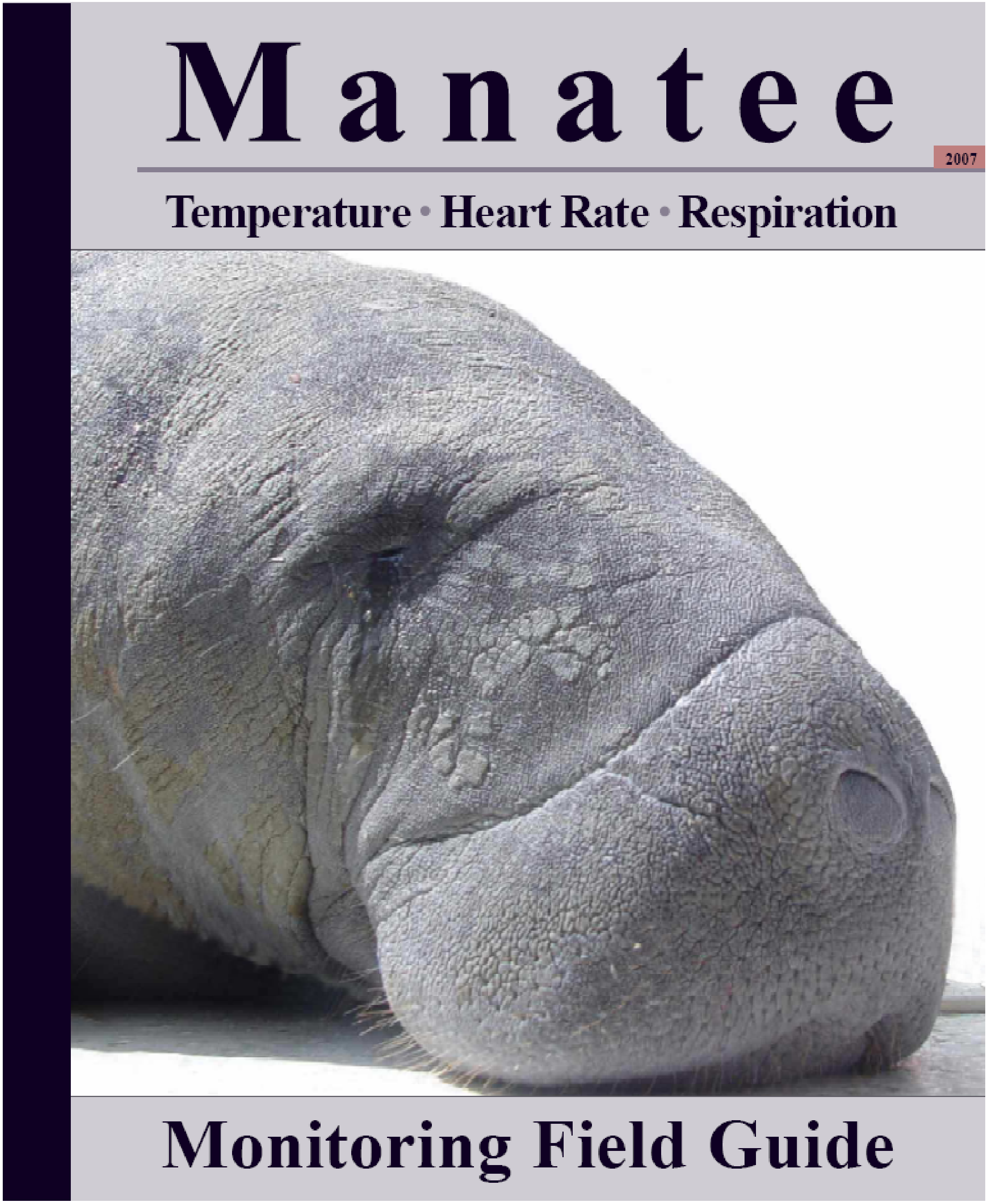
Id	Date	K^+ (mEq/L)
TPR29	04/29/05	6.1
TTB119	12/05/05	6.2
TTB132	01/05/05	6.3
TTB127	01/04/05	6.4
BZ04F72	11/21/04	6.5
BZ04M66	11/17/04	7

Table E-7: Progesterone 4 values from blood serum of heart rate monitored female manatees, determined by chemiluminescent immunoassay (Tripp *et al.* 2006)

Id	Date	Loc	Class	Sex	P4 ng/mL	Pregnancy Status
BZ03F28	11/15/05	Belize	2	F	0.17	non-preg
BZ03F28	04/19/05	Belize	2	F	0.08	non-preg
BZ03F29	04/19/05	Belize	3	F	0.11	non-preg
BZ04F67	11/17/04	Belize	2	F	0.07	non-preg
BZ04F72	11/21/04	Belize	2	F	0.12	non-preg
BZ05F80	04/17/05	Belize	2	F	0.07	non-preg
BZ05F81	04/17/05	Belize	2	F	0.22	non-preg
BZ05F86	11/13/05	Belize	2	F	0.03	non-preg
BZ05F89	11/16/05	Belize	2	F	0.13	non-preg
BZ05F91	11/17/05	Belize	2	F	0.03	non-preg
BZ97F03	11/21/04	Belize	2	F	0.07	non-preg
TEP03	06/30/05	Florida	2	F	0.035*	non-preg
TTB109	12/14/04	Florida	3	F	0.03	non-preg
TTB117	02/14/04	Florida	3	F	0.41	non-preg
TTB119	12/05/05	Florida	2	F	0.07	non-preg
TTB122	12/06/05	Florida	3	F	0.05	non-preg
TTB123	12/06/05	Florida	3	F	0.23	non-preg
TTB130	01/04/06	Florida	3	F	1.31	probable
TTB131	01/05/06	Florida	2	F	0.03	non-preg
TTB132	01/05/05	Florida	3	F	0.72	non-preg

*Value based on plasma in lithium heparin; CTB046 was not tested due to inadequate serum amount

APPENDIX F
MANATEE MONITORING FIELD GUIDE



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Introduction

This guide describes monitoring methods of oral temperature, heart rate, and respiration rate (THR) in manatees, during field rescues and research captures. The purpose of monitoring these vital signs is to better understand an individual manatee's immediate physiological condition. By monitoring THR early, often, and for the duration of handling, the ability to detect changes, such as potential deterioration in the manatee's health status may improve. Field THR reference values presented in this guide serve to assist in understanding the THR response in healthy juvenile and adult manatees following field capture. Equipment presented in this guide does not exclude the possibility of other models and brand instruments providing similar or better results.

Prolonged deviations, or lack of improvement over time, toward the reported normal THR parameter intervals should be immediately reported to an attending veterinarian.

THR Monitoring Methods

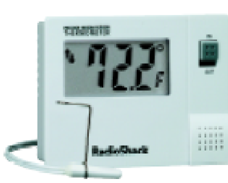
Oral Temperature

Monitoring of oral temperature should begin immediately upon physically securing the manatee. Temperature readings should be taken every 5 minutes, simultaneously with heart rate, and in conjunction with respiration monitoring.

Manatee with temperature probe

A normal juvenile to adult sized manatee oral temperature has been reported as 35.5 to 36°C (Murphy 2003). The manatee core body temperature is 35.6 to 36.4°C (Galivan et al. 1983; Irvine 1983). A steady oral temperature reading between 33.5 and 36.5°C in the field is generally normal.

Oral temperatures outside the given range should be relayed to the attending veterinarian. For example, diseased cold stress manatees will naturally show oral temperatures several degrees below the normal range. However, environmental temperatures should be carefully considered when assessing oral temperature. Healthy captured manatees monitored on land, during the cold winter seasons in Florida can show oral temperatures below the stated normal range (See "Field THR Parameters" page).



Examples of Thermometers

The thermometers pictured have been field tested. The thermometers are not water proof, and should be placed in a protective housing such as a clear plastic bag or small dry bag when using them near water.

Thermometers should be validated as some models may give clinically significant incorrect temperature readings.

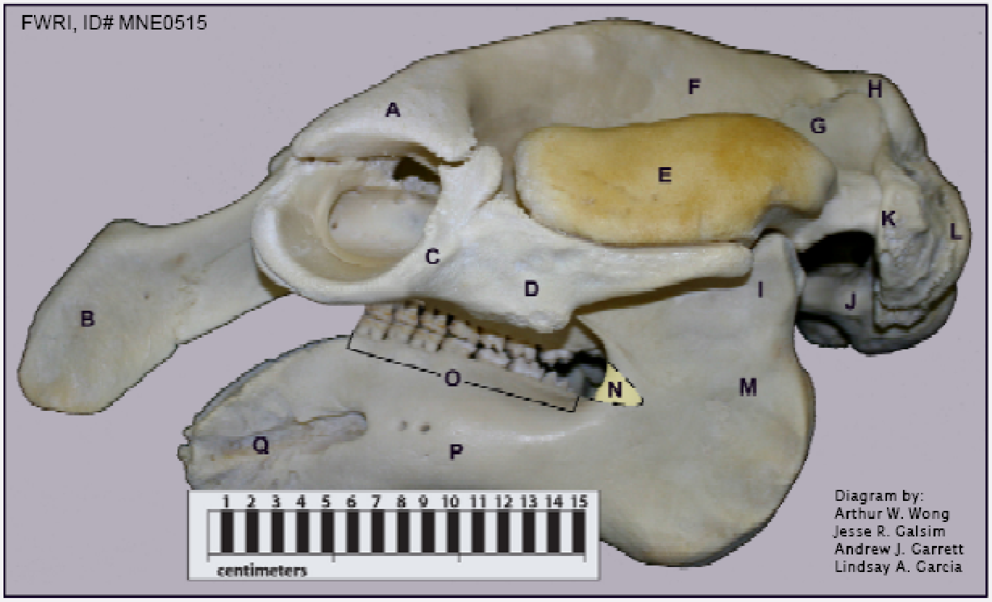
It is recommended to use thermometers which display "Out" and "In" temperatures simultaneously. When used on a manatee, these displays function as oral and ambient temperature respectively. Prior to use in the field, check or calibrate digital readings with a mercury or alcohol thermometer in a water bath, to ensure accuracy.

Placement of Temperature Probe

Hands need not be sterile, but should be free of debris and chemicals prior to insertion of the oral temperature probe. Manatees are equipped only with molariform teeth (Reep and Bonde 2006). They do not bite out of fear or aggression. Damage to probes from biting rarely occurs. However, use caution and avoid inserting fingers between occluding molars, as manatees are very capable of strong, crushing bites.

Labeled skull of an adult manatee (Rommel *et al.* 2002)

A. Frontal
B. Premaxilla
C. Zygomatic Process of the Maxilla
D. Jugal
E. Zygomatic Process of the Squamosal
F. Parietal
G. Squamosal
H. Supra Occipital
I. Temporal Mandibular Joint
J. Basi Occipital
K. Occipital
L. Occipital Condyle
M. Ramus
N. Inferior Dental Mandibulor Cavity
O. Molars
P. Mandible
Q. Mental Foramen



On either the right or left side, push the temperature probe along the outer jaw line toward the latteral cheek area, not medial. Optimal placement of the probe is in the inferior dental mandibular cavity, located buccally past the molars (See "N" in labeled skull photo). Placement of the probe within the given areas will provide more consistent temperature readings, by minimizing the occasional chewing and displacement of the probe by some animals.

THR Monitoring Methods

Heart Rate

Monitoring of heart rate should begin immediately upon physically securing the manatee. Ideally, heart rate readings should be taken every 5 minutes, simultaneously with temperature, and in conjunction with respiration monitoring.

Stethoscope

Auscultation via stethoscope may be possible on even the largest of manatees. A majority of animals will calmly tolerate the procedure. However, caution should be used as a sudden movement by an animal can potentially injure the examiner. Approach cranial or caudal of either pectoral flipper, place the stethoscope by hand in line with the axilla (or armpit) and place the bell of the stethoscope close to ventral midline. Some pushing of the hand with stethoscope may be required to get under the manatee. To better auscult, the bell of the stethoscope may be pressed firmly against the thoracic region. Once a suitable location is found, the bell can be left in place to minimize disturbance to the animal for pending follow up auscultations. To determine heart rate by number of beats per minute, count the number of heart beats heard for a 15 second period then multiply by four. Each sounding series from the closing of valves associated with atrial and ventricular contractions of the heart is counted as one beat.

Demonstration of Stethoscope Placement

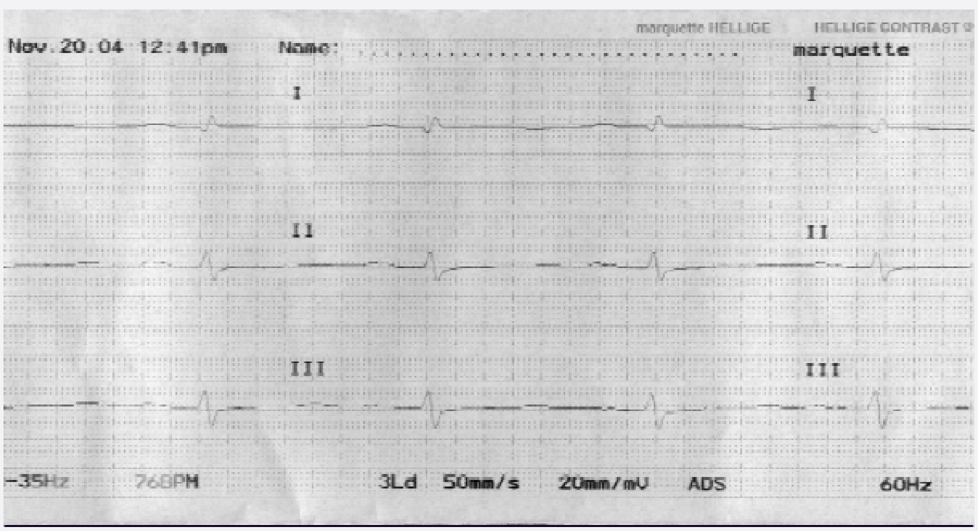
A calm manatee will typically exhibit a heart rate between 40-60 bpm (Scholander and Irving 1941, Murphy 2003). A heart rate between 40 to 70 bpm in the field is generally normal. Deviations outside of this range should be relayed to the attending veterinarian. While resting on land, a captured manatee's heart rate can initially be 80 bpm then progressively return to the stated normal range following 20-25 min. after being physically secured. However, this is not always the case with every individual. (See "Field THR Parameters").

Electrocardiogram (ECG)

Electrocardiograms should be done only under direct supervision of a trained technician and attending veterinarian.The ECG is an instrument that is useful when monitoring a manatee's heart rate for a long duration of time or when background noise inhibits auscultation via stethoscope. The ECG also provides tracings that are valuable for interpreting heart function. Bedside units used in human medicine can be used but careful validation must be exercised. Provided there is careful handling the machine will work well in the field other smaller, more modern ECG units should be considered for long term health monitoring. Displayed heart rate readings should be relatively steady, and accurate readings are verified by an associated heart symbol simultaneously displayed.

Portable human bedside unit

Tracing of BZ03F48. A 195cm long, female manatee in Belize



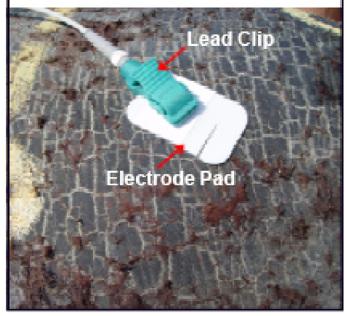
ECG tracings can be analyzed by a veterinarian as described by Siegal-Willott *et al*. (2006).

THR Monitoring Methods: Heart Rate

Lead Preparation and Placements

Sticky electrode pads are non-invasive, and with proper site preparation are effective in attaching to a manatee's skin in the field (Lead-Lok, Inc.). Lead placement areas with debry may need to be wiped with an alcohol pad, and then wiped dry with a clean paper towel. A diverse community of algae and invertebrates can be found attached to manatee skin (Bledsoe *et al.* 2006). These organisms can be removed by gently scraping with your finger nail or plucking by hand. The areas for lead placement should be dried extensively with an absorbent sponge, heavy duty paper towels, or beach towel, to ensure proper adherence of the sticky electrode pads. Ultra sound gel may be used at the connection point of the lead clip and the electrode pad to help improve electrical conductivity. A small amount of tissue glue can safely be applied directly to the area, to help ensure firm attachment of the electrode pad to the manatee's skin. Keep the lead connections free from sand and water, as this will seriously inhibit the ability to obtain accurate readings.

Cleaned lead electrode site



Examples of ECG lead placement

Lead placement need not be exact, but following the described location descriptions will provide optimal readings and tracings. Two forelimb leads are placed dorsally on the right and left side, approximately 2-7 cm cranial to the pectoral flipper, anterior to the scapula. Two hind-limb leads are placed laterally approximately 10 cm caudal to the pectoral flippers at the level of the manatee's shoulder (Siegal-Willott *et al.* 2006).

The four leads form a rectangle around the heart (Note the permissible variation in lead placement between the two photos).
Left Photo: Forelimb leads are cranial of pit tag sites and shoulder regions.
Right Photo: Forelimb leads are located directly on the shoulder regions.

Respiration Rate

The times of each respiration should be noted with a watch, in consecutive 5 minute intervals. During handling on land, respirations should be continuously monitored.

Monitoring respiration rate is a versatile method of assessing a manatee that is swimming in the water or being examined on land. The normal respiration rate of manatees is reported to be 2 to 4 breaths per 5 min interval (Scholander and Irving 1941, Murphy 2003, Bossart 2001). In its aquatic environment manatees have displayed extended submergence periods between 4 and 12 minutes, with larger animals capable of holding their breaths longer than smaller ones (Parker 1922, Hartman 1979). On land, respirations can range between 3 and 15 breaths per 5 minute interval (Walsh and Bossart 1999). Under field capture conditions, manatees can have initial respiration rates as high as 17 breaths per minute. While resting on land, a captured manatee's respiration rate can progressively return to the stated normal range, but this is not always the case with every individual. (See "Field THR Parameters").

Manatee with opened nares

Hyperventilation, respirations >5 breaths per 5 min interval, occurring for a duration of three consecutive 5 minute intervals should be considered abnormal, and an attending veterinarian should be notified. A period of apnea progressing beyond 5 minutes can occur in a deeply resting individual. However, in animals assessed on land, this behavior should raise some concern, and an attending veterinarian should be informed of its occurrence. In such a case, a manatee can be prompted to breathe by pouring water over the top of the head. If necessary place the animal back into the water, support in a stretcher, and monitor closely for a breathing response.

When monitoring respiration rate of manatees on land, opening of the nares alone does not constitute a breath. Evidence of respiration should be seen, heard, or felt. Visible respiratory exchange can be determined by a rising and lowering of the thorax during the opening of the nares. The sound of strong respiratory exchanges can be heard. A hand can be placed in front of a manatee's nares to feel for a breath. Respiratory abnormalities such as air leaking out of closed nares or any discharge from the nostrils should be noted. The following terms can be used to further describe quality of breaths.

- Shallow breath: A relatively short respiratory exchange time is observed, heard, or felt. There is little rising or lowering of the manatee's thoracic region.
- Deep breath: A relatively long respiratory exchange time is observed, heard, or felt. The rising and lowering of the manatee's thoracic region is quite obvious and great.
- Stuttering breath: Not a smooth inhalation. Obvious, short, rapid cessations of inhalation occur during inhalation. No exhalation during the cessations of inhalation.

Field THR Parameters:

For Florida and Antillean Manatees
Following Capture and During Handling in the Field

Air and water temperatures associated with manatees sampled for oral temperature, heart rate, and respiration rate.

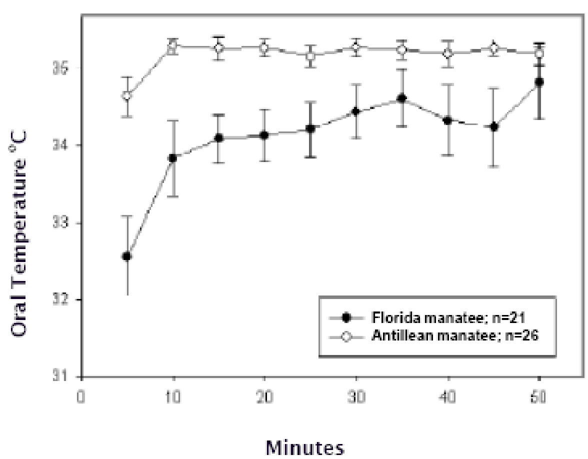
	Belize and Puerto Rico			Florida		
	Mean (±SD)	Median	Range	Mean (±SD)	Median	Range
Air °C	30.7 (±3.9)	30.4	24.2-40.8	23.8 (±6.0)	22.0	14.7-31.9
Water °C	28.9 (±1.5)	28.9	25.1-31.5	25.9 (±4.2)	25.6	21.5-30.9

Descriptive Statistics of Oral Temperatures

Florida Manatees				
Time (min)	Mean (±SD)	Median	Range	Std. Error
5	32.6 (±1.8)	32.8	29.5-35.1	0.5
15	34.1 (±1.4)	34.5	29.8-35.7	0.3
25	34.2 (±1.4)	34.7	30.1-35.7	0.4
40	34.323 (±1.6)	34.9	30.7-35.8	0.5
50	34.8 (±1.5)	35.1	30.5-35.9	0.5

Antillean Manatees				
Time (min)	Mean (±SD)	Median	Range	Std. Error
5	34.6 (±0.9)	34.9	32.6-36	0.3
15	35.3 (±0.6)	35.3	34.1-36.2	0.1
25	35.2 (±0.7)	35.3	33.6-36.2	0.1
40	35.2 (±1.6)	35.3	33.3-36.2	0.2
50	35.2 (±0.6)	35.25	34.0-36.1	0.1

Mean Oral Temperatures with Standard Error Bars



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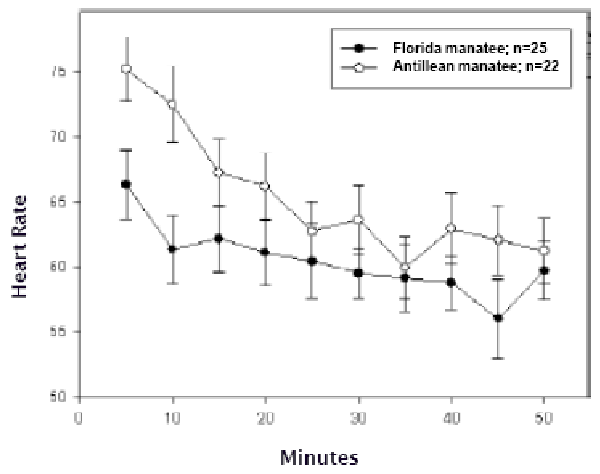
Descriptive Statistics of Heart Rates

Florida Manatees				
Time (min)	Mean (±SD)	Median	Range	Std. Error
5	66 (±9.7)	70	48-80	2.7
15	62 (±11.1)	64	32-84	2.5
25	60 (±12.6)	57	40-80	2.9
40	59 (±8.7)	60	44-72	2.0
50	60 (±6.9)	62	45-68	2.2

Antillean Manatees				
Time (min)	Mean (±SD)	Median	Range	Std. Error
5	75 (±7.7)	74	64-88	2.4
15	67 (±9.4)	68	52-80	2.6
25	63 (±10.1)	63	44-80	2.3
40	63 (±10.9)	63	44-80	2.7
50	61 (±10.5)	60	48-84	2.5

Mean Heart Rates with Standard Error Bars

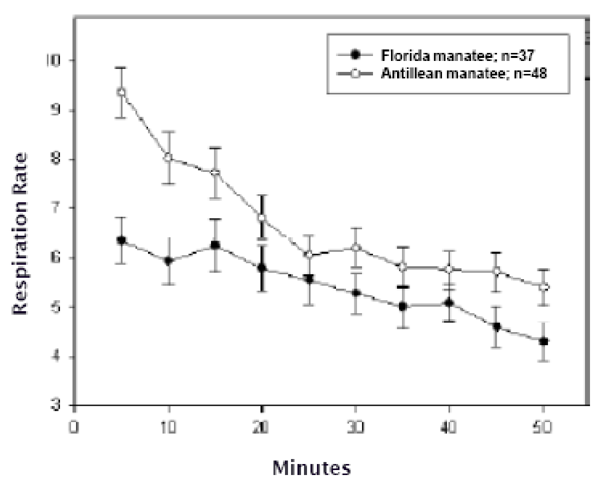


Descriptive Statistics of Respiration Rates

Florida Manatees				
Time (min)	Mean (±SD)	Median	Range	Std. Error
5	6 (±2.9)	6	1-13	0.5
15	6 (±3.3)	6	0-17	0.5
25	6 (±3.1)	5	0-17	0.5
40	5 (±2.2)	5	1-10	0.4
50	4 (±2.0)	4	0-8	0.4

Antillean Manatees				
Time (min)	Mean (±SD)	Median	Range	Std. Error
5	9 (±3.5)	9	3-17	0.5
15	7 (±3.5)	8	2-15	0.5
25	6 (±2.7)	6	0-11	0.4
40	6 (±2.5)	5.5	2-12	0.4
50	5 (±2.3)	5	2-12	0.4

Mean Respirations with Standard Error Bars



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BIOGRAPHICAL SKETCH

Arthur William Wong was born in Brooklyn , New York. He received a B.S. in biology at Southampton College, Long Island University in 2000. During his undergraduate studies he worked in animal husbandry and training at the Zeehondencreche Seal Rescue and Rehabilitation Center in the Netherlands, the New York Aquarium, and Central Park Zoo. In his final year he studied aboard a sailing vessel in the Caribbean. After graduation Arthur pursued field research in Hawaii, Maine, and the Dry Tortugas. In 2003, he was hired as a marine mammal biologist with the Florida Fish and Wildlife Conservation Commission. Within months of his employment, the University of Florida accepted him as a graduate student. This thesis is a product of his time while working as a state biologist and simultaneously pursuing his master's degree. This thesis was supported by the University of Florida-College of Veterinary Medicine-Aquatic Animal Health Program, and the Florida Fish and Wildlife Conservation Commission.