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EFFECT OF HORMONAL AND TEMPORAL FACTORS ON CAPTIVE FEMALE MANATEE (*Trichechus manatus latirostris*) BEHAVIOR

by

CHIFUYU HORIKOSHI

(Under the Direction of Professor Bruce A. Schulte)

ABSTRACT

Activity patterns, spatial use and reproductive hormones of nine adult-female captive manatees at Homosassa Springs Wildlife State Park (HSWSP) were studied from January 6 to August 10, 2003. This study probed two main topics: 1) activity pattern and spatial use of the facility by manatees over three times of day and three seasons (winter, spring and summer), and 2) correlation between manatees behavior and reproductive hormone concentrations collected via fecal samples. Activity patterns and spatial use of the manatees were affected by provisioned food availability over the day and natural vegetation over the study period. Five manatees had estrous cycle patterns. Two individual behaviors, blowing bubbles and inverted posture, and the level of interaction were positively associated with the estrous cycles. Further study is required to see if similar behavior and endocrine relationships are evident in wild manatees.

INDEX WORDS: Manatee, Behavior, Activity, Spatial use, Reproduction, Hormone, Feeding

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by

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B.S., Eastern Oregon University, 2001

M.S., Georgia Southern University 2004

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

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2004

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David Rostal

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CHAPTER 1

BACKGROUND

Knowledge of external (environmental condition) and internal (physiological state) factors of endangered species is fundamental because it can help model species survival and assist management strategies applied to specific instances of need. For example, an external or internal stimulus might evolve as a signal for a specific behavior related to mating (Crews and Moore 1986). Therefore, understanding the relationship between external factors such as food availability or internal factors such as hormonal status should improve our ability to predict the species specific situations in which these stimuli trigger reproductive behavior.

The Florida manatee (*Trichechus manatus latirostris*) is listed as endangered under the Endangered Species Act with a total population of some 2861 to 3113 animals (FMRI 2003). Each year, many manatees are struck by motorboats, subjected to cold stress or otherwise incapacitated. In an effort to maintain manatee numbers, injured manatees or orphaned calves are rescued and transported to one of four rehabilitation centers in Florida (Young 2001). Some manatees are released into the wild in a relatively short time, but other manatees, including those that are captive born, remain in captivity for longer periods. These captive manatees are educational ambassadors to the public (Young 2001), and can provide crucial biological information to researchers and managers, such as determination of gestation and estrous cycling, behavior of estrous females, mating, parturition, nursing, and calf growth and development (Odell et al. 1995).

The physiological examination of large aquatic animals presents a number of obstacles. Conventional physiological examination on untrained manatee requires an animal to be dry-docked, and numerous people need to forcibly restrain the animal (Colbert 2001). This procedure is associated with risk of injury and stress for both the animal and handlers because of the large body size of the animals (Colbert and Bauer 1999, Colbert et al. 2001). Recently, animal husbandry training has been performed with captive manatees as a safe alternative. Using positive reinforcement and operant conditioning techniques, husbandry training allows an animal to voluntarily participate in the acquisition of behavioral, physical and/or physiological samples (Colbert and Bauer 1999, Colbert et al 2001).

In this thesis, I addressed two broad topics. First, I determined the spatial use of the enclosure and related behavioral changes by nine captive female manatees at Homosassa Springs Wildlife State Park (HSWSP) for three allotted periods of a day (noon, mid-afternoon, and late-afternoon) through the three seasons (winter, spring, and summer). Second, I determined the relation between behavior and the estrous cycles of the manatees. For physiological sample collection, husbandry-training techniques were used to obtain fecal, urine and blood samples from two manatees as well as vulva measurements from a single manatee.

Homosassa Springs Wildlife State Park, Florida, has a unique manatee exhibit that consists of a natural river and a man-made pool that is used for medical examinations. The two components of the exhibit allowed me to train two manatees in a controlled setting and observe all nine manatees in a reasonably naturalistic environment.

Because the manatees are exposed to a naturalistic environment that includes natural food resources, the examination of various aspects of behavioral and physiological parameters of these captive manatees is especially valuable in order to contrast and understand free ranging manatees.

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CHAPTER 2

ACTIVITY PATTERNS AND SPATIAL USE OF FACILITY BY A GROUP OF CAPTIVE FEMALE MANATEES

INTRODUCTION

Florida manatees (*Trichechus manatus latirostris*) are opportunistic and non-aggressive herbivores (Hartman 1979, Best 1981, Reynolds and Odell 1991) that consume more than 60 species of both fresh and marine vegetation (Hartman 1979, Bengtson 1983, Reynolds and Odell 1991, Wells at el. 1999, Marshall 2000). Adult manatees feed for 6 to 8 hours a day (Hartman 1979, Best 1981, Reynolds 1981, Marshall et al. 2000), ingesting approximately 7% of their body weight in aquatic vegetation daily (Bengtson 1981, Etheridge et al. 1985). Manatees select particular habitats for specific activities such as feeding or resting (Koelsch 1997). Reynolds (1977) and Hartman (1979) reported that manatees returned to a preferred feeding site and used it continuously until resources had been diminished or until they found another favorable site. Between feeding bouts, manatees normally rest for two to four hours (daily total range 2-12 h)(Hartman 1979).

Environmental constraints affect daily and seasonal patterns of herbivorous animals, while energy and nutrient intake directly regulates specific activity budgets (Nielson 1984, Pepin at el. 1990, Risenhoover 1986). Changes in the relative energy intake and expenditure affect activity patterns (Robbins 1983, Fancy and White 1985, Green and Bear 1990). From an evolutionary standpoint, animals may allocate their activity period to optimize energy intake (Cederlund et al. 1989); therefore, time that is spent on foraging must surpass the costs of activity and provide sufficient energy to withstand non-foraging periods (MacArthur and Pianka 1966, Norberg 1977). As a result, quantity and quality of forage are probably the key components in determining activity budgets in large herbivore species (Owen-Smith 1979) like manatees.

Daily and seasonal activities of manatees consist of feeding, resting, idling, traveling, and socializing (Hartman 1979). Manatees may select these behaviors depending upon efficiency and availability of energy and nutrient intake. Foraging strategies involve the selection of plant species and the nutritive parts of the selected vegetation, as well as the mode of feeding, such as grazing or rooting (Lefebvre et al. 2000). On a daily basis, manatees move between foraging and resting areas when remaining in one region over a period of days or weeks, and are apt to use specific pathways repeatedly (Koelsch 1997). Seasonal migration of the ecological range between fresh and marine water environments may be triggered by an increase in the abundance of forage in spring when the water temperature allows manatees to disperse (Wells at el. 1999, Deutsch et al. 2000). Direct behavioral observations of the same manatees in the wild over diurnal and seasonal time scales are difficult to acquire (Hartman 1979, Reynolds 1981, Koelsch 1997). Studying manatees in captivity assures finding the animals and controls for numerous environmental variables, albeit introducing potential artifacts. In addition, knowledge of daily and seasonal activity patterns and spatial use of facility by captive manatees provides a basis of activity whose understanding will help to perceive atypical behavior when animals express internal (physiological) changes in endocrinology.

The environmental condition of the manatee exhibit at Homosassa Springs Wildlife State Park (HSWSP) is unique for a zoological facility. The manatee enclosure holds nine female-adult manatees. It consists of a fenced natural river; thus, the manatees are exposed to a similar environment as wild manatees for at least part of the year. The nine female manatees were rescued from cold water stress, injured by motorboat collisions, or have physiological problems, and are candidates for release into the wild in the future. These manatees have been in captivity from 9 to 42 years, yet Young (2001) showed that time in captivity had little affect on their activity patterns before, during and after a provisioned feeding period. The manatees are regularly fed by the park personnel at designated upstream locations which are deeper than other areas of the enclosure and consist of a rocky substrate. However, they also have access to natural resources on the downstream end of the enclosure where it is shallower and mainly consists of a sandy riverbed. During the warm season, the sandy riverbed is covered by algae and aquatic vegetation.

I investigated activity patterns and spatial use of the facility by the nine female captive manatees at HSWSP. The manatees were fed by the park personnel at four scheduled times during the day and at a certain area of the enclosure. I was especially interested in how their feeding behavior changed before and after scheduled feeding times. I also was interested in their seasonal change of activity and spatial use because over during spring and summer periods natural vegetation became more available. Therefore, I hypothesized that activity patterns and spatial use of the facility would vary by time of day, as well as over the three seasons (winter, spring, and summer) of the study period. Specifically, I predicted that the manatees would become more active and shift their use of the enclosure from the provisioned food area to other regions after the last daily feeding. In addition, I predicted seasonal change in spatial use of the enclosure with a greater use of the areas of natural vegetation in the spring and summer months.

Materials and Methods

Study Site and Subjects

The study was conducted at Homosassa Spring Wildlife State Park (HSWSP) in Homosassa Springs, Florida, which is approximately 70 miles north of Tampa. The manatee exhibit is part of the Homosassa River. The boundary between the Homosassa River and the exhibit consists of a number of poles embedded in the riverbed as a fence. This fence prevents the captive manatees from escaping. The water level in the exhibit is influenced by not only the tide of the river, but also the direction of wind. Wind from west brings water into the exhibit and keeps the water level high. In 2002, a small manmade pool (ca. 95,000 L), designed for medical examinations, was completed. It was connected to the northwest side of the enclosure by a narrow passage. The gate was usually open allowing the manatees to enter the pool freely.

I created a scaled map of the enclosure and divided it into seven areas, labeled A-1, A-2, B-1, B-2, C-1, C-2 and the medical pool (Figure 2.1). The area A-1 incorporated a spring that lies beneath the Underwater Fishbowl Observatory. The area of spring encompasses approximately 0.2 ha and reaches depths of 13.5 m with an estimated volume of 1.1×10^7 L (Young 2001). The spring provides 1.14×10^5 L of water every hour and maintains a water temperature in the enclosure around 23 degrees Celsius for all seasons (HSWSP record). The riverbed of the region A mainly consisted of a rocky substrate while regions B and C consisted of a sandy riverbed. The spring water flows from area A-1 through region B and goes out though the fence at area C-1 and into the open river (personal observation). Therefore, the middle part of region B and area C-1 were exposed to strong water currents and the riverbed of those areas was deeper than the other areas. The area C-2 was shallow with a weak river current and near the effluent of the hippopotamus exhibit; the C-2 often received debris from this exhibit, especially after heavy rains. When tidal current was coming into the enclosure through the fence at the C-1, the effluent remained in the regions B-2 and C-2.

Daily feeding occurred at a designated location, called the Manatee Salad Bar, near the Fishbowl Underwater Observatory in the area A-1. The manatees were fed the commercial vegetables such as heads of lettuce, cabbage, kale, and/or green peppers before the park opened at 0800 hour. As part of educational demonstrations for park visitors, additional feeding times were held at hours of 1045, 1300, and 1515. Initially, carrots were thrown into the water in shallow area of A-2. In this way, the audience could watch the manatees feed during the program. During the program, the ranger entered the water and handfed a small bucket (ca. 0.5L) of vitamin tablets (made for livestock) to the manatees. After feeding all vitamins, the manatees were fed the main vegetables at the Manatee Salad Bar in the A-1. The medical pool was used for feeding small portion of vegetables (kale and/or green pepper) twice a day after the daily feeding times at hours of 1315 and 1530. This was done to accustom the manatees to enter the pool.

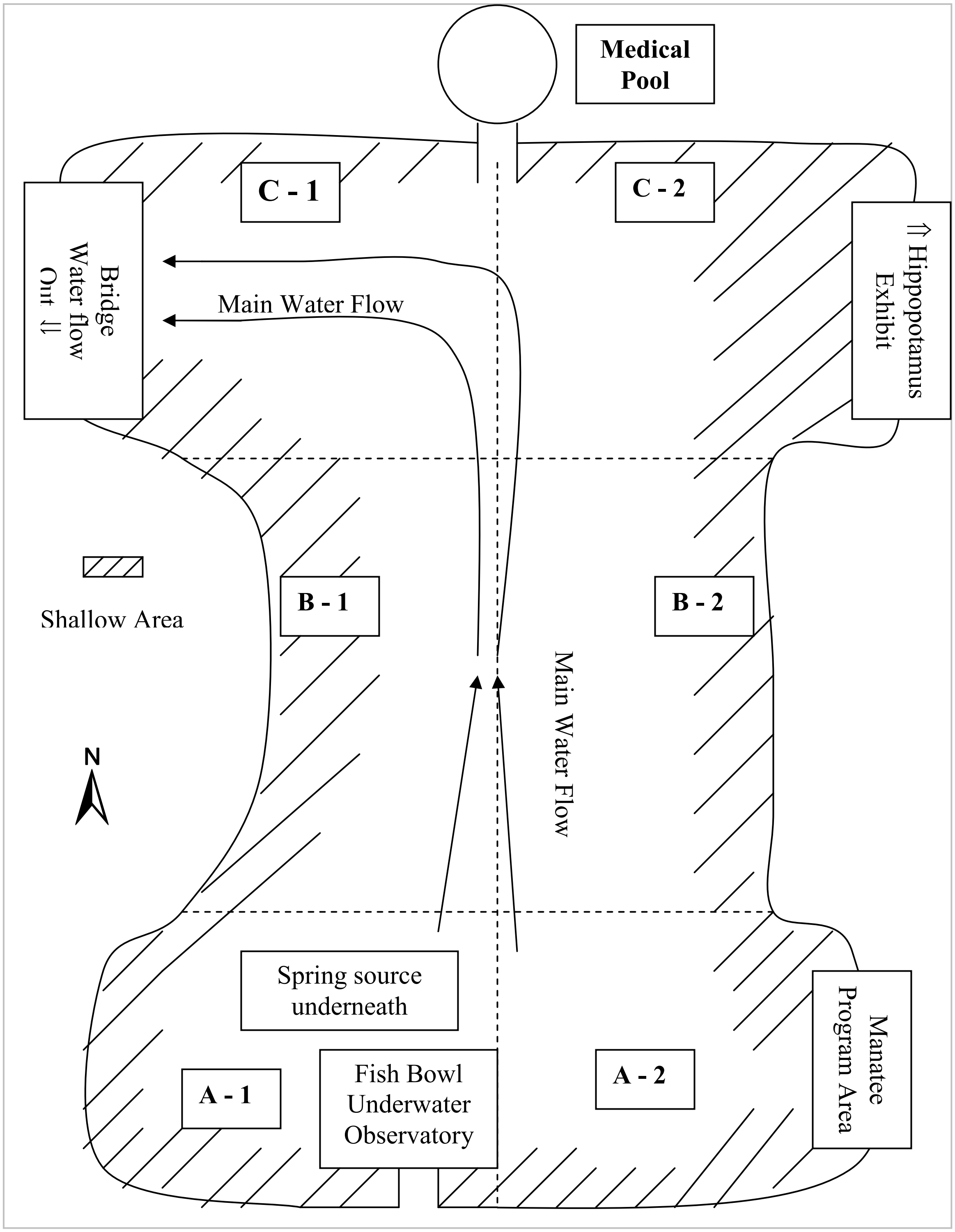

Figure 2.1. A map of the manatee enclosure with seven delineated areas at Homosassa Springs Wildlife State Park. The length (North to South) of the enclosure is approximately 100 meters.

Study Animals

Homosassa Springs Wildlife State Park retains nine female-manatees in the enclosure (Table 2.1). Seven of the manatees were born in the wild. Three were rescued because of cold stress (Holly, Oakley and Willoughby), two because of boat injury (Amanda and Electra) and one because of an unidentified physiological problem (Rosie). Ariel was found with Amanda as a yearling calf. In 1990, Betsy was born to Amanda in captivity (Amanda mated with a male in the facility before the breeding of manatee became prohibited). In 1975, Lorelei was born in captivity at Miami Seaquarium and moved to HSWSP in 1994.

Behavioral Observations

The study was conducted from January 6 to August 10, 2003 (Julian dates 6-222). Behavioral data were collected three days a week over 31 weeks. I constructed an ethogram in order to define mutually exclusive behaviors (Table 2.2). Using scan sampling and instantaneous recording, I noted the behavior and location of each manatee in one of the seven delineated areas every 25 minutes (Martin and Bateson 1993). I observed manatee behavior from the walking path around the exhibit. During the study period, a total of 7429 scan samples were recorded from all the nine manatees with an average of 825 scans per manatee over 77 days.

Observations were made from the hours of 1115 to 1700 in three time periods: noon (1115 – 1255), mid afternoon (1350 - 1505), and late afternoon (1610 - 1700). The number of scans per time period was five, four, and three for noon, mid afternoon, and late afternoon, respectively. Uneven scan numbers per time period were unavoidable

Table 2.1. The nine captive female manatees at Homosassa Springs Wildlife State Park in 2003.

Manatee	**Age**	**Captive (years)**	**Comment**
Amanda	37	30	Mother of Ariel & Betsy
Ariel	30	30	Rescued when < 1 year old
Betsy	13	13	Captive born
Electra	10	9	Physical Disability
Holly	9	9	Rescued when < 1 year old
Lorelei	28	28	Captive born
Oakley	9	9	Rescued when < 1 year old
Rosie	42	36	Largest body Size (>3000lbs)
Willoughby	9	9	Rescued when 1 year old

because of the scheduled feeding periods of manatees and my own husbandry training of two manatees each morning and evening. I divided my study period into three equal seasonal periods: Julian date 6 -77 (January 6 to March 18, winter), 78-149 (March 19 to May 29, spring), and 150 - 222 (May 30 to August 10, summer).

The number of days in which data was collected was uneven for the three daily time periods as well as the three seasonal periods. This was due to the schedule for husbandry training initiated from April 16 (106 Julian), when the manatees were either trained in the morning or evening. This was in contrast with the previous schedule (Jan 6 – April 15) in which each manatee was trained every morning and every evening. Therefore, I created two time phases for behavioral observation. Phase I initiated from the hours 1115 through 1700. Phase II initiated from the hours 1350 through 1840. I used these time phases alternatively by day. However, data after hours 1700 from phase II were included in the analysis in the next chapter (Captive female manatee behavior and social interaction associated with reproductive hormones) but not this chapter. This was due to unify hours of daily observation over the study period since data were collected between 1115 and 1700 until April 16.

The Manatee Educational Program at HSWSP presented three daily shows at the hours of 1045, 1300, and 1515 at area A-2. The manatees were fed in their daily feeding area in A-1 after each afternoon Manatee Educational Program at 1300 and 1515. All nine manatees congregated at the feeding area throughout a show and vantage points for viewing the manatees were crowded with tourists. This prevented reliable behavioral observations. Therefore, I did not obtain any behavioral sample during the afternoon feeding periods and for approximately 45 - 55 minutes after each feeding time. In addition, preliminary study in the summer 2002 showed that manatee activities did not change significantly during early morning period (between the hours of 0800 and 1045) and late morning to early afternoon period (between the hours of 1115 and 1300). The manatees were mostly inactive around the feeding areas (A-1 and A-2). Consequently, I did not observe their behavior during early morning period (between the hours of 0800 and 1045) and used this morning period for manatee husbandry training for behavior and hormone study (see Chapter 3).

A total of 32 behaviors per manatee were recorded and those were placed into three behavior categories: 1) Inactive: resting or sleeping at bottom, 2) Eat: feeding, and 3) Active: summary of other 29 behaviors (Table 2.2). Feeding behavior was divided into two categories: eat provisioned food and eat natural vegetation in order to determine usage of seasonal food resources.

Data Analysis

Daily proportion of spatial use in the manatee enclosure by the nine manatees was analyzed for each manatee over the three time periods within a day and over the three seasons. All statistical analyses were performed using the JMP statistical software (version 4) with an alpha level of $p < 0.05$ for the fiducial level of significance. Both daily and seasonal spatial uses of the facility were examined by repeated measures analysis of variance (ANOVA). When the data did not meet normality, the Friedman's Test was used for non-parametric analysis. Seasonal changes of food sources also were analyzed using repeated measures ANOVA.

Table 2.2. Ethogram used for scan sampling of female captive manatees at Homosassa Springs Wildlife State Park. The behaviors observed were classified as Eat (one behavior stratified by type of forage consumed), Inactive (two) or Active (29).

Behavior	**Description**
Eat	
Eat provisioned food	Manatee consumes lettuce or other commercial vegetables provided by park personnel
Eat natural vegetation	Manatee consumes aquatic vegetation in water, or browse at edge of exhibit
Inactive Behaviors	
Float	Manatee rests or sleeps in water column (back facing up)
Rest	Manatee rests or sleeps at bottom
Active Behaviors	
Aggressive movement	Manatee abruptly swims, walks, or stops
Breathe	Manatee inhales and exhales through nostrils
Bubble	Manatee release air from nostrils without surfacing
Contact	Manatee touches another manatee with parts of body; an obvious inadvertent contact was not counted
Displace	Manatee displaces another manatee from a location without contact
Dive	Manatee swims down into deeper area of the exhibit (underneath of Fishbowl Observatory).

Flaring	Manatee opens mouth about half way and oscillates upper perioral lip
Flipper splash	Manatee splashes water with flippers
Head splash	Manatee nods head to splash water
Inverted	Manatee remains in an inverted posture in shallow area while touching other manatees, but not resting or asleep
Inverted surface	Manatee surfaces with an inverted posture for a short period of time
Inverted Swimming	Manatee swims in an inverted position
Lap swim	Manatee swims in circle in certain area
Mounting	Abdomen of a manatee touches another/others manatee(s) back side, or abdomen; inadvertent contact was not counted
Mounting received	Manatee receives mounting from another/ other manatee(s)
Mouthing	Two manatees touch each other with their mouths
Nostril movement	Manatee moves muscle around nostrils without opening and closing nostrils.
Open Mouth	Manatee opens mouth in the water
Pushing	Manatee touches another manatee aggressively with body parts noted
Rolling over	Manatee rolls over laterally while moving through water
Rub	Manatee rubs its body against a rock or other objects in the exhibit

Spinning swim	Manatee pivots in the water by using flippers
Swim	Manatee moves through water by using tail
Swim Walk	Manatee swims and walks at the same time
Tilt body	Manatee tilts its body side way while inactive in shallow area with back facing up posture
Touch	Manatee touches objects, other than manatees, with her mouth
Turning half	Manatee turns its body half laterally
Walk	Manatee moves along bottom by using flippers to move forward
Other	Behavior not described, if becomes common, create term
Not visible	Cannot see manatees at all
Unknown Manatee	Can see manatees but cannot ID manatee
Zip on behavior	Can see and ID manatee but cannot tell what it is doing

RESULTS

During the total observation period from January 6 to August 10, 2003, the manatees predominantly used region A, accounting for over 50% of all scans (Figure 2.2). They used area A-2 the most at 30% and area B-1 the least at 3% of all seven areas. B-2 and C-2 were used fairly equally (14% and 16% respectively). However, the manatees occupied these seven locations differentially within the three periods of the day: noon (1115 - 1255), mid afternoon (1350-1515), and late afternoon (1610-1700). At the noon period, the manatees used region A more than 70% with nearly equal proportion between the areas of A-1 (37%) and A-2 (36%). The manatees were located in area B-2 for 13% of the scans, yet only 3 % in B-1. The manatees were rarely present in the region C, occupied for only 6 % of the scans. During the mid afternoon time period, the manatees were still found in region A (55%). However, the proportion of occupation shifted towards A-2 (43%) and away from A-1 (12%). During the mid afternoon period, use of B-2 increased 19% and region C only 14% for all scans. In contrast, during the late afternoon period, the manatees dramatically shifted their location towards region C (46%) compared to that of region A (27%). Use of the medical pool increased over the day from noon (5%) to mid afternoon (7%) and late afternoon (15%).

Use of all seven locations was contrasted using Friedman's Test and the result indicated that the manatees used the seven locations differently throughout of a day (Friedman's Test X^2 = 31.1, df = 6, P <0.001). The use of all seven locations over the three time periods (noon, mid afternoon, and late afternoon) was contrasted using Friedman's Test and the result showed no significant difference (Friedman's Test X^2 =

1.143, df = 2, $P > 0.5$). However, use of region A (provisioned food area) and region C (natural food area) over the three daytime periods was significantly different (Specified contrast using Friedman's Test $X^2 = 129.5$, df = 2, $P < 0.0001$).

Seasonal variation of the spatial usage was contrasted using repeated measures ANOVA. Daily use of regions A, B, and C was significantly influenced by the three seasons ($F_{2,16} = 129.12$, $P < 0.0001$), ($F_{2,16} = 4.26$, $P = 0.0328$) and ($F_{2,16} = 99.50$, $P < 0.0001$), respectively (Figure 2.3). The manatees mostly used region A during the winter period, and gradually shifted towards regions B and C during the spring, increasingly in region C during the summer (Table 2.3).

During the noon period, the manatees were located in area A for the majority of scans (winter 79%, spring 72%, and summer 61%) (Figure 2.4), yet statistical analysis showed significant difference over the three seasons ($F_{2,16} = 13.49$, $P = 0.0004$). This may be a result from the reduced sample data set after Julian date of 106. The seasonal change during the mid afternoon period was increased use of area C-2 from the winter (3%) to the summer (12%). During the late afternoon period, the manatees significantly increased use of region C from the winter season (19%) to the spring season (44%) to the summer season (68%) ($F_{2,16} = 63.9$, $P < 0.0001$) while the manatees significantly decreased use of region A from the winter season (50%) to the spring season (23%) to the summer season (11%) ($F_{2,16} = 45.53$, $P < 0.0001$).

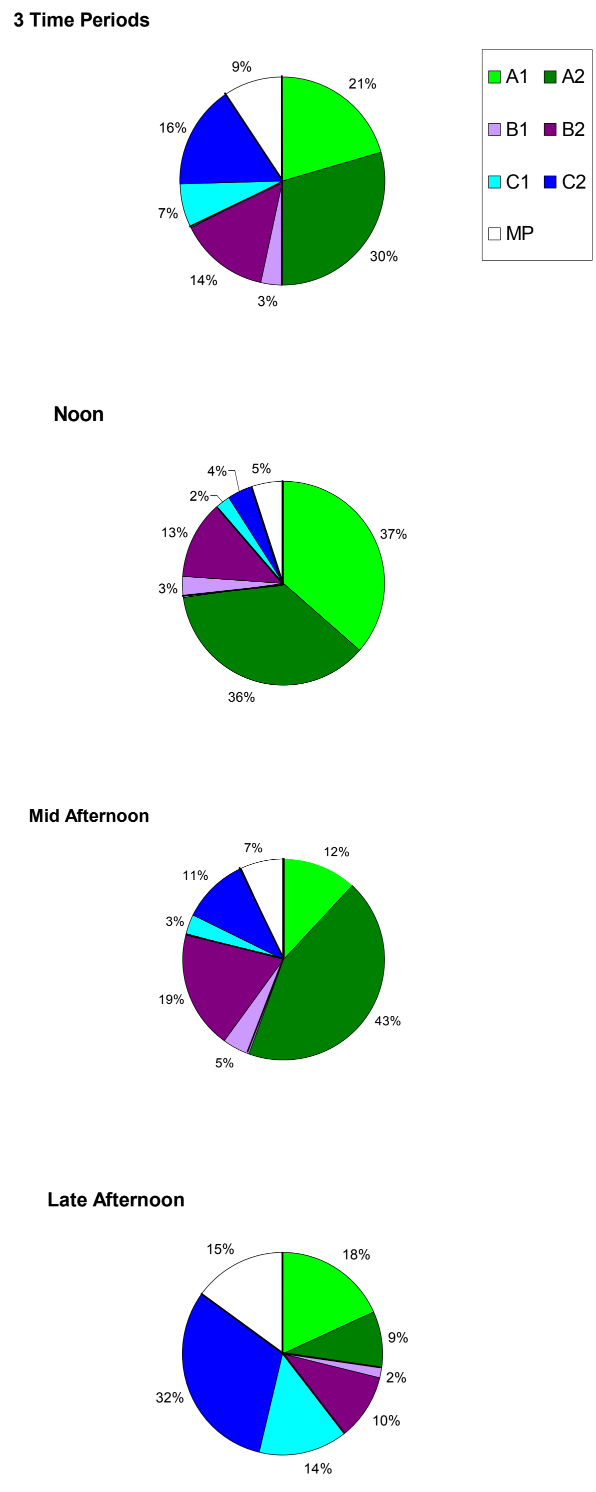

Figure 2.2. Summary of habitat use in the manatee enclosure by nine captive manatees at Homosassa Springs Wildlife State during the three time periods combined and separately (noon (1115-1250), mid afternoon (1350-1505) and late afternoon periods (1610-1700)) Park from January 6 to August 10, 2003 (6 to 222 Julian).

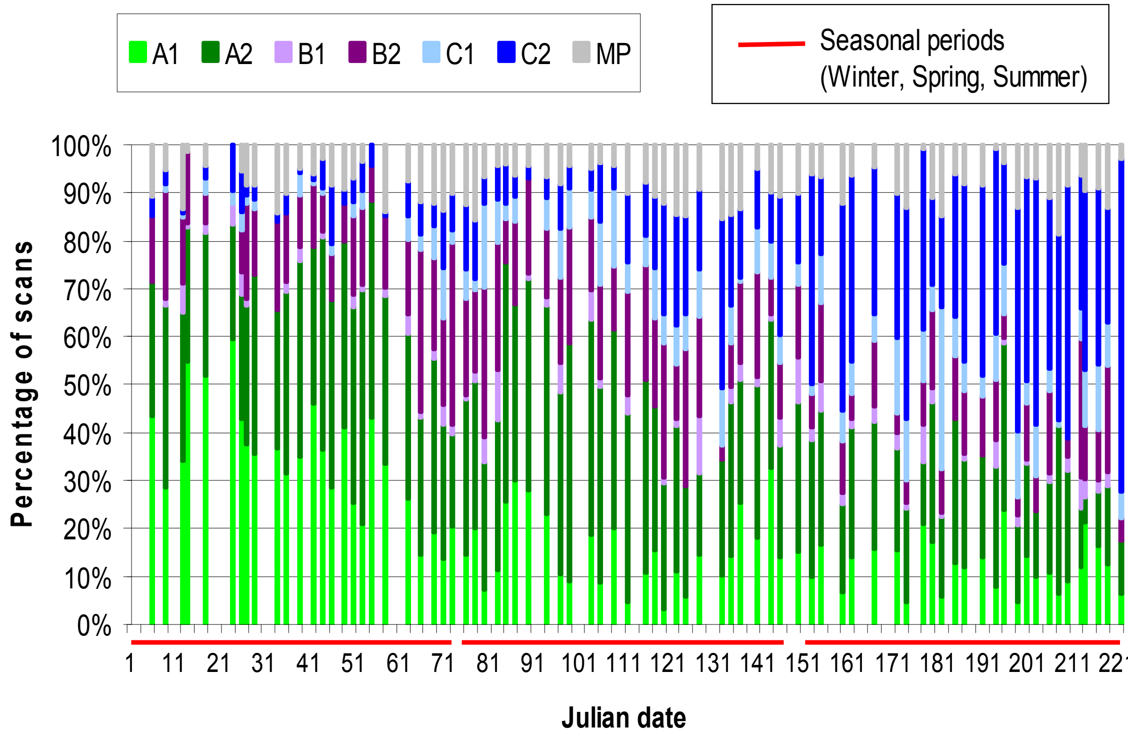

Figure 2.3. Percentage of habitat use in the manatee enclosure by nine captive manatees at HSWSP per observation day (1115 – 1700) over the study period from January 6 to August 10, 2003 (6 to 222 Julian).

Table 2.3. Proportion of manatee-location scans for the seven delineated areas in the enclosure by nine captive female manatees at HSWSP over the three seasons (Winter, Spring and Summer) from January 6 to August 10, 2003. The most heavily used area for each season is in bold type.

	Seasons (Julian date)		
Area	**Winter (6-77)**	**Spring (78-149)**	**Summer (150-222)**
A	**67**	**49**	33
B	17	21	14
C	8	21	**45**
Medical Pool	8	9	8
Total	100	100	100

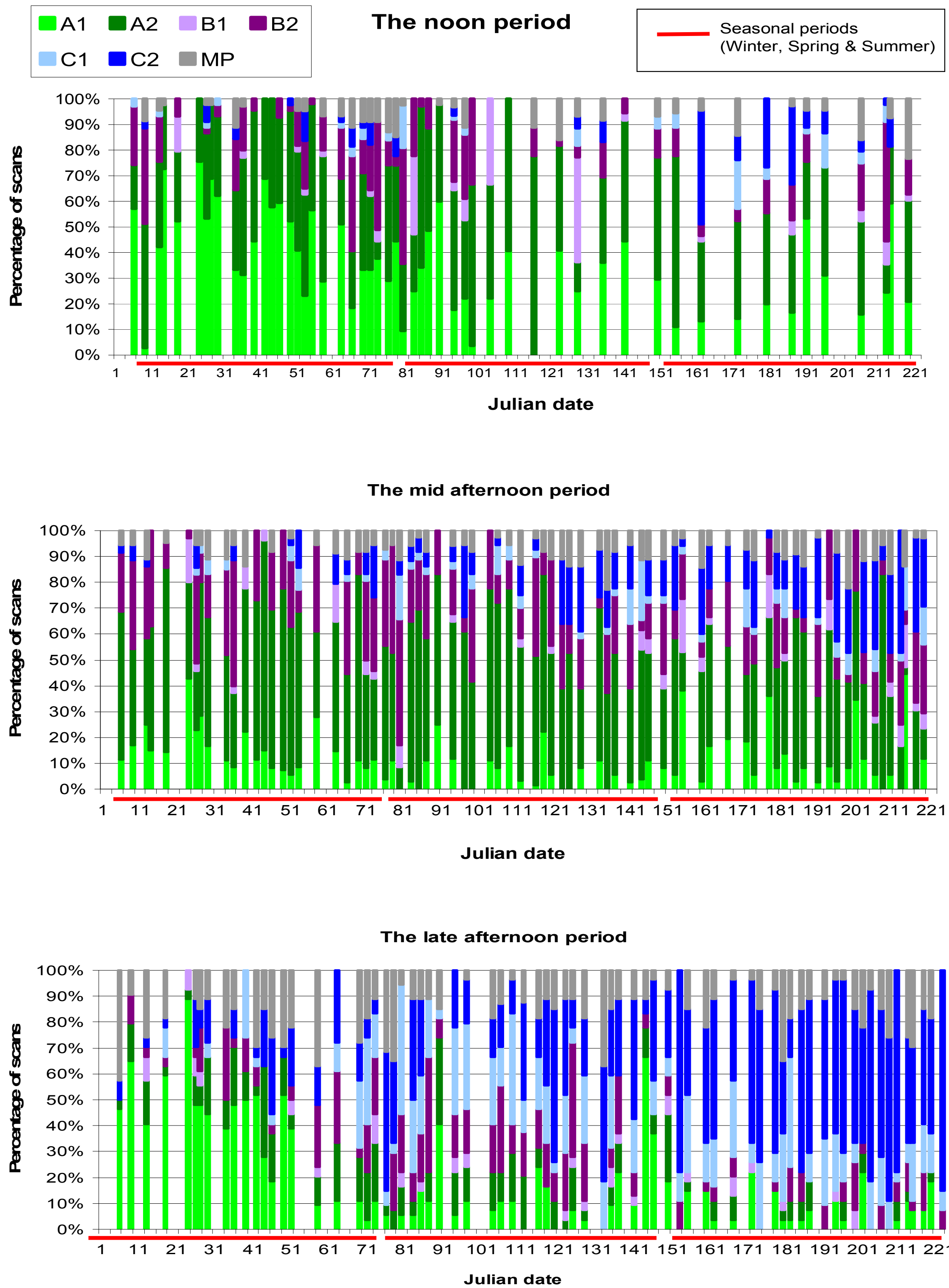

Figure 2.4. Percentage of habitat use in the manatee enclosure by nine captive manatees at HSWSP during the noon (1115-1255), the mid afternoon (1350-1505), and the late afternoon (1610-1700) over the study period from January 6 to August 10, 2003 (6 to 222 Julian).

Over the total study period, the manatees showed an even distribution of "inactive" and "active" behaviors (40% and 43% respectively) (Figure 2.5). However, activity patterns were specific to the time periods within a day. The manatees decreased "inactive" behavior significantly from the noon, the mid afternoon and the late afternoon periods, occurring 57%, 42%, and to 18% respectively ($F_{2,16} = 94.07$, $P < 0.0001$). Conversely, the proportion of "eat" increased from the noon, the mid afternoon and the late afternoon periods, occurring 6%, 10%, to 38% respectively ($F_{2,16} = 213.50$, $P < 0.0001$).

Seasonal activity pattern was contrasted using Friedman's Test and the result indicated that the manatees did not vary the activity pattern throughout the day (Friedman's Test $X^2 = 1.5$, df = 2, $P > 0.25$) (Figure 2.6). However, feeding activity had a transitional change from winter to summer. The manatees increased feeding on natural vegetation ($F_{2,16} = 153.42$, $P < 0.0001$) and decreased feeding on provisioned food ($F_{2,16} = 72.62$, $P < 0.0001$) from the winter to the summer period. The percent occurrence of "eat" behavior slightly increased from the winter season (16%) through the summer season (23%).

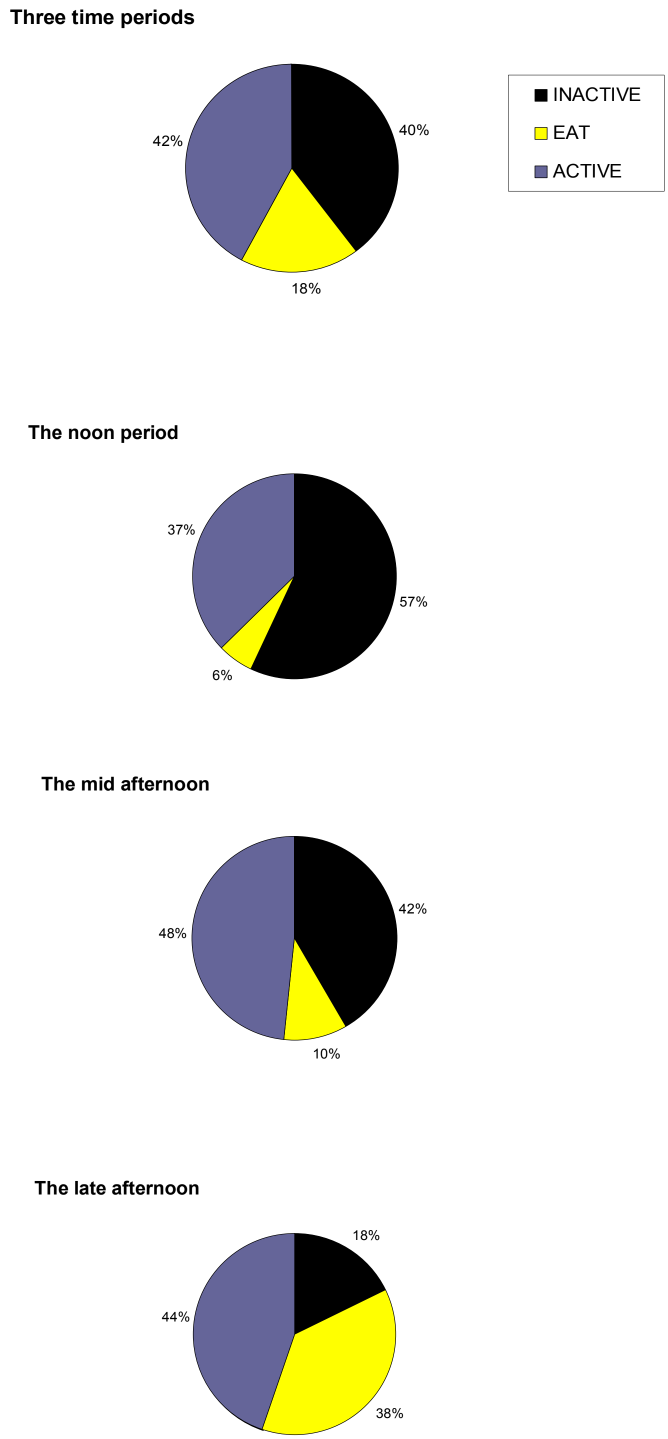

Figure 2.5. Summary of activities by nine captive manatees during the three time periods combined and separately (noon (1115-1250), mid afternoon (1350-1505) and late afternoon periods (1610-1700)) at Homosassa Springs Wildlife State Park from January 6 to August 10, 2003 (6 to 222 Julian).

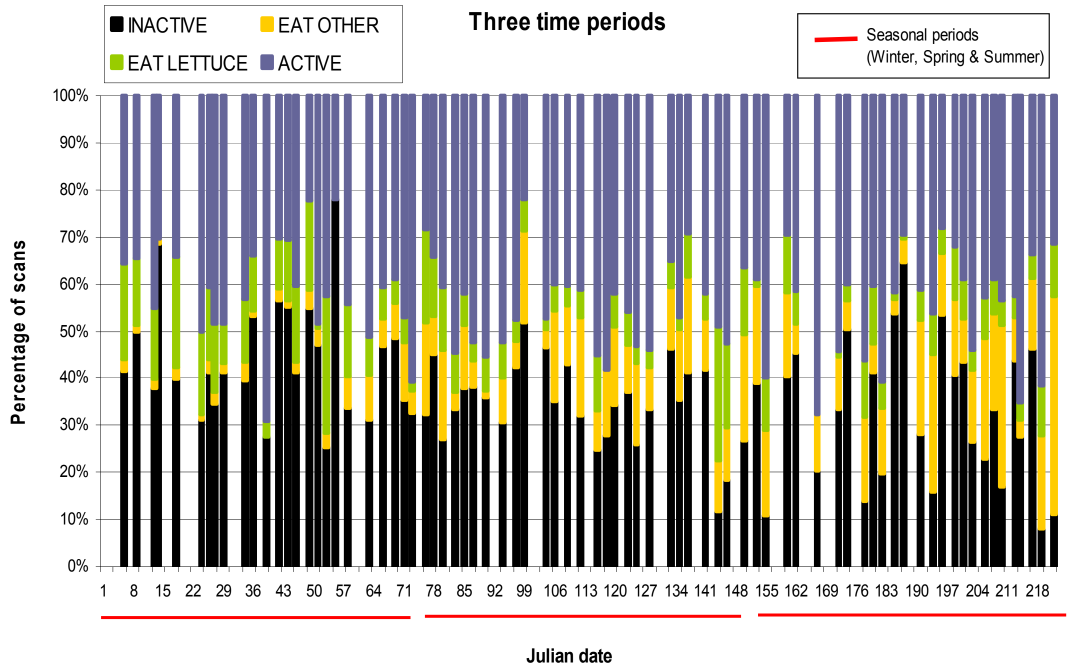

Figure 2.6. Percentage of three behaviors by nine captive manatees at Homosassa Springs Wildlife State Park over the entire daily observation period (1115 – 1700) from January 6 to August 10, 2003 (6 to 222 Julian).

During the noon period, the manatees were inactive for a large portion of all scans (winter: 48 %, spring: 47 %, summer: 45%) (Figure 2.7). The manatees did not feed much during the noon period throughout the three seasons (range from 1% to 5%). On Julian dates 206, 213 and 219, slightly increased activity was found that was probably the result of unintentional manipulation by the park. The park scheduled educational programs on the hippopotamus at different times from the manatee programs. They used individual speakers for each program until approximately two weeks from the last day of the total study period. Once the speaker at the manatee program was on during a hippopotamus program, all the manatees congregated in the area of A-2 and swam intensively rather than rested, which was the usual behavior at this time period. This act of intensive swimming was repeated every time (maximum of twice per day during my daily observation) the speaker was on without food.

During the mid afternoon period, the manatee altered food sources throughout the three seasons significantly. The manatees increased feeding on natural food gradually from the winter season (2%) to the summer season (10%) ($F_{2,16} = 22.65$, $P < 0.0001$) while they fed on lettuce the most during winter (5%) and the least during spring (2%) (Friedman's test $X^2 = 9.39$, df = 2, $P < 0.01$). The percentage of foraging increased remarkably during the late afternoon period. However, the manatees were persistent in foraging throughout the three seasons, accounting for 41%, 39%, and 35% of total scans during the winter, spring, and summer periods, respectively ($F_{2,16} = 0.79$, $P = 0.485$). Nevertheless, the manatees evidently shifted their food source from leftover heads of lettuce supplied by the park to natural vegetation from winter to summer. They fed on lettuce 31% of total scans during the winter season and reduced this to 9% in the summer season (Friedman's test $X^2 = 10.9$, df = 2, $P < 0.005$). On the other hand, they fed on natural food 10% of total scans during the winter season, but increased to 25% during the summer season ($F_{2,16} = 4.52$, $P = 0.0278$). "Inactive" behavior varied over the three seasons. Manatees were "inactive" the least (10%) during the spring period and the most (28%) during the summer period (Friedman's test $X^2 = 10.67$, df = 2, $P < 0.005$).

Overall, seasonal change of activity budgets and spatial use did not vary much during the noon and the mid afternoon periods (Figure 2.8 and Figure 2.9). However, during the late afternoon, not only did use of area C-2 increase, but the proportion of "eat" increased remarkably during the summer period (Figure 2.10).

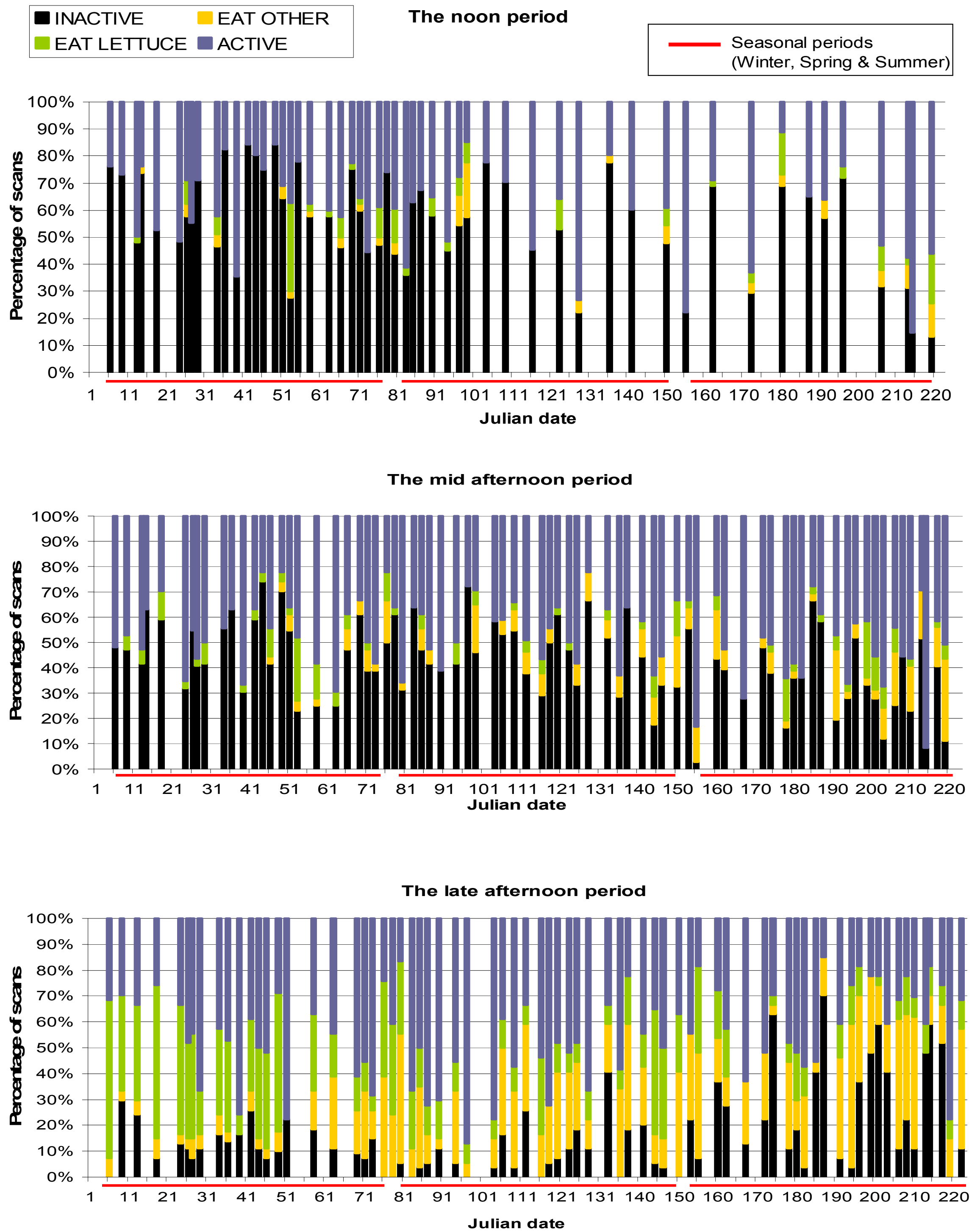

Figure 2.7. Percentage of three behaviors by nine captive manatees at Homosassa Springs Wildlife State Park during the noon (1115-1250), mid afternoon (1350-1505) and late afternoon (1610-1700) periods during the study conducted from January 6 to August 10, 2003 (6 to 222 Julian).

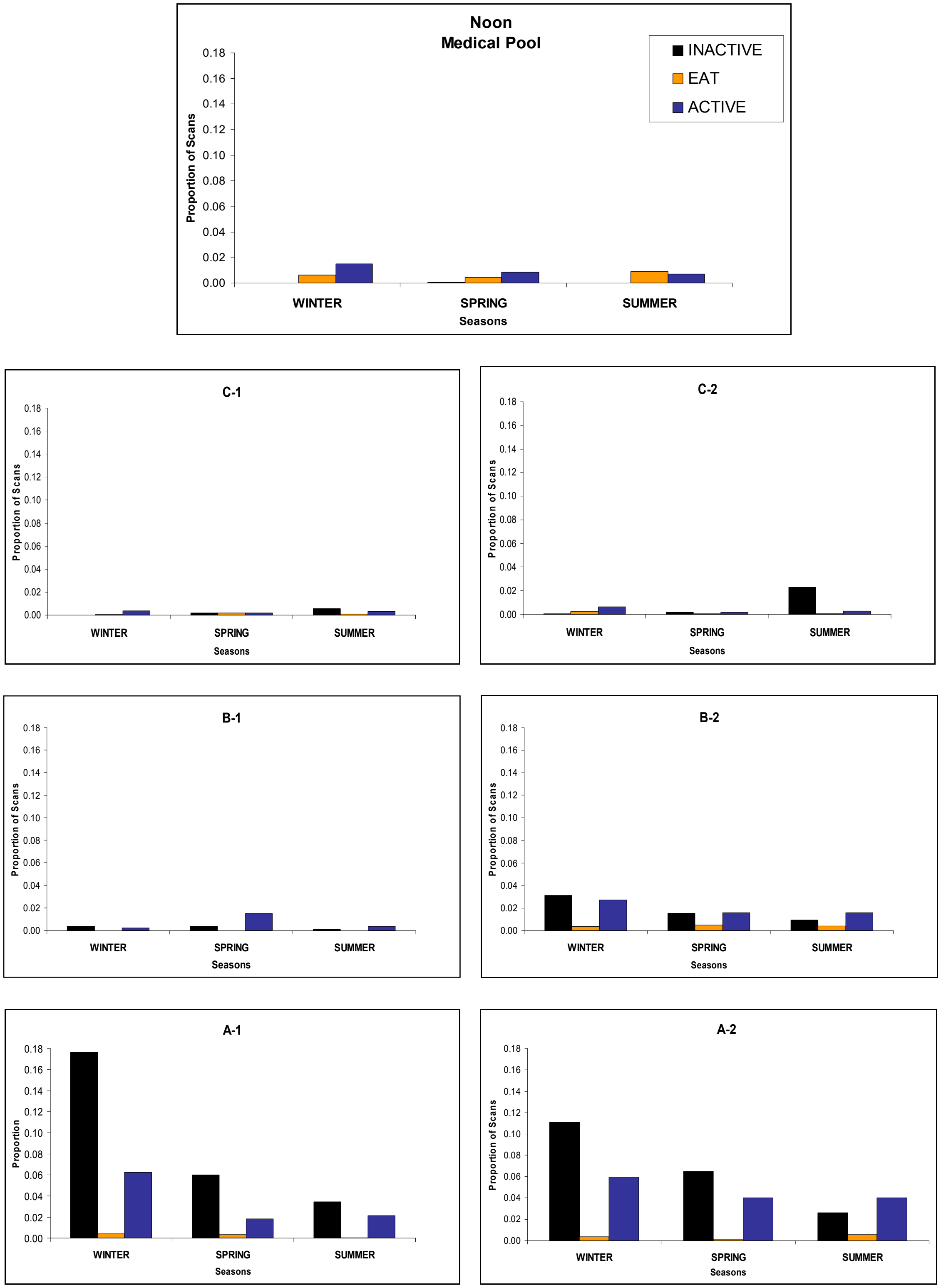

Figure 2.8. Spatial distribution in the manatee enclosure associated with three behaviors by nine captive manatees at HSWSP during noon period (1115 – 1250). Note that total proportion of all three activities and three seasons for all 7 locations adds up to 100% (i.e., all 63 bars add up to 100%).

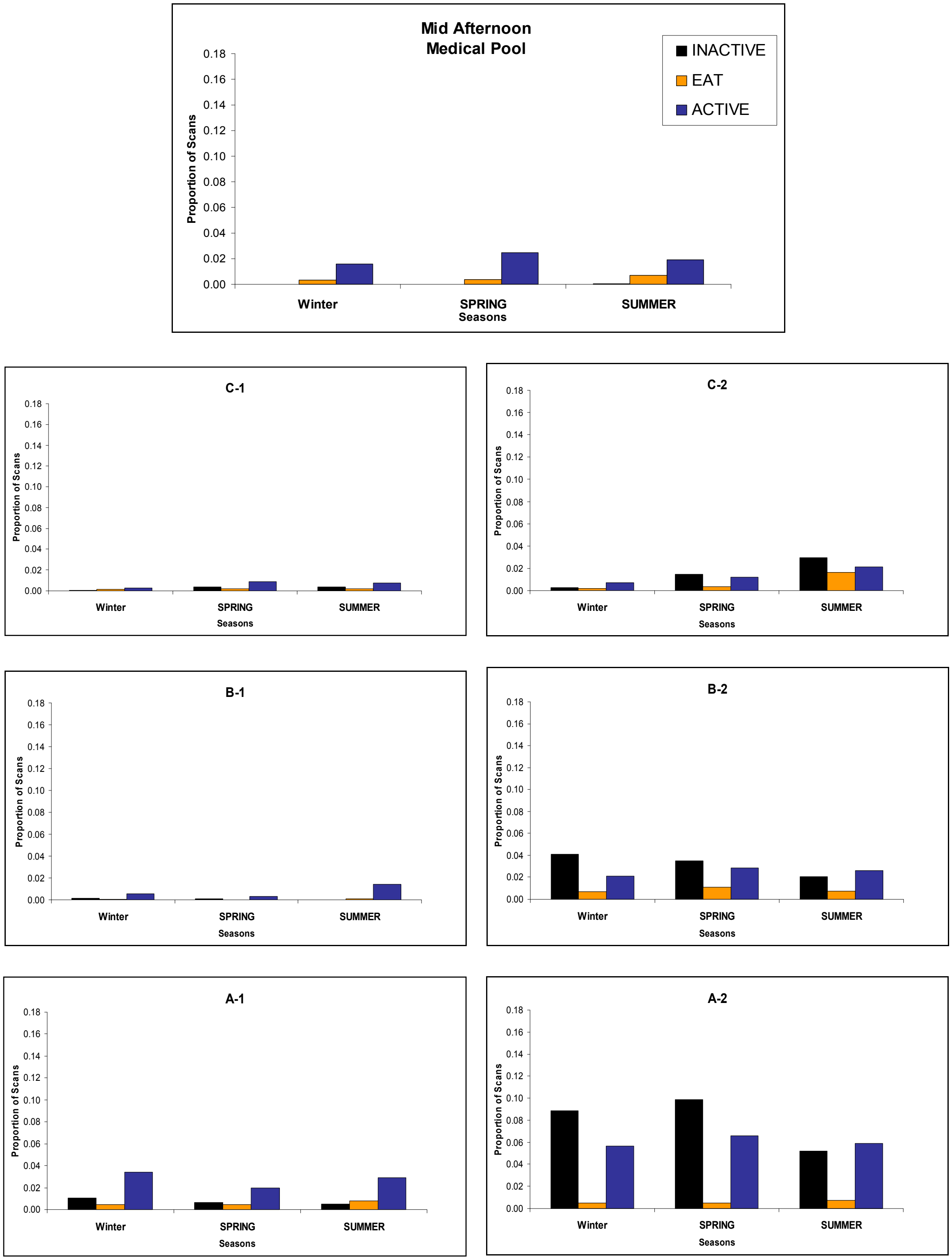

Figure 2.9. Spatial distribution in the manatee enclosure associated with three behaviors by nine captive manatees at HSWSP during mid afternoon period (1350 – 1505). Note that total proportion of all three activities and three seasons for all 7 locations adds up to 100%.

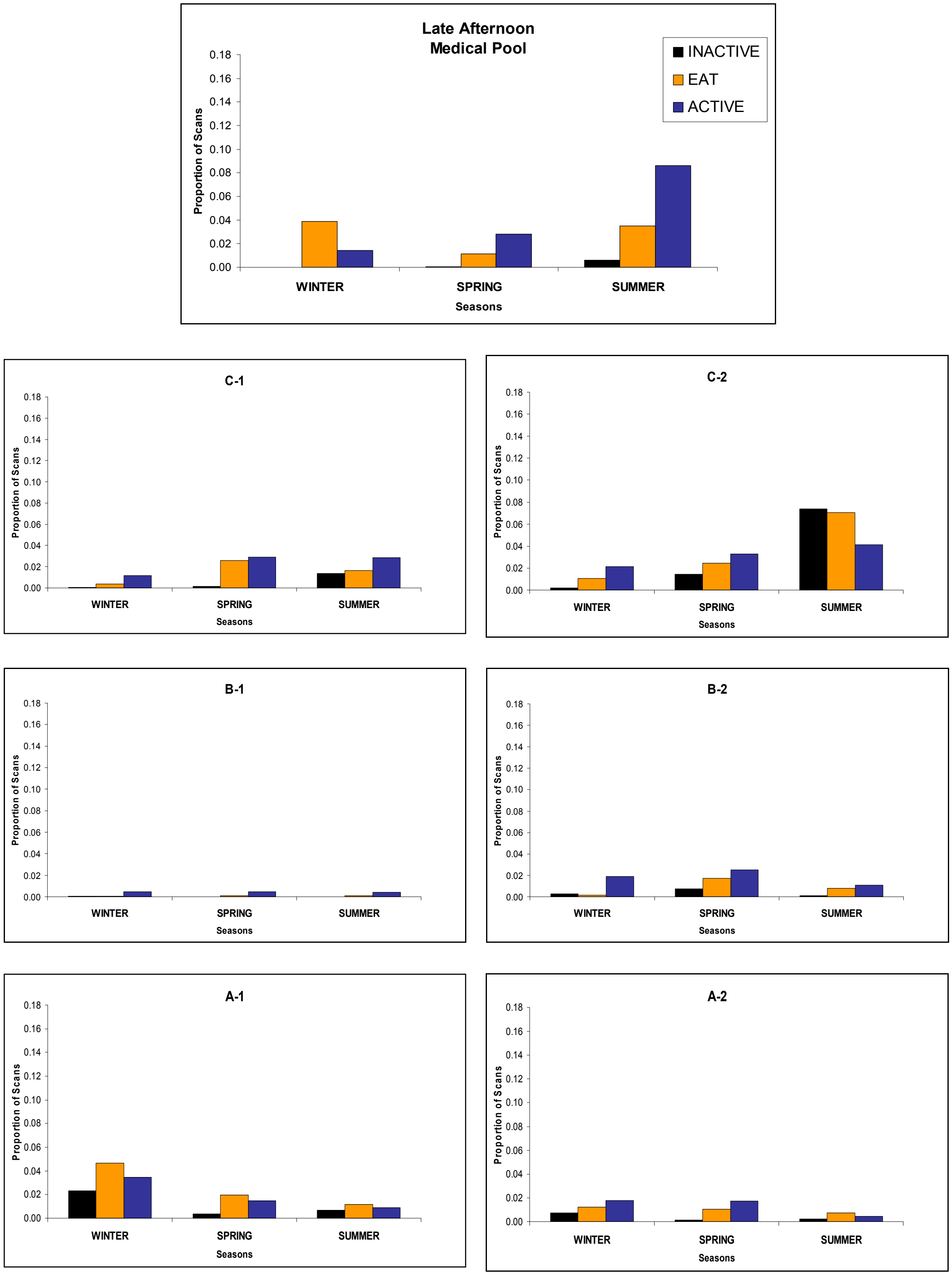

Figure 2.10. Spatial distribution in the manatee enclosure associated with three behaviors by nine captive manatees at HSWSP during late afternoon period (1610 - 1700). Note that total proportion of all three activities and three seasons for all 7 locations adds up to 100%.

DISCUSSION

The spatial use of the facility by and activity budgets of the nine female manatees were unique in the three observation periods within a day as well as the three seasonal periods. The variations of the daily activity and spatial use were mainly a result of the feeding schedules of the park. The seasonal variations were due to changes in forage availability in the enclosure.

Daily activity and spatial use

Koelsch (1997) reported that manatees used areas of calmer and/or shallow water for resting or low energy activities in order to conserve their energetic demands. Accordingly, my results indicate that the manatees did not spend energy on searching for natural food, but remained less active to await the next feeding time until the last daily feeding was completed (Figure 2.7). The manatees appeared to anticipate the daily feeding times and the locations (region A). Approximately 30 minutes before each educational program about the manatees by park personnel, the manatees in A-1 moved towards A-2 or B-2 individually. They gathered around the areas where the manatee program occurred and stayed there exhibiting mostly inactive behaviors.

Another means to conserve energy involves basking when a manatee exposes its back to the sun and remains less active. Although manatees are marine mammals, their metabolic rate is 50% lower than other studied marine mammals (Irving 1973, Irvine 1983) and 15-22% lower than similarly sized terrestrial mammalian species (Reynolds and Odell 1991). Consequently, absorbing radiant energy from direct sun may reduce loss of body heat. After the noon observation period, there was a shift in spatial distribution of the manatees from A-1 to A-2 and B-2 (Figure 2.2). This shift may have been in response to the alteration of direct sunlight from the area of A-1 to the areas of A-2 and B-2. Although depth of water in the enclosure depended upon the tide, the manatees typically positioned their bodies in areas that were shallow enough to bask and breathed by lifting up only their heads. Moore (1956), Shane (1983) and Koelsch (1997) reported similar behaviors from free-ranging manatees.

After the last feeding by the park, the majority of manatees fed on either leftover provisioned food in region A, or natural vegetation in regions B and C. Region C was relatively shallow with a weak river current and a sandy riverbed rather than the rocky substrate found in region A. Region C also contained a variety of natural food sources such as the roots of aquatic plants or trees, and aquatic grasses. The manatees sometimes fed on terrestrial forage such as Palmyra palm tree leaves (*Borassus*) by extending the upper part of their body out of the water and grabbing leaves using the perioral bristles on the muzzle (personal observation). Supplementing grazing by browsing may increase total protein intake (Hobbs et al. 1981). In addition, the area of C-2 received an effluent from the hippopotamus exhibit, especially after heavy rain. I frequently observed that the manatees fed on the effluent. The effluent was brownish in color and probably contained minerals, B-complex, protein and non protein-N compounds that primarily covered the area of C-2 (Best 1981).

Seasonal activity and spatial use

Numerous studies of ungulates in their natural environments suggest that seasonal availability of energy and nutrients from their diet have great influence on activity patterns (Green and Bear 1990, Moncorps et al. 1997). Bengtson (1983) documented that seasonal changes in nutritional requirement, quality of food, or temperature may influence feeding duration of manatees. My results indicated that the seasonal change of habitat use was mainly derived by change in availability of natural food resources. Proportion of feeding behavior was similar throughout the seasons of this study period. Nonetheless, the source of forage altered from leftover, floating lettuce found in region A to natural vegetation and debris found in region C when these resources became available. Aquatic vegetation started growing in region C and its concentration intensified as the photoperiod length increased. I often observed the manatees feeding on the riverbed where new aquatic grass appeared. As the photoperiod increased, I also noticed that there remained a relatively high concentration of aquatic grass on the other side of the bridge that the manatees could not reach, as it was outside the enclosure. In April, a month after most wild manatees left Homosassa Springs for the coast, I occasionally observed some free-ranging manatees returned to feed on the aquatic vegetation outside the enclosure on the other side of the bridge.

Conclusions

Spatial use and activity pattern of the HSWSP captive manatees were apparently affected by energy constraints and nutrient intake. Behavioral strategies of the manatees were 1) to remain “inactive,” probably to conserve energy, while the park provisioned food, 2) to alter locations along with direction of sun, possibly to assist with thermoregulation, and 3) to change location seasonally where and when food resources became abundant, showing a preference for natural vegetation when available. Specific to these captive manatees, understanding their activity patterns could assist in manatee care. For example, foraging on natural vegetation could be encouraged by both enhancing its growth and reducing the amount and frequency of provisioned food. The middle region (area B) of the exhibit is largely a travel corridor or resting area; yet this is an important feature of the exhibit because it encourages movement between the two feeding areas. On a broader scale, this study demonstrated that for these captive manatees the activity patterns and spatial use were comparable to that of free-ranging manatees in relation to the availability of food resources.

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CHAPTER 3

CAPTIVE FEMALE MANATEE BEHAVIOR AND SOCIAL INTERACTION ASSOCIATED WITH REPRODUCTIVE HORMONES

INTRODUCTION

Although several researchers have observed and described the reproductive behavior of the Florida manatee (*Trichechus manatus latirostris*), the field observations have not been accompanied by relevant endocrinological data. An adult female manatee in a group of adult males is assumed to be in estrus, but the correspondence between behavior and endocrinology has not been verified. Marmontel et al (1992) described vulva swelling in females pursued by several males, suggesting that the swelling is a sign of estrus. Hartman (1979) illustrated copulatory position between a female and a male as follows: a male mounts and embraces the back of a female and then approaches under her abdomen to achieve belly-to-belly contact. If the female is not receptive, she rolls over to expose her back to the male in order to escape. However, relations between behaviors and concentrations of reproductive hormones have not measured for good reason. Although gentle, manatees are large animals (ca. 3 m and 500 kg as average size adults) (Reynolds and Odell 1991) and the aggregation of males around a female often results in quick movements and intensive interactions (Hartman 1979). A researcher could be injured in such a situation. Moreover, their engagements often bring up debris from the river bottom, reducing visibility. Although attempts to collect fecal samples for the examination of endocrine hormones has been conducted in such situations, success is extremely low (Dr. Larkin, personal communication, Horikoshi, personal experience).

To address these problems, Florida manatees housed in captivity are extremely useful subjects for study. By studying captive manatees, only recently has the reproductive physiology of female manatees been elucidated. Larkin (2000) reported that length of the estrous cycle of the Florida manatee is between 28 and 42 days. Furthermore, vulva swellings were observed in association with increased estradiol and/or low progesterone (Larkin 2000).

The current study tracked the behavior and hormone levels of two captive manatees intensively using collection of feces, urine, and blood samples through husbandry training at Homosassa Spring Wildlife State Park (HSWSP). I also observed the other seven captive manatees and made opportunistic fecal collections at HSWSP. I determined behavioral correlates for distinct phases of the estrous cycle in captive manatees.

I selected particular behaviors (blowing bubbles, inverted posture and mounting) performed by the captive manatees as potentially related to hormonal changes based on past observations of captive and wild manatees. A park ranger at HSWSP observed a captive female manatee blowing bubbles when a male manatee approached the females in the exhibit when the park held male manatees more than a decade ago. Inverted posture was observed during my preliminary study at HSWSP in 2002. A manatee approached with the inverted posture to move underside another manatee. Larkin (2000, p. 178) documented mounting behavior among the captive-female manatees, defined as “a situation where one manatee is on her back holding on and being held by another manatee who is on top of the first manatee." Such interactions also have been observed between wild female and male manatees and among male manatees (Hartman 1979, Randall et al. 1999).

Based on these observations, I made three hypothesizes: 1) individual behavior would differ during estrous versus non-estrous periods. Specifically, I predicted that during an estrous cycle, particularly in the pre-ovulatory period, manatees would show blowing bubbles, inverted posture, and behaviors associated with interaction such as mounting and mouthing. These behaviors would be less prevalent during the non-cycle period of the same individuals and in non-cycling manatees. 2) Interactive behavior would differ during estrous versus non-estrous periods. I predicted that manatees would interact more during an estrous cycle. 3) The number of manatees simultaneously cycling would vary over the three seasons (winter, spring, and summer) of the study. I predicted that the number of manatees in estrus would be lowest in the winter and highest in the spring in accordance with observed patterns in wild manatees and a previous study on captive manatees (Harman 1979, Rathbun, et al. 1995, Larkin 2000). 4) A positive association between vulva size and reproductive hormone concentrations (progestins and estradiol). Based on field observations of female manatees in mating herds, I predicted that vulva size would increase during pre-ovulation.

MATERIALS AND METHODS

Study Site and Subjects

The study was conducted at Homosassa Spring Wildlife State Park (HSWSP) in Homosassa Springs, Florida, which is approximately 70 miles north of Tampa. The manatee exhibit is part of the Homosassa River. The boundary between the Homosassa River and the exhibit consists of a number of poles embedded in the riverbed as a fence. This fence prevents the captive manatees from escaping. Above the fence, there is a bridge, which allows visitors across the river. The water level in the exhibit is influenced by not only the tide of the river but also the direction of wind. Wind from the west brings water into the exhibit and keeps the water level high. In 2002, a small manmade pool (ca. 95,000 L), designed for medical examinations, was completed. The medical pool is connected to the northwest side of the enclosure by a narrow passageway with a length of 14.1 m. There are two gates in the passage between the pool and the enclosure. One gate is made of wire fence and is used to isolate a manatee. The other gate is made of a white board that weighs 136 kg and is used to seal the passage completely. The water level in the pool is controlled by closing this gate and draining or adding water. The gate was usually open allowing the manatees to enter the pool freely.

The south side of the enclosure incorporates a spring that lies beneath the Underwater Fishbowl Observatory. The area of spring encompasses approximately 0.2 ha and reaches depths of 13.5 m with an estimated volume of 1.1×10^7 L (Young 2001). The spring provides 1.14×10^5 L of water every hour and maintains a water temperature in the enclosure around 23 degrees Celsius for all seasons (HSWSP record). The spring water flows from the south to the northwest side of the enclosure and goes out though the fence under the bridge and into the open river. The other side from the bridge was shallow with a weak river current and near the effluent of the single male hippopotamus exhibit. This area often received debris from this exhibit, especially after heavy rains. Water temperature of the manatee enclosure was monitored by a total of ten thermometers located throughout the exhibit. Collection of water temperature data from nine locations in the manatee enclosure was performed by the park personnel.

Daily feeding occurred at a designated location, called the Manatee Salad Bar, near the Fishbowl Underwater Observatory in the south side of the enclosure. The manatees were fed the main vegetables such as heads of lettuce, cabbage, kale, and/or green peppers before the park opened at 0800 hour. As part of educational demonstrations for park visitors, additional feeding times were held at hours of 1045, 1300, and 1515. The manatees were fed the main vegetables at the Manatee Salad Bar after each afternoon Manatee Educational Program at 1300 and 1515. The medical pool was used for feeding small portions of vegetables (kale and/or green paper) twice a day after the daily feeding times at hours of 1315 and 1530. This was done to accustom the manatees to enter the pool.

Study Animals

HSWSP retains nine female-manatees in the enclosure (Table 3.1). Seven of the manatees were born in the wild. Three were rescued because of cold stress (Holly, Oakley, and Willoughby), two because of boat injury (Amanda and Electra) and one with an undefined physiological problem (Rosie). Ariel was found with Amanda as a yearling calf. In 1990, Betsy was born to Amanda in captivity (Amanda mated with a male in the facility before the breeding of manatee became prohibited). In 1975, Lorelei was born in captivity at Miami Seaquarium and moved to HSWSP in 1994.

Behavioral Observations

The study was conducted from January 6 to August 10, 2003 (Julian dates 6 – 222). Behavioral data were collected three days a week over 31 weeks.

I constructed an ethogram in order to define mutually exclusive behaviors (Table 3.2). Using scan sampling and instantaneous recording, I noted the location of each manatee in one of the seven delineated areas every 25 minutes (Martin and Bateson 1993). Individual activity at each scan was recorded. Focal animal sampling with continuous recording was conducted for each manatee during the intervening 15 minutes between scans. At each 15-minute focal observation, I obtained frequency and calculated a rate (number of events/15 minutes) of activities and the social interaction of each animal as both the sender and the receiver. Frequency of activities was documented as states and events (Martin and Bateson 1993). States were exclusive, extended behaviors or body postures. Events took place during any state behavior and were relatively instantaneous activities such as discrete body movements. I observed manatee behavior from the walking path around the exhibit. The order in which manatees were observed for the focal samples was determined by random selection without replacement for any given day of observation. During the study period, focal samples were recorded a total of 696 times with an average of 77 focal samples per manatee, and scan samples were recorded a total of 7429 scan samples with an average of 825 scans per manatee from all nine manatees over 77 days. The study was conducted three times a week from 1115 to 1700 or 1350 to 1840 alternatively by day. This complemented the husbandry training, which was conducted either before or after the behavioral observation.

The Manatee Educational Program at HSWSP presented three daily shows at the hours of 1045, 1300, and 1515 at the shallow area by the spring. The manatees were fed in their daily feeding area in the Manatee Salad Bar after the two afternoon manatee programs 1300 and 1515. All nine manatees congregated at the feeding area throughout a show and vantage points for viewing the manatees were crowded with tourists. This prevented reliable behavioral observations. Therefore, I did not obtain any behavioral sample during the afternoon feeding periods and for approximately 45 - 55 minutes after each feeding time. In addition, preliminary study in the summer 2002 showed that manatee activities did not change significantly during early morning period (between the hours of 0800 and 1045) and late morning to early afternoon period (between the hours of 1045 and 1300). The manatees were mostly inactive around the feeding and the manatee program areas. Consequently, I did not observe their behavior during the early morning (between the hours of 0800 and 1045) and used this time for manatee husbandry training and physiological sample collections.

Table 3.1. The nine female-captive manatees at Homosassa Springs Wildlife State Park during the study period from January to August 2003.

Manatee	Age	Captive (years)	Comment
Amanda	37	30	Mother of Ariel & Betsy
Ariel	30	30	Rescued when < 1 year old
Betsy	13	13	Captive born
Electra	10	9	Physical Disability
Holly	9	9	Rescued when < 1 year old
Lorelei	28	28	Captive born
Oakley	9	9	Rescued when < 1 year old
Rosie	42	36	Largest body size (>3000lbs)
Willoughby	9	9	Rescued when 1 year old

Table 3.2. Ethogram used for focal sampling of female captive manatees at Homosassa Springs Wildlife State Park.

Behavior	**Description**
Aggressive movement	Manatee abruptly swims, walks, or stops
Breathe	Manatee inhales and exhales through nostrils
Bubble	Manatee release air from nostrils without surfacing
Contact	Manatee touches another manatee with parts of body; obvious inadvertent contact was not counted (esp. when manatees aggregate at a small space)
Displace	Manatee displaces another manatee from a location without contact
Dive	Manatee swims down into deeper area of the exhibit (underneath of Fishbowl Observatory).
Eat	Manatee consumes lettuce, aquatic vegetation in water or browse at edge of exhibit
Flaring	Manatee opens mouth about half way and oscillates upper perioral lip
Flipper splash	Manatee splashes water with flippers
Float	Manatee rests or asleep in water column (back facing up)
Head splash	Manatee nods head to splash water
Inverted	Manatee remains in an inverted posture in shallow area while touching other manatees, but not resting or asleep
Inverted surface	Manatee surfaces with an inverted posture for a short period of time

Inverted Swimming	Manatee swims in an inverted position
Lap swim	Manatee swim in circle in certain area
Mounting	Abdomen of a manatee touch another/others manatee(s) back side, or abdomen; inadvertent contact was not counted
Mounting received	Manatee receives mounting from another/ other manatee(s)
Mouthing	Two manatees touch each other with their mouths
Nostril movement	Manatee moves muscle around nostrils without opening and closing nostrils.
Open Mouth	Manatee opens mouth in the water
Pushing	Manatee touches another manatee aggressively with body parts noted
Rest	Manatee rests or sleeping at bottom
Rolling over	Manatee rolls over laterally while moving through water
Rub	Manatee rubs its body against a rock or other objects in the exhibit
Receiver	Manatee receives contact as indicated in sender description
Sender	Manatee initiates contact, usually social interaction such as mounting, mouthing, chest to chest contact, chest to genital region contact, mouth to chest or genital region contact
Swim	Manatee moves through water by using tail

Swim Walk	Manatee swims and walks at the same time
Tilt body	Manatee tilts its body side way while inactive in shallow area with back facing up posture
Touch	Manatee touches objects, other than manatees, with her mouth
Turning half	Manatee turns its body half laterally
Walk	Manatee moves along bottom by using flippers to move forward
Other	Behavior not described, if becomes common, create term
Not visible	Cannot see manatees at all
Unknown Manatee	Can see manatees but cannot ID manatee
Zip on behavior	Can see and ID manatee but cannot tell what it is doing

Physiological Sample Collections by Husbandry Training

A complete description of the husbandry training process is provided in the appendix.

Fecal collection

Fresh fecal samples were obtained five days a week beginning on January 14, 2003 from Willoughby and January 21, 2003 from Lorelei. A fecal sample was collected only when it emerged from a manatee in the ventral-up posture. When I saw feces emerging from her anus, I used a plastic bag to collect the sample while giving multiple medium-length whistle blows. In addition to the whistles, food was constantly provided by the assistant as long as the manatee remained relaxed. Once I obtained a sample, the manatee was released from the station. When I did not see feces, I terminated the procedure.

Urine collection

Urine samples were obtained five days a week beginning on January 27, 2003 from Willoughby and February 11, 2003 from Lorelei. A similar procedure was followed for urine collection as used for fecal collection. I attached a floating plate to my arm that contained two distilled collection cups. I kept the floating plate close to my body in the water. I positioned a knee underneath the manatee and lifted the base of the tail to expose the urogenital slit above the water. A collection session lasted for two minutes. I applied light pressure for the first 30 sec, moderate pressure for the next minute and then heavy pressure for the final 30 sec to the animal's bladder area. The procedure was repeated after a short break or diversion if the animal did not provide a sample. The maximum number of repetitions per collection session was six. When the animal provided a sample, I reinforced with multiple short whistles and this was a signal to the assistant to reinforce with the animal's favorite food such as apple and sweet potato. I pressed the sterilized collection cup against the urogenital slit to obtain up to two cups of urine. I continued giving short whiles the animal urinated and maintained the hand position on the animal's abdomen until urination stopped. When the manatee finished providing a sample, she was reinforced with multiple short whistles and food. I also gave the animal reinforcing vocal praise and rubbed her abdomen. If the manatee did not provide a sample in the allotted time, I gave a signal to the assistant to push the animal away from the station without providing either whistles or food.

Urine pH measurement

A small portion of collected urine was used for pH test using a urinalysis reagent strip from Bayer. Approximately 2 ml of urine sample was separated into another urine collection cup and a urinalysis reagent strip was dipped into the urine sample immediately after urine was collected. The strip was kept in the urine for one minute and the color recorded as a converted pH value.

Vulva size and looseness measurement

Vulva size (length, width, and height) measurements were taken from the two manatees beginning on March 14, 2003. I used a caliper to measure length (from the side of the umbilicus to the side of the anus) and width (from the right outer edge to the left outer edge) of the urogenital slit. Two rulers were used for measuring height of the thickest part of the urogenital slit. I placed one of the rulers vertically against the lowest level of the body next to the thickest part of the slit, and then placed the other ruler horizontally on the top of the slit. I recorded the thickness of the slit at the point where the two rulers intersected.

The categorical measure of either loose or tight vulva was determined by the amount of force needed to insert a swab into the vulva slit. I put a small amount of lubrication gel on the vulva slit before a sample swab was inserted. I recorded loose vulva when a sample swab was inserted smoothly without any force and tight vulva when the tip of sample swab was impeded and unable to penetrate unless I applied some amount of force gradually. Originally, this process was performed to acquire vaginal smears; however, I did not obtain enough samples for statistical analysis.

Opportunistic fecal sample collections

Fecal samples from the other six manatees were obtained by simply following an animal in the water and collecting the samples opportunistically. I repeated this process daily in order to collect at least two fecal samples per female per week. I could not obtain feces from Electra because of her physical disability from a motorboat collision and entanglement from a crab trap. She could not float; therefore, she dragged her body on the bottom of shallow areas of the river.

Preservation of physiological samples

Each sample was temporarily stored on ice in the field at maximum of 3 hours. The samples were then transferred into containers and labeled with the name and date. Urine samples from the two trained manatees were collected using an average of two sterilized cups per time. Until a training session was terminated, the urine samples also were stored on ice temporarily. The samples were transferred into glass containers and labeled with name, date, and time of a day. Plasma samples were directly collected into heparin vials and were mixed by inverting them repeatedly. The samples were temporarily stored on ice in the field in an upright position. Each plasma sample solution was centrifuged for 10 minutes. The supernatant was transposed into 1.5 ml vials and labeled with heparin, name of manatee, and date. All those samples were frozen in a -20 °C freezer at HSWSP until they were sent to Dr. Iske Larkin at University of Florida. Analysis of fecal samples was conducted using radioimmunoassay by Dr. Larkin at University of Florida. Urine samples were sent to Dr. Brown at the Smithsonian Conservation & Research Center for analysis by enzyme immunoassay.

Hormone Analysis

Fecal sample assay

Frozen manatee fecal samples were freeze-dried in a lyophilizer, VirTis Freeze Mobile 12XL. From each dried fecal sample, 0.25g was weighed, solubilized with 5 ml citrate buffer at a pH of 3.7 and 5ml 100% ethanol, and then mixed overnight at room temperature rotating the samples end over end. The sample solution was centrifuged for 30 min. The supernatant, 100 µl for the estradiol assay or 300 µl for the progestin assay, was transferred into a test tube. The samples were then double extracted by adding 5 ml of ethyl ether, vortexing for 1 min, letting sit for 2 min, then snapping frozen in a cooling bath with dry ice and methanol, decanting with ethyl ether (plus lipids and steroids in the organic phase) into smaller tubes to dry under air in a warm water bath, and then repeating the process.

The estradiol assay used an E-6-17β antibody from US Biological at a working concentration of 1:30,000 and for the progestine assay a progestine monoclonal antiserum CL425 from the Clinical Endocrinology Laboratory at UC Davis was used at a working concentration of 1:10,000. All samples and standards were run in duplicate. After extraction the dried samples were re-suspended with 100 µl borate buffer (0.5 *M*, pH

8.0). The appropriate antibody (100 µl), BSA/ borate buffer (100 µl; 0.5 *M* borate buffer, 7.5% BSA), and radiolabeled estradiol (100 µl of 15,000 cpm) or progesterone (100 µl of 8,500 cpm) were added and the tubes vortexed and incubated overnight at 4°C. The standard curve consisted of tubes for non-specific binding (NSB), baseline values (Bo), total counts (Tc) and increasing concentrations of the hormone being measured: for estradiol 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800 ng/100 µl; for progesterone 0.009, 0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5.0 ng/µl. Bound-free separation was accomplished by adding 500 µl of 5% charcoal/0.5% dextran and centrifuging the tubes for 30 min at 2000 G. The supernatant (500 µl) was added to 5 ml of scintillation cocktail, and counted on a Beckman LS 5801 scintillation counter. Concentrations were estimated by commercially available software (Microplate Manager). The percentage of maximum binding was calculated using the formula (B0 – NSB / Tc (total count)) x 100).

The passage time of progesterone level between serum, urine and feces are different. The time-line for blood represents that moment at the time of collection, and metabolites in the urine may take a few hours to be processed by the kidneys and excreted; therefore, both serum and urine concentrations represent values from the same day (Larkin 2000). However, fecal wastes take six to seven days to transit through the digestive system. Thus, estradiol and progestin concentration levels from fecal samples are expected to represent serum or urine levels from six days before fecal collection.

Data Analysis

I identified the estrous cycle pattern, estradiol curves and progestin curves of the female manatees at HSWSP based on the criteria defined by Dr. Larkin (2000) as follows: an estrous pattern was defined as a pattern in which increased estradiol values are followed by increased progestin values. The estradiol peaks chosen were described as the highest estradiol concentration at or above an individual's mean. An estradiol curve began and ended with concentrations that fell at or below an individual's mean preceding and following the curve. A curve of progestin (luteal phase) began with at least two consecutive sample values near or above one standard deviation of an animal's mean and ended when two consecutive values fell to at or below the mean. I looked at each behavior over the estrous cycle as well as these two components of the cycles and the non-cycle periods. Only exception was occurred in Amanda that an increased estradiol curve (Julian date 160 – 194) followed by an increased progestin curve (Julian date 185 – 202) in which consists of only one high value, not two consecutive sample values near or above its one standard deviation value. Lack of sample collection within these days may explain why there was only one sample value. Hence, I included the estradiol curve but excluded the progestin curve for the analysis.

I divided my study period into three equal seasonal periods: winter (Julian date 20-87), spring (88-155), and summer (156-222) for analysis of seasonal change in hormone concentrations. I also divided the period of hormone sample collection (from January 20 to August 10) into six equal periods (with 32 - 33 days per period). The mean of the estradiol or progestin values from estrous cycling manatees were calculated to see monthly change of hormone concentrations. Dates of the six periods were: 1 = January 20 – February 22 (20 - 53), 2 = February 23 – March 28 (54 - 87), 3 = March 29 – May 1 (88-121), 4 = May 2 – June 4 (122-155), 5 = June 5 – July 8 (156 - 189), and 6 = July 9 – August 10 (190 - 222). Periods 1 and 2 corresponded with winter; periods 3 and 4 corresponded with spring; and periods 5 and 6 corresponded with summer.

Statistical analyses were performed using the JMP statistical software (version 4) with an alpha level of $p < 0.05$ for the fiducial level of significance. The Shapiro-Wilk W test was used to test for normality of distribution.

I used repeated measures ANOVA to test whether there was concordance of each behavior (blowing bubble, contact, float, inverted, mounting, mounting received, mouthing, rest, roll over, turn half, and swim) reflected by each of the four phases (luteal, estradiol, both, and non-cycle) across all of the cycling manatees. Julian dates of the hormone data from fecal samples were shifted for 6 days earlier to contrast behavioral data. To determine whether individual behavior would differ between cycling and non-cycling periods within cycling manatees, I counted the number of days that individual behavior appeared within each of the four periods (luteal, estrous, both and non) for each manatee. To determine whether interactive behavior would differ between cycling and non-cycling animals, the number of days per phase during each of the three phases (luteal, estrous, and both) was used per manatee. A paired T-test was used for parametric or Wilcoxon Signed Ranks test was used for non-parametric analysis to determine whether interactive behaviors (contact, sender, or receiver) changed during estrous versus non-estrous periods. Contrasting levels of interaction between cycling and non-cycling animals during non-cycling periods was not determined because of small sample size.

Using one way analysis of variance (ANOVA) with the raw data when assumptions were met or with the data transformed to the natural log when assumptions were not met, estrous periods and vulva sizes were contrasted as follows: 1) To determine whether changes in vulva size (length, width, and height separately) differed with each period of the four phases (luteal, estradiol, both and non-cycle). 2) To determine whether changes in vulva size (length, width, and height separately) during periods when I recorded the condition of loose vulva differed by phase. Tukey-Kramer was performed to compare each pair of the data set. 3) To contrast estradiol values at times I recorded loose vulva versus times I did not recorded loose vulva.

RESULTS

Betsy (13 years old), Rosie (42 yrs), and Willoughby (9 yrs) did not show estrous patterns (Figure 3.1), while the other five manatees, Amanda (37 yrs), Arial (30 yrs), Holly (9 yrs), Lorelei (28 yrs), and Oakley (9 yrs) did show estrous patterns (Figures 3.2a and 3.2b). The range of estradiol concentrations between non-cycling and cycling animals was similar (5-120.64 ng/g and 13.12-128.19, respectively), but the range for progestins was greater for the cycling animals (8-61.33 ng/g and 5 to 102.66 ng/g, respectively). Progestin concentrations on the non-cycling manatees did not meet the defined criteria for an estrous cycle. Betsy had fluctuations in estradiol; yet, her progestin concentration did not exceed the mean value plus one standard deviation (Figure 3.1 and Table 3.3). For Rosie and Willoughby, the level of each hormone met its criteria; yet, progestin concentrations did not exceed one standard deviation of the mean for more than two consecutive samples.

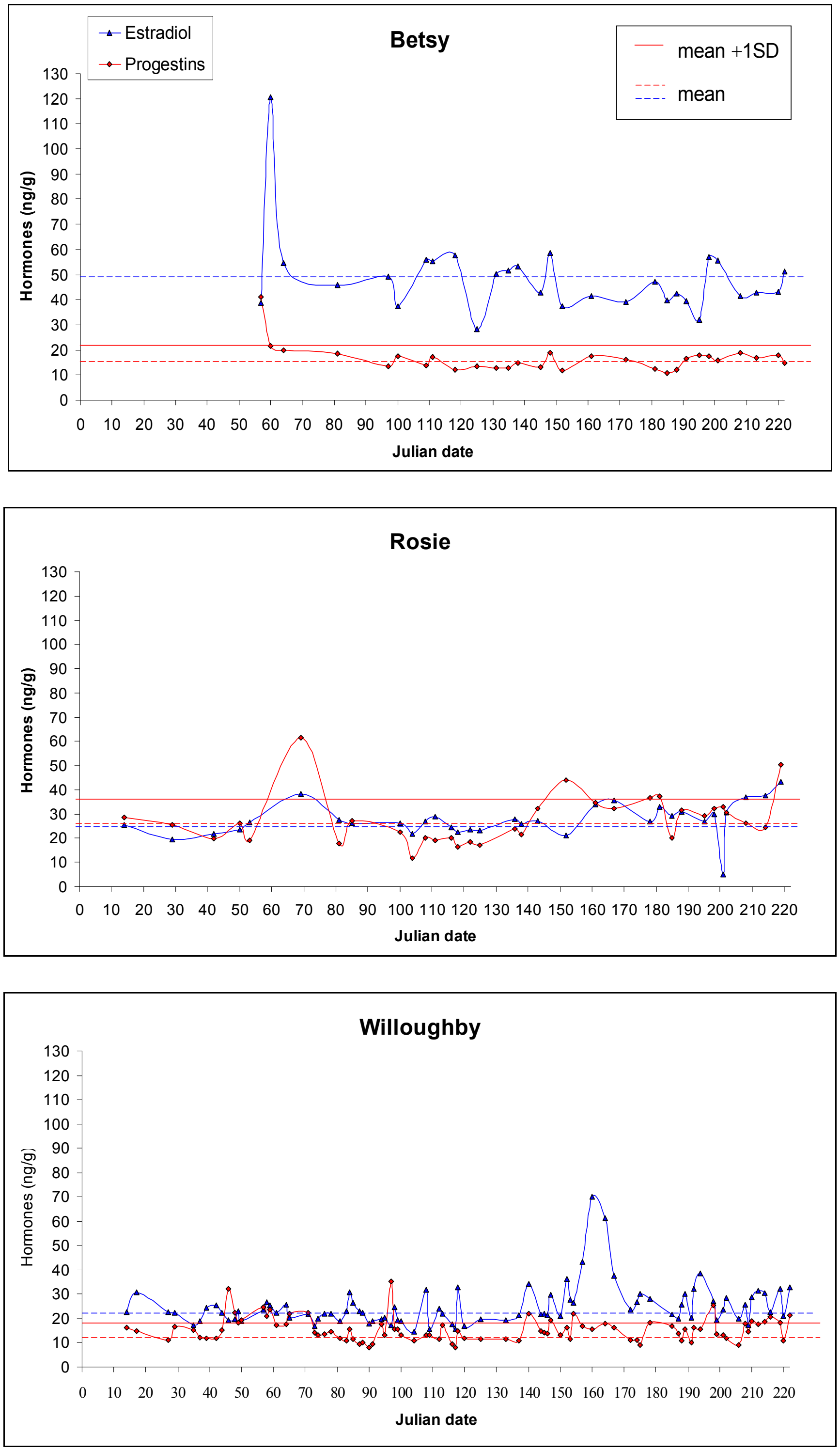


Figure 3.1. Estradiol and progestin fecal hormone concentrations (ng/g) of non-cycling manatees; Betsy, Rosie, and Willoughby at Homosassa Springs Wildlife State Park. A dash line indicates mean value of each hormone concentration while a solid line indicates mean value plus one standard deviation of each hormone concentration.

Table 3.3. Summary of fecal hormone concentrations from January to August 2003 for eight captive female manatees at Homosassa Springs Wildlife State Park.

Estrous Cycle	Manatee	Hormones	Mean + 1 SD (ng/g)	Max	Min
Non Cycling	**Betsy**	**Estradiol**	48.58 + 16.04	120.64	28.15
		Progestins	16.46 + 5.47	40.93	10.84
	Rosie	**Estradiol**	27.49 + 6.74	43.37	5.00
		Progestins	27.63 + 10.30	61.33	11.73
	Willoughby	**Estradiol**	24.98 + 8.47	69.98	14.48
		Progestins	15.41 + 4.88	35.20	8.00
Cycling	**Amanda**	**Estradiol**	28.48 + 3.41	34.72	17.69
		Progestins	36.98+ 14.45	74.93	20.00
	Arial	**Estradiol**	39.15 + 9.85	67.98	21.20
		Progestins	26.08 + 14.30	63.14	5.06
	Holly	**Estradiol**	28.73 + 6.42	42.21	18.83
		Progestins	48.65 + 21.51	103.46	21.06
	Lorelei	**Estradiol**	28.90 + 11.29	128.19	19.90
		Progestins	27.82 + 22.37	102.66	8.80
	Oakley	**Estradiol**	32.17 + 6.41	49.12	13.12
		Progestins	38.46 + 21.63	90.92	12.00

Amanda had one clear luteal phase of 32 days (Julian date from 146 - 178), but she did not have a clear estrous cycle pattern in which an increased estradiol value followed an increased progestin value (Figure 3.2a and Table 3.4). A slightly increased estradiol curve was found for 34 days (Julian date between 160 and 194) within the luteal phase (progestin curve) and this estradiol curve met the criteria. Following this estradiol curve, there was another progestin curve for 17 days, between 185 and 202 (with only one high sample value). Nevertheless, I did not consider this progestin curve as luteal phase for the analysis. Although Amanda did not have a clear estrous cycle pattern, I consider Amanda as a cycling manatee because a progestin curve and an estradiol curve met the criteria individually. Arial showed two estradiol curves and two increased progestins curves followed by each estradiol curve (Figure 3.2a and Table 3.4). These two cycles were 49 days apart. Lorelei also showed a total of four estradiol and progestin curves, however, there was one anestrous period for 61 days between the second and the third cycles. Holly had a total of four estradiol curves and four progestin curves followed by each estradiol curve. Holly had fairly regular cycles throughout the study period (Figure 3.2b and Table 3.4). Oakley had only two clear estradiol curves and three clear progestin curves followed by these two estradiol curves (Figure 3.2b and Table 3.4). Like Lorelei, Oakley also had one anestrous period of 48 days between the first and the second cycles.

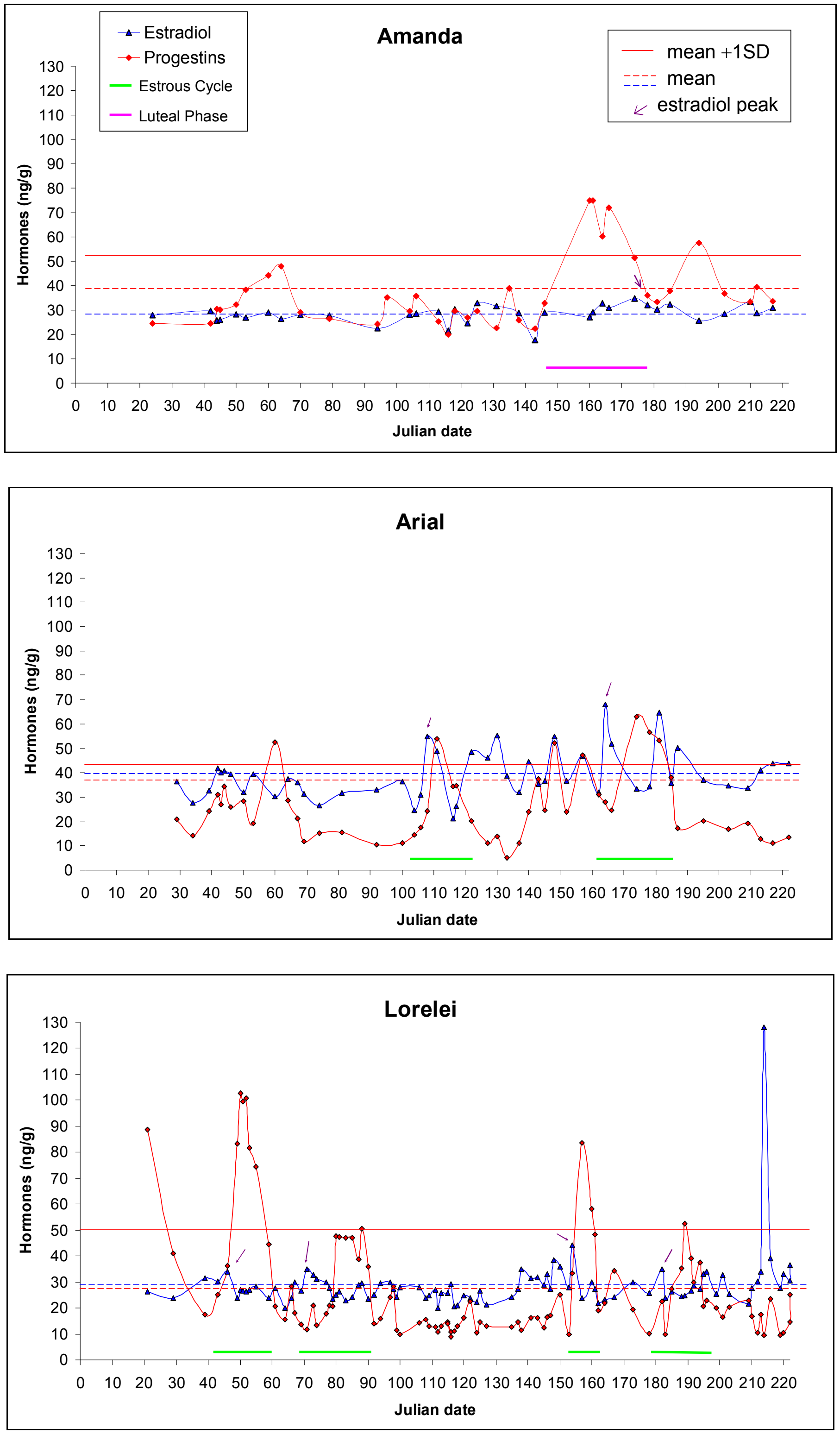
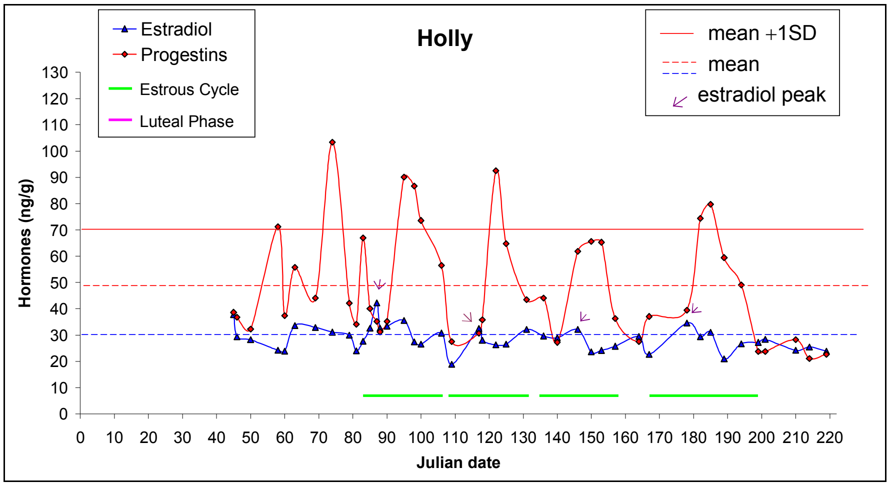


Figure 3.2a. Estradiol and progestin fecal hormone concentrations (ng/g) of cycling manatees; Amanda, Arial and Lorelei at Homosassa Springs Wildlife State Park. A dash line indicates mean value of each hormone concentration while a solid line indicates mean value plus one standard deviation of each hormone concentration.



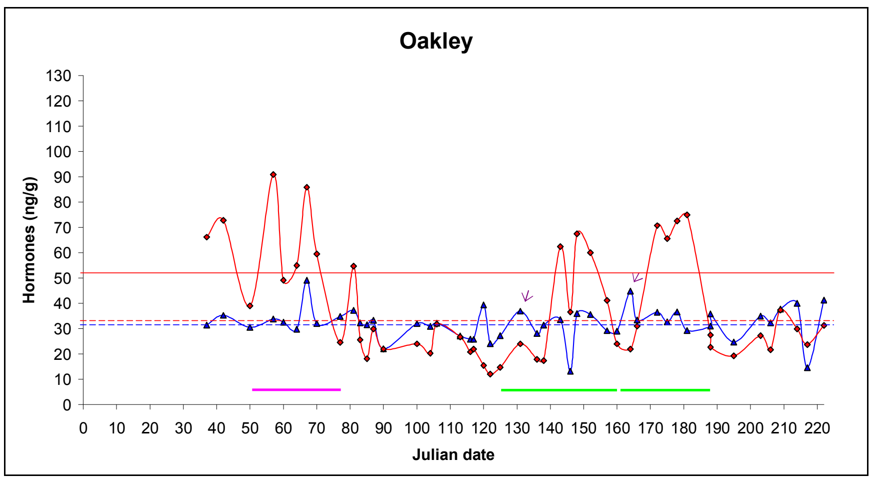


Figure 3.2b. Estradiol and progestin fecal hormone concentrations (ng/g) of cycling manatees; Holly and Oakley at Homosassa Springs Wildlife State Park. A dash line indicates mean value of each hormone concentration while a solid line indicates mean value plus one standard deviation of each hormone concentration.

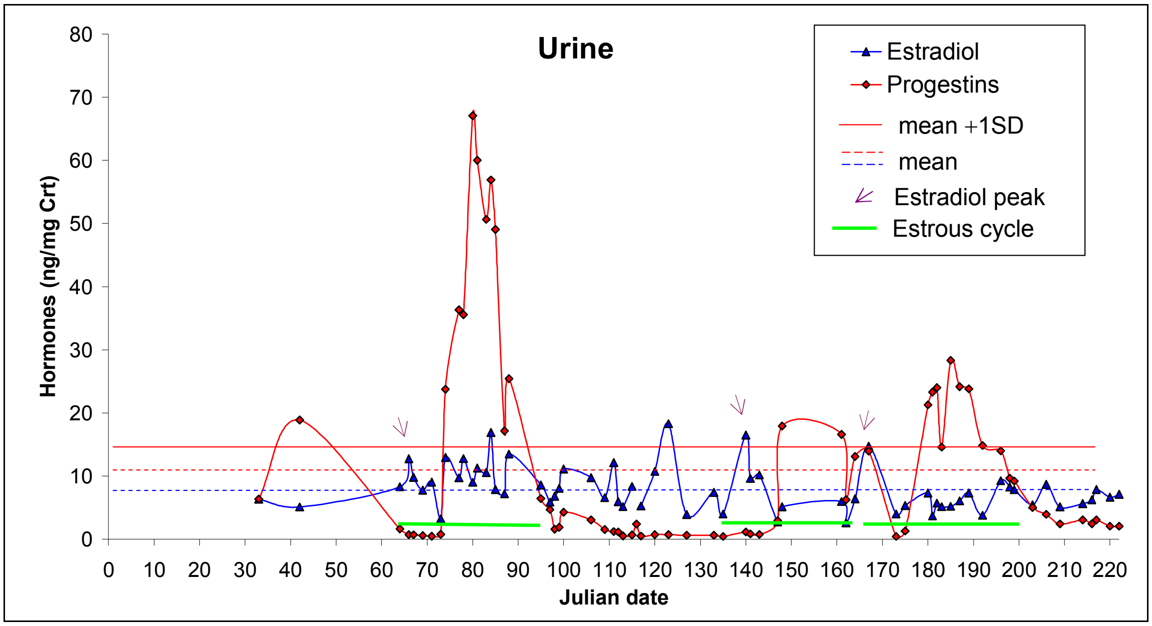
Table 3.4. Summary of fecal estradiol curves and luteal phases (progestin curves) for five female captive manatees at Homosassa Springs Wildlife State Park. The grand mean ± SD was calculated using the four manatees: Arial, Holly, Lorelei, and Oakley.

Manatee	# of est-radiol curve	# of day	Date	Peak date	# of luteal phase	# of day	Date	Estrous cycle length
Amanda	1	34	160-194	-	1	32	146-178	-
Arial	2	10	106-116	108	2	16	106-122	16
		12	162-174	164		21	166-187	25
mean								21
Holly	4	15	83-98	87	4	19	90-109	26
		9	109-118			13	118-131	22
		10	140-150			17	140-157	17
		22	167-189	178		21	178-199	32
mean								24
Lorelei	4	20	29-49	46	4	18	43-61	32
		9	69-78	71		13	79-92	23
		4	153-157	154		9	153-162	9
		5	178-183	182		12	183-195	17
mean								20
Oakley	2	-	-	-	3	27	50-77	
		11	125-136	131		22	138-160	35
		6	160-166	164		22	166-188	28
mean								32
Grand mean								**24 ± 5.4**
Total	13				14			12

The hormone concentration patterns were similar between urinary and fecal progestin from Lorelei (Figure 3.3). Urinary estradiol showed clearer delineation of the cycles by exceeding one standard deviation of the mean value compared to fecal estradiol levels (see exception on Julian date 148 for the fecal estradiol, Figure 3.3).

A total of 14 luteal phases from five manatees were found and 12 phases out of the 14 overlapped throughout the study period (Table 3.4). The grand mean of the estrous cycle from the four manatees: Arial, Holly, Lorelei, and Oakley was found 24 days ± 5. The number of the luteal phase overlapped for each month increased from a minimum in February (2 phases) to a maximum in June (6 phases) (Figure 3.4).

Slightly increased mean estradiol was found from the last half of the study period (May 2 – Aug 10), yet, it did not show seasonal fluctuation (range from 28.50 ng/g to 33.28 ng/g) throughout the study period (Figure 3.5). Mean progestin value showed two peaks at the first and second period (Jan 20 – Mar 28 at 44.10ng/g and 38.84, respectively) and at the fifth period (Jun 5 – July 8 at 42.20 ng/g) and it dropped at the third and fourth period (Mar 29 – Jun 4 at 27.00 ng/g and 28.92 ng/g, respectively).



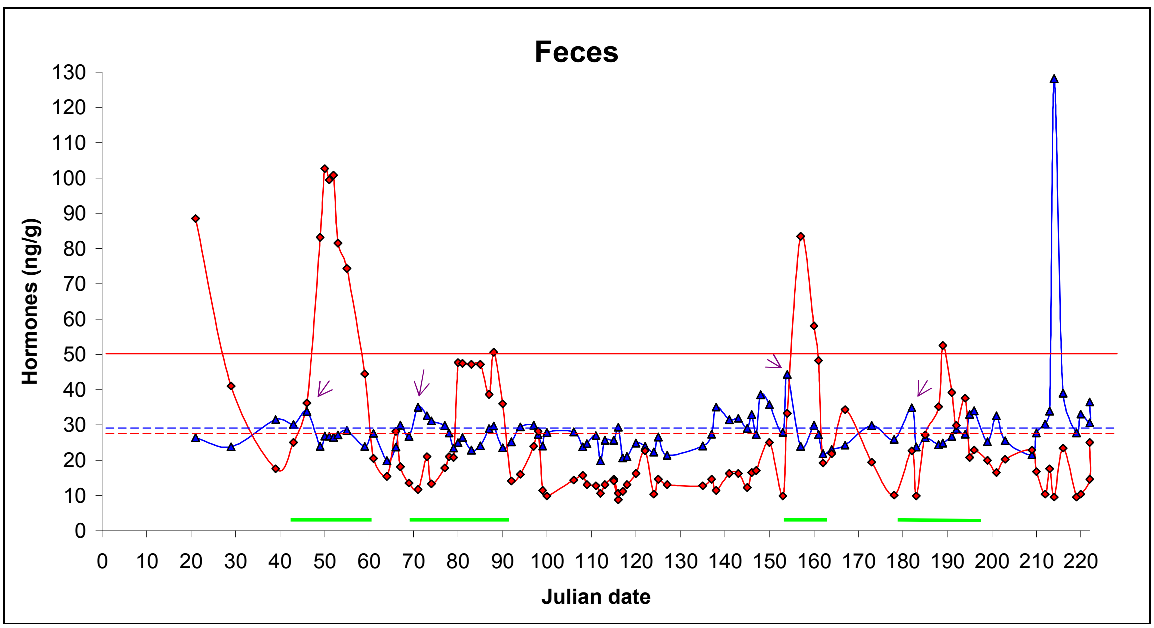


Figure 3.3. Estradiol and progestin concentrations from fecal and urine samples of Lorelei at HSWSP. A dash line indicates mean value of each hormone concentration while a solid line indicates mean value plus one standard deviation of each hormone concentration.

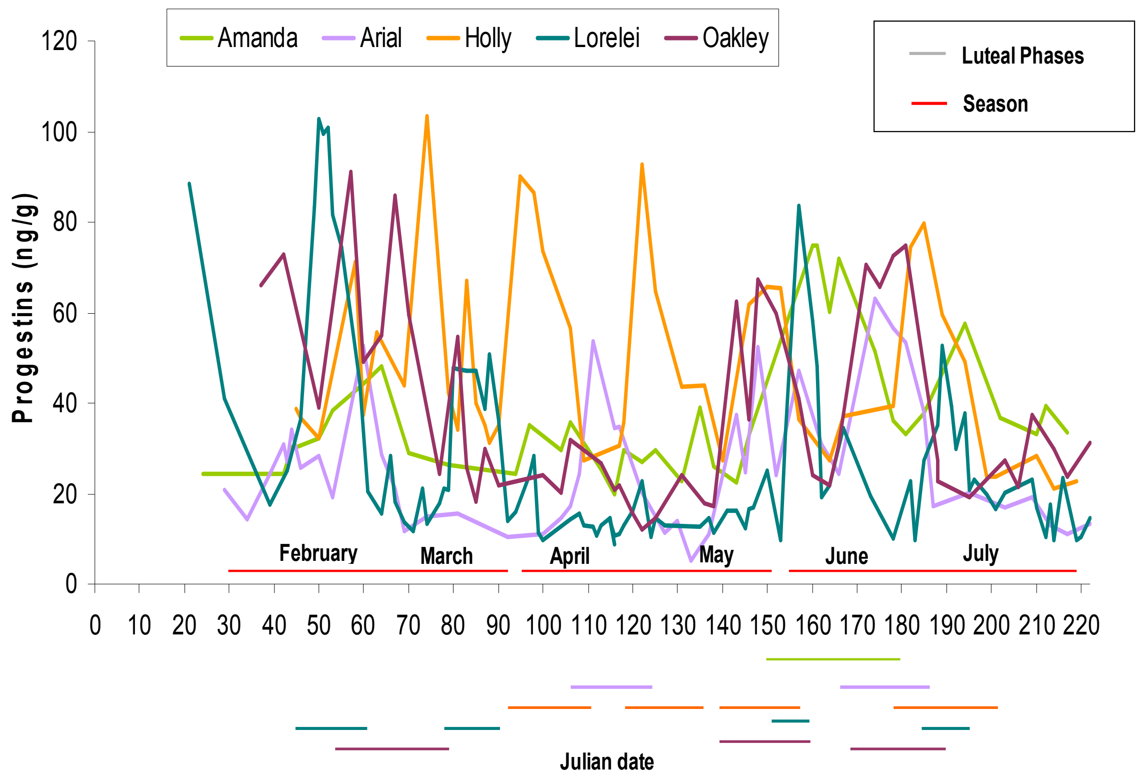


Figure 3.4. Fecal luteal phases of five female captive manatees at Homosassa Springs Wildlife State Park over seven month study period from January to August, 2003.

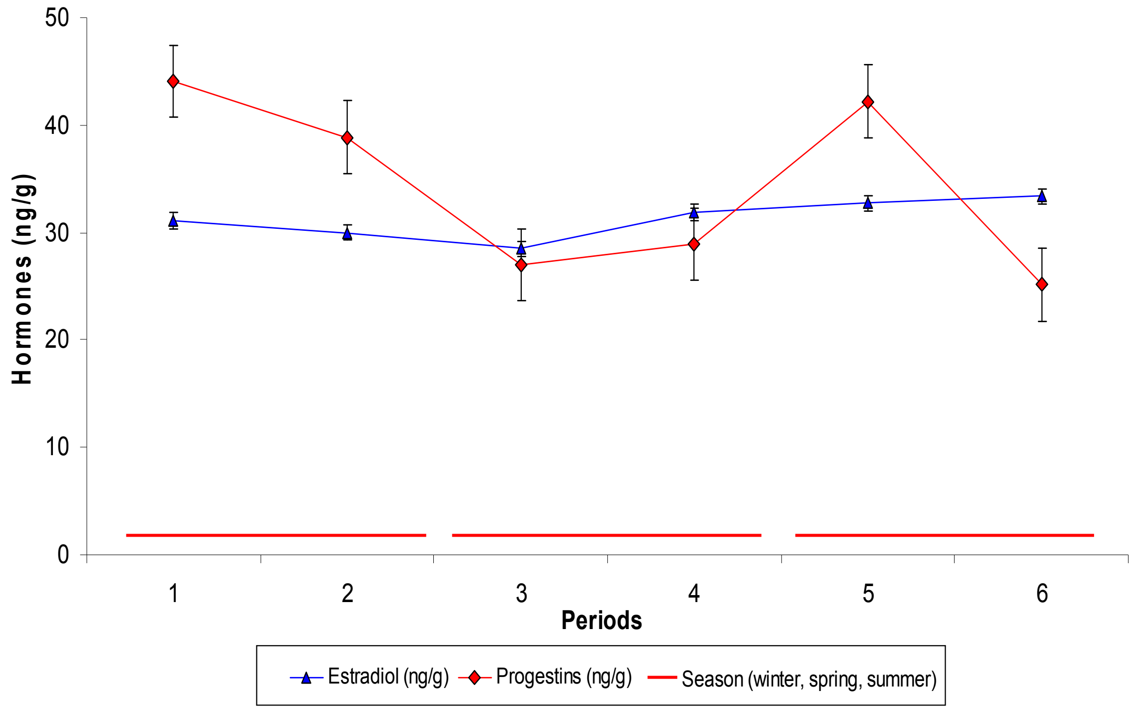


Figure 3.5. Monthly mean values of fecal estradiol and progestin concentrations from five cycling female captive manatees at Homosassa Springs Wildlife State Park. Each period represents 32 - 33 days of the mean value over 6 periods: 1 = January 20 – February 22, 2 = February 23 – March 28, 3 = March 29 – May 1, 4 = May 2 – June 4, 5 = June 5 – July 8, and 6 = July 9 – August 10. Period 1 – 2, 3 – 4 , and 5 – 6 corresponds winter, spring, summer respectively.

No relationship was evident between vulva size and the estrous periods in Lorelei. However, there was a period of 18 days where she exhibited a loose vulva and 8 days out of the 18 were within her estrous cycles (44%). Estradiol values were significantly different between the days I recorded loose vulva (mean of 39.13 (ng/g) ± 27.23 SD) and the days I did not record loose vulva (mean of 23.26 (ng/g) ± 4.14) ($F_{1,92}$ = 17.67, P = <0.0001). Estradiol values were especially high when loose vulva was recorded during the luteal phase (mean of 67.10 (ng/g) ± 63.04) ($F_{3,9}$ = 3.9218, P = 0.048). Moreover, for the days with loose vulva, I found the width of the vulva was positively affected by phase of the estrous cycle ($F_{3,14} = 10.78$, $P = 0.0006$). Urine pH values ranged from pH 7.5 to 8.3 (7.8 ± 0.24 SD). The pH fluctuation did not change with the estrous patterns ($F_{3,24} = 0.89$, $P = 0.46$). Vulva size and urine pH of Willoughby were not contrasted with her hormone concentrations because of her lack of estrous cycle patterns.

Water temperature of the manatee enclosure showed little variation throughout the study period (range from 21.95° Celsius in the lowest mean minimum to 24.36° Celsius in the highest mean maximum) (Figure 3.6).

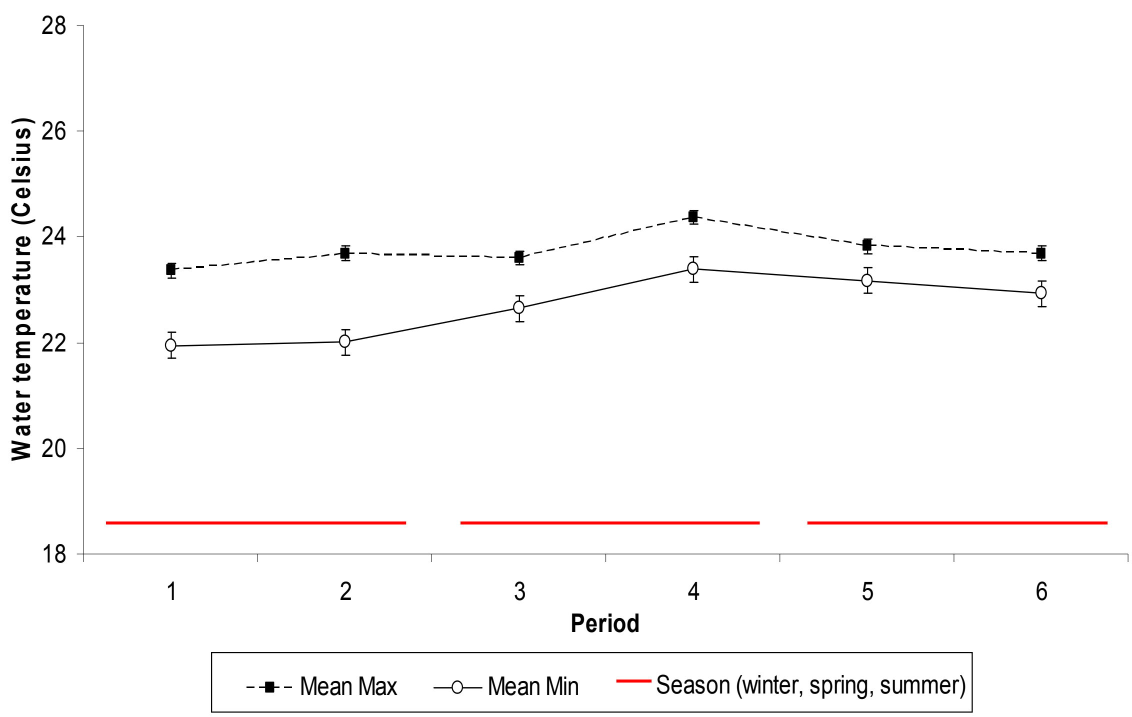


Figure 3.6. Mean maximum and minimum water temperature per collection period from a total of 10 locations of the manatee exhibit. Period 1 = January 4 – February 16, 2 = February 16 – March 9, 3 = March 10 – April 30, 4 = May 1 – May 26, 5 = May 27 – June 29, and 6 = June 30 – August 17. Length of each period was set up by the park.

Hormone and Behavior

Among the cycling manatees, some individual behaviors were associated with particular phases of estrous cycle or the non-cycling periods. During the luteal phases, blowing bubbles (data from focal observations) was most prevalent ($F_{3,12}$ = 5.1746, P = 0.0159) (Table 3.5). Manatees inverted (data from focal observations) on the greatest relative number of days during estradiol phase (Friedman's test X^2 = 8.91, df = 3, $P <$ 0.05) (Table 3.5). During non-cycling periods, the relative day-count (days a behavior occurred in a phase/phase length) for the following behaviors was greater: float (data from focal observations) (Friedman's test X^2= 11.22, df =3, $P < 0.025$) and scans (Friedman's test X^2 = 13.08, df= 3, $P < 0.005$), rest (data from focal observations) (Friedman's test X^2 = 12.42, df = 3, $P < 0.01$) and scans (Friedman's test X^2 = 13.5, df = 3, $P < 0.005$), and swim (data from focal observations) ($F_{3,12}$ = 6.05, P = 0.0095).

Among the cycling manatees, the relative day-count (days behavior occurred / phase length) for both the sender (t = 3.67, df = 4, P = 0.021) and the receiver (t = - 3.49, df = 4, P = 0.025) was significantly greater during their cycling periods. On the other hand, among non-cycling manatees, the relative day-count for the sender (t = - 4.96, df = 3, P = 0.0157) and the receiver (t = -10.74, df = 3, P = 0.0017) was also greater during the cycling period of the cycling manatees.

Table 3.5. Relative day-count (days behavior exhibited in a phase/phase length in days) for blowing bubbles and inverted posture per phase by five cycling manatees over the study period (January 6 to August 10, 2003) at HSWSP.

Phase	Blowing Bubbles	Inverted posture
Estradiol	1.25	1.05
Luteal	1.70	0.38
Estradiol and Luteal	0.94	0.20
Non	0.72	0.38

DISCUSSION

Two behaviors blowing bubbles and inverted posture, appeared to be associated with reproductive hormone concentrations. My results showed an increased number of blowing bubble days during the luteal phase but not during pre-ovulatory period among the estrus cycling manatees. Thus, blowing bubbles cannot be considered as an estrous behavior. Inverted posture displays the genital region of a manatee and it was positively associated with during pre-ovulatory period among the cycling manatees. This behavior seems comparable to mating behavior in manatees and sexual exhibitions such as lordosis behaviors (arching the back and raising the hindquarters) in a number of female terrestrial mammals (Morali and Beyer 1979). Estradiol may be an activator of this behavior (1979). The other behaviors such as swim, inactive and float were performed at a significantly greater relative frequency on a day-count basis during the non-cycling period. The greater relative occurrence of these behaviors may be explained by the absence of behaviors that were more frequent during estrous cycle phases. I saw no pattern between estradiol or progestins levels and the mounting or mouthing behaviors. A larger sample size may be required to elucidate such a relationship if one exists.

The results of social interaction (sender and receiver) clearly supported my hypotheses in that the manatees displayed interaction on a greater number of days during an estrous cycle. The exclusive increase in day-count for contact behavior among the estrous cycling manatees suggested that these manatees interacted with each other more frequently than they interacted with non-cycling manatees or among non-cycling manatees. Moreover, interactive behaviors by the cycling manatees influence the behavior of the non-cycling manatees during the estrous cycling periods. Larkin (2000) reported a positive association between social interactions and an increased estradiol and/or decreased progesterone level among the HSWSP manatees during her study in 1996.

Non-reproductive social interaction is commonly observed in many other marine mammals and it consists of either inter- or intra-sexual contacts (Wells et al. 1999). Hartman (1979) observed male to male interaction at Crystal River in that “the two males tumbled to the bottom where they remained tightly clasped, thrusting, and wallowing” (p. 108). Remarkable interactions among the HSWSP females were similar to his description of the male-to-male interaction. This interaction usually occurred between Holly and Oakley (cycling), as well as Willoughby (non-cycling) and Electra (reproductive status not available). Holly often led a group interaction. On some occasions, such involvement was quite intense. I observed a delayed response time of about 3-5 minutes for a Manatee Educational Program (March 14 and May 8) or they did not go to the program at all (one time on March 14) and continuously embraced or chased each other. In addition to this intensive interaction, the HSWSP females occasionally embraced chest to chest or chest to genital region and lay on the riverbed until they were interrupted by other manatees (Figure 3.8). I once recorded more than 10 minutes of such an engagement until they were interrupted. Through my observations, Holly and Oakley were usually on top of Electra and Willoughby while these two manatees lay inverted underneath of Holly and Oakley (Figure 3.9). This involvement seems similar to what Hartman (1979) described as belly-to-belly embraces or "play" among young males and cows through his field observation. My results showed that manatees who were cycling may have influenced the interactive behavior of the non-cycling manatees. However, these four manatees may have two separate purposes for this interaction: one for intra-sexual contacts and the other for play. According to Hartman (1979), manatee "play" probably allows individuals to form essential social associations and occurred when animals were replete, rested and absence of environmental restraint. Therefore, the intensive interaction among the HSWSP captive manatees perhaps galvanizes the social bond and also acts to comfort of the unique captive living conditions at HSWSP.

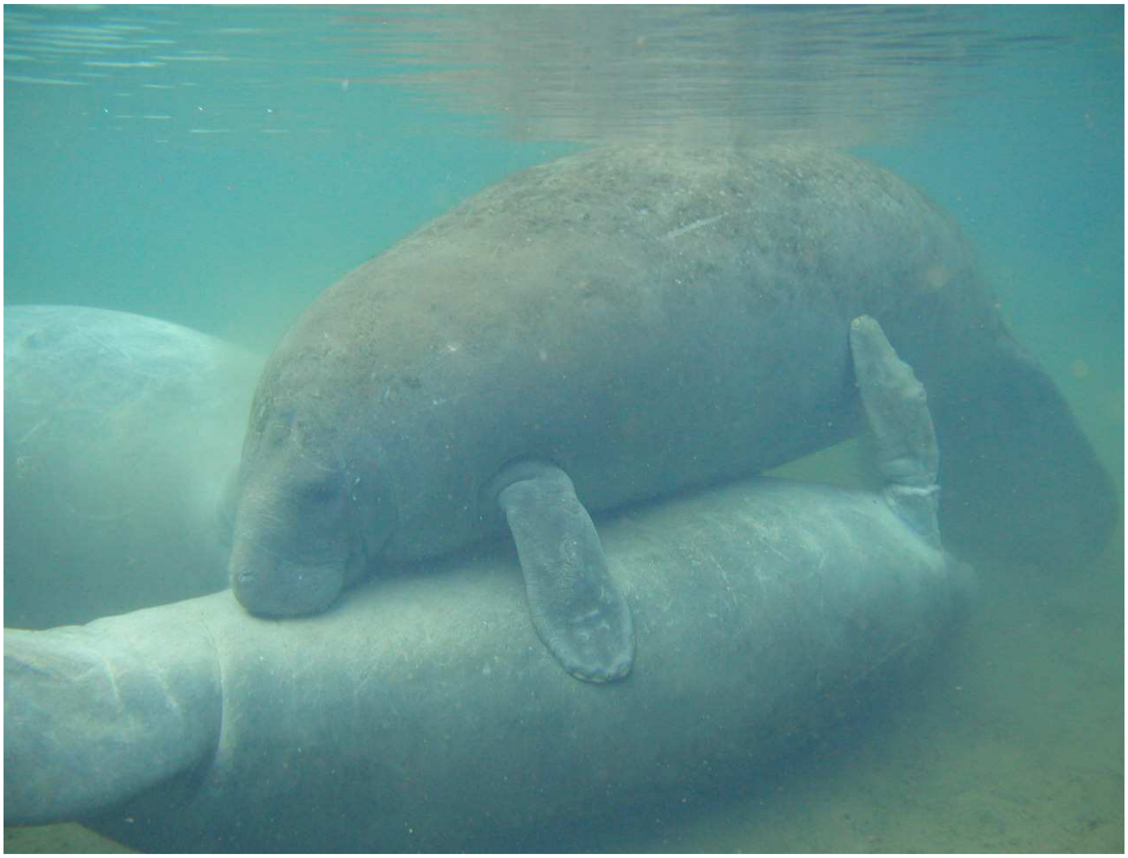


Figure 3.8. An engagement of embrace among younger females at Homosassa Springs Wildlife State Park. Holly on top, Electra (front) and Willoughby (back) inverted.

Figure 3.9. An engagement of embrace among younger females at Homosassa Springs Wildlife State Park. Holly and Oakley on top, and Electra (front) inverted.

Breeding season of the Florida manatee has been suggested to be year-round since manatees are polyovular (Marsh et al 1984, Marmontel 1988). However, field observations indicate spring (April – May) as representing the peak period from breeding (Rathbun et al. 1995). Larkin (2000) documented seasonality of reproductive hormone concentrations as high in either spring or fall based on 12 female manatees at HSWSP and Sea World in 1996. However, my results from HSWSP captive females in 2003 recorded low mean progestin during the spring but a high mean progestin during winter and summer. During the summer, the highest number of luteal phases was recorded from the four manatees twice (June 2-6 and July 3-6). This indicated that June to July represents the peak of reproductive seasonality over my study period. Because my study ended on August 10, this study did not contain the reproductive activities in fall season. Variations of reproductive seasonality peak at spring and fall in the previous study and the summer peak in this study may be reflected by weak reproductive seasonality of Florida manatees.

Generally, the constraint of mammalian reproductive activities during the cold seasons is explained as a means to conserve energy. The metabolic rate of manatees is 50% lower than other studied marine mammals (Irving 1973, Irvine 1983) and 15 – 22% lower than that of estimated similar body size of a terrestrial mammalian species (Reynolds and Odell 1991). Therefore, until water temperatures reach about 20°C, free-ranging manatees aggregate at warm water springs or man-made locations, where they can obtain effluents of warm water from power plants and industrial units (Irvine, 1983). As a result, manatee's energy intake is restricted during the cold season from a limitation of feeding sites. Captivity, however, usually imposes a rigid diet in which animals can predict a certain level of nutrient quantity thus having a lack of seasonal variation (Fernandes 1996). Such environmental stimuli can indirectly have an effect on an animal's physiological condition such as on reproduction (Best 1981). In addition to their daily diet, the unique natural environment at HSWSP can provide some seasonal variation of natural vegetation. My research results from spatial occupancy and activity of the HSWSP manatees (see chapter 2) showed an increased usage of natural vegetation from spring towards the summer season. This trend may correspond to the increased tendency to cycle among the HSWSP manatees during the summer. On the other hand, changes in water temperature in the manatee exhibit would not be likely to influence hormone concentrations because of the low variability over the seasons. Although frequent (ca. hourly) and absolute water temperature in the manatee exhibit were not available to contrast their hormone concentrations, the mean minimum water temperature ranged from approximately 22°C to 24°C, which was above the minimum suitable water temperature (20°C) for manatees (Irvine 1983). Therefore, fluctuation of seasonal mean hormone values was not likely to relate to changes in water temperature.

Lorelei's vulva size was not related to her estrous cycle phase. However, loose vulva was associated with high estradiol values. The measurement of vulva width may be affected by looseness of the vulva more than a change in vulva width itself. Larkin (2000) documented a positive relation of vulva swelling with increased estradiol concentration from captive female manatees. She measured vulva size using 3 ranks: 0 = flat or indented, 1 = slight to total swelling of the vulva area, 2 = swelling includes surrounding area. Bearing in mind that by measuring vulva size alone using a caliper, my samples excluded any indication of swelling of the surrounding area. I did, however, notice on occasion swelling in the vicinity of her vulva. Thus, for the future it may be pertinent to use both a swelling ranking system and measure the actual vulva size for determination of vulva change relative to hormone levels. Lorelei was relatively calm and quite acceptable to the swab insertion when the loose vulva was recorded. On the other hand, she was not calm and quite sensitive for a swab insertion when the tight vulva was recorded. She moved her body quickly by contracting her stomach to avoid me to insert a swab deeper.

There was no association between urine pH and hormone fluctuations from Lorelei. In female Asian elephants, a pre-ovulatory urinary pheromone ((Z)-7-dodecenyl acetate) is used as a signal of impending ovulation to males (Rasmussen 2001). Changing pH serves to optimize the pheromone synthesis, observed from the stage of pre-ovulatory (pH 8.34) to the luteal phase (pH 7.67). Thus, the absence of urinary pH change in Lorelei suggests that manatees may not use a urinary pheromone for reproductive communication, but many more animals and samples are needed before this conclusion can be drawn.

The nine female captive manatees at HSWSP consist of a younger group, Betsy, Electra, Holly, Oakley, and Willoughby, and an elder group, Amanda, Arial, Lorelei, and Rosie. The manatees in the younger group shared some aspects in that they have similar body sizes and close ages, were brought into captivity at an age of one except for Betsy (captive born), and occasionally interacted intensively with each other. On the other hand, the manatees in the elderly group also shared several aspects, for instance, larger body size, longer length of captivity, performance of some particular behaviors such as rolling over, and the experience of reproduction in the past (Larkin 2000).

Despite the similar characteristics in each group, these aspects did not have any association with their hormone fluctuations. Holly, Oakley, and Willoughby are roughly the same age and they were all brought into the facility at about the same time. With this in mind, it is notable that Willoughby had very small estradiol and progestin fluctuations while Holly and Oakley had sufficient fluctuations to classify as cycling. Rosie, the oldest manatee at HSWSP, did not show any cycle and overall her hormone concentration was low while Lorelei had four cycles during the study period. In contrast to my findings, Larkin (2000) observed that Amanda and Rosie had regular estrous cycles throughout her study period and Lorelei did not have any fluctuation of estradiol and had a low progesterone level in her 12 month study in 1996.

Because the environmental conditions in the wild and with captive manatees are dissimilar, information of reproductive condition from captive manatees may not quite elucidate reproductive status of free-ranging manatees for several reasons. A number of external features, including, environmental stressors, social influences, nutrition level, and temperature, have an effect on the estrous phase of animals (Estep and Dewsbury 1996). Thus, the irregularity of the reproductive cycle among the HSWSP female manatees is possibly particular to their captive status. For instance, environmental conditions in captivity allow for the presence of humans and/or heterospecifics in or near the animal's enclosure. Such situations can inhibit or interfere with normal reproductive behavior, perhaps as a result of stress on reproductive physiology (Estep and Dewsbury 1996). Furthermore, a variety of social cues such as specific behavioral, neural mechanism, chemosignals, or physical contact play an important role for the regulation of reproductive function in mammals (Bronson 1987, McClintock 1987). For that reason, the major cause of reproductive irregularly or nonappearance of estrous cycle among HSWSP manatees could be an absence of a male in their habitat. For instance, sexual stimulus such as copulation and pheromone by conspecific males induces ovulation in female Asian musk shrew (*Suncus murinus*) (Dryden and Anderson 1977) or modulate reproductive physiology and behavior of the female in other vertebrate species (Crews and Moore 1986). Generally, free-ranging manatees are exposed to the opposite sex and once they reach sexual maturity at approximately age 3 (Marmontel, et al. 1992, Marmontel 1995, Rathbun et al 1995, O'Shea and Hartley 1995), reproduction cessation among them is rare (Rathbun et al. 1995).

The absence of an estrous pattern in Betsy and Willoughby may be influenced by the presence of adult cycling females or absence of an adult male in the enclosure. Social stimuli can trigger reproductive suppression among females (Wasser and Barash 1983, Abbott 1987) or delay or accelerate puberty (Drickarmer 1974). For example, female tamarins remaining in their natal groups exhibited low urinary estrogen levels and are unlikely to have regular cyclic patterns as well as sexual behavior, however, increased estrogen concentrations were observed by pairing an adult male or the removal of natal family, especially in adult cycling females (Epple and Katz 1984, French et al. 1984). An absence of an estrous cycle in Rosie may be explained by reproductive senescence when considering the age of Rosie (42 yrs). Although reproductive senescence is rare in mammals and has not been reported in West Indian manatees, it is perhaps because none of the female captive manatees have reached senescent age yet. In fact, occurrence of reproductive senescence was suggested in dugongs (Marsh et al. 1984). Marmontel et al. (1992) mentioned no appearance of reproductive senescence for the two manatees at Miami Seaquarium; however, these manatees were aged at 34 and 35 at the time.

Contrasting urine and fecal samples from Lorelei, the urinary estradiol revealed a clearer variation than the fecal estradiol; hence, the values of urinary estradiol verified each estradiol peak from the fecal samples. The length of estrous cycle in Lorelei extended between Julian date 147 and 156 to 135 and 162 in fecal samples to urine samples respectively. However, fecal progestins were more reliable than urine progestin; this was due to frequent fecal sample collection. The plasma progestin indicated a positive correlation with urinary progestin. For the future research, behavior together with frequent fecal, urine and blood serum sample collection using husbandry training methods helps in the verification of estrous cycle and its relation with behavior.

Conclusion

Blowing bubbles and inverted posture were associated with estrous cycles. Inverted posture was related to the pre-ovulatory period. The interactive behaviors showed significant variation between the periods of the estrous versus non-estrous among the cycling manatees. In addition, these cycling manatees were likely to influence interaction level of the non-cycling manatees.

The number of manatees in an estrous cycle simultaneously varied seasonally, yet, the summer (esp. June – July) was the season during my study period with the greatest number of simultaneous (but not synchronous) cycles. A positive relationship between loose vulva and increased estradiol concentration was found in the trained manatee, Lorelei. The size of vulva width was also positively associated during days when loose vulva was recorded. For the future, measuring both the actual vulva size and vulva swelling using ranking system is suggested to determine vulva change associated with hormone levels.

State of reproductive hormone among the HSWSP captive manatees was not generally influenced by age, body size, length of captivity, or previous reproductive status. Mean length of an estrous cycle among the cycling manatees was found 24

(± 5.4) days. This was a similar estrous length (28 – 42 days) from Larkin (2000) defined in her research in 1996. Environmental influences such as lack of social cues may effect the irregularity or absence of estrous cycles among these manatees.

Further research on reproductive status and behavior of captive manatees will not only improve proper captive management of manatees, but also assist our understanding of the mating system in free ranging manatees, especially timing of estrus in a focal female through its behavior. This study provides verification in length of estrous cycle among captive manatees, supports weak reproductive seasonality, and shows some positive relationships between estrous cycle phases and individual or interactive behaviors by a group of captive female Florida manatees.

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CHAPTER 4

SUMMARY

The external (environmental conditions) and internal (physiological conditions) factors of the HSWSP female captive manatees were studied. Spatial use and the activity pattern of the manatees were evidently derived by energy expense and nutrient intake rather than an internal factor, specifically reproductive hormone concentrations. Five of the eight manatees examined for estrous cycles exhibited some cycling. Four of these manatees experienced two or more cycles with a mean duration (24 ± 5.4) similar to that of the only other study to measure reproductive hormones (28-42 days, Larkin 2000). Two individual behaviors, blowing bubbles and inverted posture, were found to vary with hormonal changes. The interactive behavior among the cycling manatees was positively related with their estrous cycles. Simultaneous cycling of different manatees peaked during the months of June and July. This pattern reflected the availability of natural vegetation from the spring to summer seasons but whether this was a causative factor is inconclusive from this study.

Conservation Implications

All sirenian species are listed as vulnerable to extinct and the Florida manatee is listed as an endangered species by the IUCN (IUCN 1990). Adult Florida manatees do not have many natural predators, but human impacts such as watercraft activity, entanglement by nets and lines, ingestion of fishing gear or debris, flood-control gates and navigation-canal locks, and destruction of habitat and food resources are responsible for the high mortality rate of the Florida manatee (O'shea et al. 1985, Laist 1987, Beck and Barros 1991, Langtimm et al. 1998). The major cause of death of the Florida manatees is watercraft collision (Ackerman and Wright. 1995, Marsh and Lefebvre 1994, Wright et al. 1995). Furthermore, the greatest proportion of all deaths from identifiable causes was human-related (Ackerman and Wright. 1995). As a result of this, their population dynamics are expected to be disturbed (O'Shea and Ackerman 1995). Because of the relatively high incidence of human related fatalities, the number of Florida manatees could drop precipitously without protection by Federal legislation. These acts include the U. S. Marine Mammal Protection Act of 1972, the Endangered Species Act of 1973, the Florida Endangered and Threatened Species Act of 1977, and the Florida Manatee Sanctuary Act of 1978 (Marmontel et al. 1997, Marsh and Lefebvre 1994). Current estimation of the population number by synoptic aerial surveys is approximately 2861 to 3113 (FMRI 2003). This count indicates that the Florida manatee's population increased over 50% in the past decade although the possibility also exists that the means of counting manatees also has improved.

Nevertheless, manatee fatalities by watercraft increased from 38 in 1992 to 95 in 2002 (FMRI 2003). According to carcass-recovery data, there was a significant correlation between increasing boat registrations and increasing boat-related death of manatees (Ackerman and Wright 1995). Marmontel et al (1997, p. 475) reported, "If [Florida] manatee mortality increases by 10%, the population should trend toward extinction." Under such condition, captive manatee breeding will be required. Thus, continuous research and understanding on the basis of estrous behavior and verification of individual estrous cycle and its seasonality for captive manatees are essential for future management of the Florida manatee and could potentially improve wild population numbers.

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APPENDICES

Appendix A: Husbandry Training

In December 2002, I visited Living Seas in Epcot, Orlando to observe an on-going manatee-husbandry training project. I learned the basic husbandry training protocols such as the fundamental approaches and the rate of trained behavior acquisition. During February through June 2003, I observed manatee husbandry trainings at Mote Marine Laboratory in Sarasota and Sea World in Orlando to expand my repertoire of training techniques. Published husbandry training papers by Colbert and Bauer (1999) and Colbert et al. (2001) also were used as references.

From January 4 to August 10, 2003, I conducted husbandry training with two female manatees five days a week at Homosassa Springs Wildlife State Park (HSWSP). Based on information from the preliminary study in the summer 2002, I selected Lorelei because she was likely to stay in the medical pool longer and more often than the other manatees so that it was easier to isolate her in the medical pool. Willoughby was chosen because she was the most sociable among these nine manatees and I often observed her remaining in a ventral-up posture, which is a necessary posture for fecal and urine sample collection.

Training was conducted using positive reinforcement. Before each training session, apples, cantaloupes, carrots, sweet potatoes, and watermelon were cut into pieces to use as reinforcement. Accompanied with a piece of food, a whistle was blown each time the animal completed a trained behavior such as targeting, stationing, or remaining ventral-up posture. A handful of apples or sweet potatoes were used as a reward for completing each new behavior. Each manatee was trained in separate sessions by isolating a manatee in the medical pool. The medical pool at HSWSP was connected to the northwest side of the manatee enclosure. There were two gates in the passage between the pool and the enclosure. One gate was made of wire fence, thus, it was used for manatee isolation. The other gate was made of a white board that weighted 136 kg and used to seal the passage completely. The water level in the pool was controlled by closing this gate and draining or adding water. I and any assistants accessed the medical pool by a set of steps, which were designated as the stationing area for the manatee husbandry training. The end signal for each training session was a sound created by knocking an empty metal container in the water. Once a week, the training was recorded on videotape from which data were collected to assess progress.

Appendix B: Targeting and Stationing

Two objects were used as targets in order to have the manatees station and follow the targets to designated area of the medical pool. First, I held a piece of food (fruit or vegetable) with my thumb and opened my hand in front of the manatee's face. When the manatee touched her lips to my hand, I immediately whistled once and gave the piece of food from my hand as positive reinforcement. I repeated this procedure while changing the location of my hand side to side until the manatee followed my hand 100% of the time. Snapping fingers for three times in the water was used as signal of an individual target for Lorelei. When she located my snapping fingers in the water, I opened my hand as a target object for her. A yellow large sponge was used as the individual target for Willoughby. When Willoughby touched her lips to the yellow sponge, I whistled once and gave a piece of food from my other hand. I repeated these procedures while changing the location of the individual target from side to side until each manatee consistently accomplished the behavior at a 90% success rate, which occurred quite rapidly (Figure 1). This procedure was carried out only in the medical pool without separating the trainee manatee from the other manatees in the exhibit. When other manatees interrupted the targeting and stationing training, I terminated the procedure.

Responses from each manatee reached 100% by the third trial. This prompt success was probably because the manatees were accustomed to being fed by hand by park personnel.

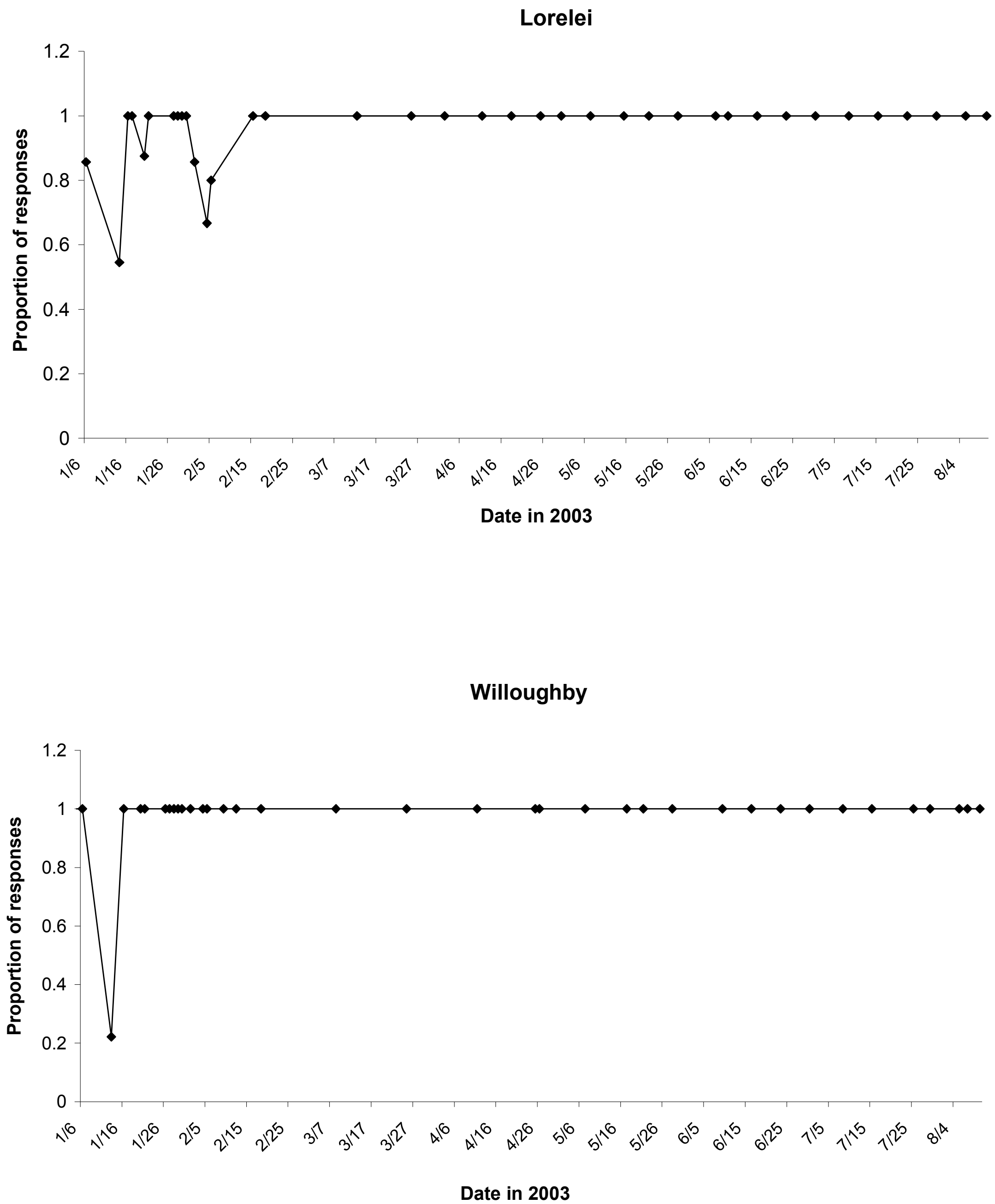
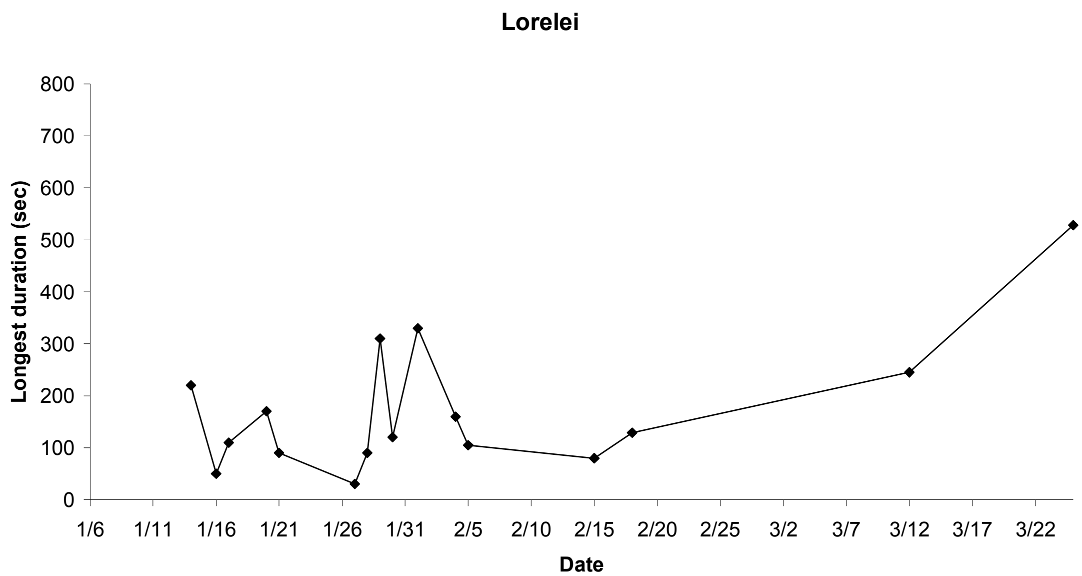


Figure 1. Proportion of responses from an individual signal for targeting behavior by Lorelei and Willoughby during husbandry training over the study period at Homosassa Springs Wildlife State Park.

Appendix C: Ventral-Up Positioning

After a manatee was stationed in the medical pool by the steps, I placed my hand in the water just in front of the manatee's eye. I moved my hand to the shoulder and back of a manatee and rubbed to signal that a manatee should turn over. I rubbed one side of her shoulder three times in a back and forward motion while pulling the same side of her flipper to signal for her to allow me to turn her body. When she permitted me to do so, I reinforced her with a single blow on the whistle. I repeated this procedure while accelerating the intensity of dragging her flipper. When the manatee turned over completely to the ventral-up position, I held the other side of her flipper to support her position and gave a handful of food with multiple short whistles as a reward. After the manatee accomplished the turning over behavior more than two to three times, I reduced the amount of reinforcement (both food and whistle) to a single reward period following turning over. As long as the animal remained relax in the ventral-up position, I provided a blow on the whistle and moderate reinforcement with a piece of food approximately every 10 seconds. I recorded the duration of the ventral-up position. My goal was for the manatee to remain in this position for five to eight minutes.

Individual variation was found between these two manatees. Lorelei attained the tuning over behavior on the first day of the training (January 6^{th}, 2003), while Willoughby required several days of step-by-step learning before she accomplished the behavior on January 20, 2003 (Figure 2). On the other hand, Willoughby could maintain the ventral-up position for longer (310 ± 234 seconds, mean ± SD) than Lorelei (173 ± 129 seconds). Willoughby was able to breathe while maintaining the ventral-up posture whereas Lorelei could not. Willoughby might have learned how to breathe from the ventral up posture in the past. I often saw her inverted or inverted swimming while I did not observe Lorelei in any of these postures during my behavioral observation periods.



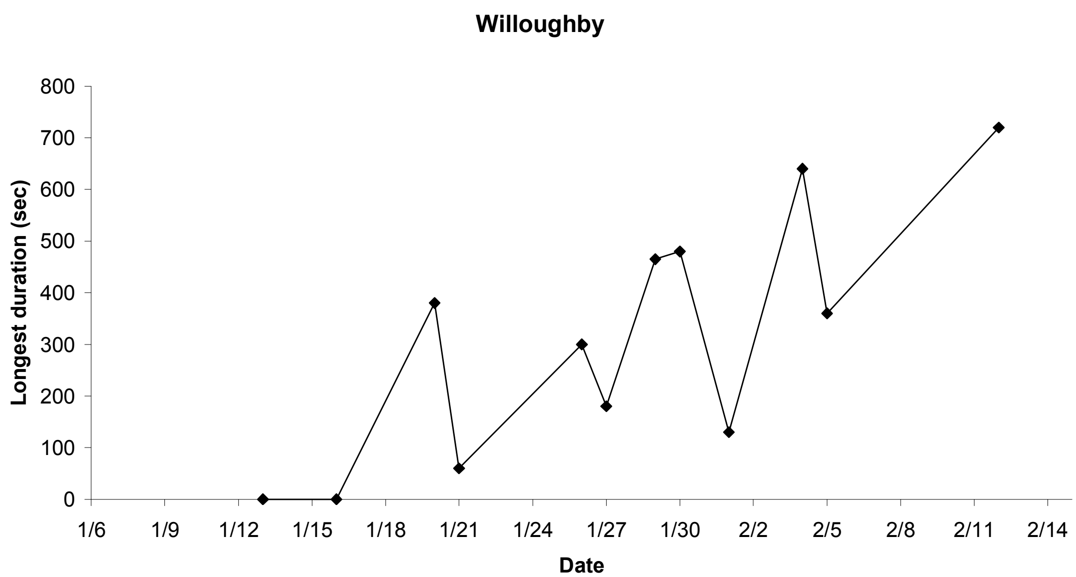


Figure 2. Longest duration (second) of ventral-up posture per training session by Lorelei and Willoughby. The duration was recorded until the procedure of needle acclimation was initiated.

Appendix D: Breathing from ventral-up position

With longer duration in the ventral-up position, a manatee needs to respire. While using my hand to hold her flipper for support, I elevated the head of the manatee with my other arm approximately every 2 minutes to expose her nostrils. I repeated this procedure until the manatee breathed. Three to four pieces of food and multiple short whistles were given when the manatee accomplish this behavior. This reinforcement was switched to three whistle blows only when the manatee breathed without my support and at her own pace.

This behavior was conducted by only Lorelei since Willoughby already knew how to breathe in a ventral up position. Lorelei took 34 days to accomplish this behavior and the success rate was increased gradually to 100% on April 25th, 2003 (Figure 3).

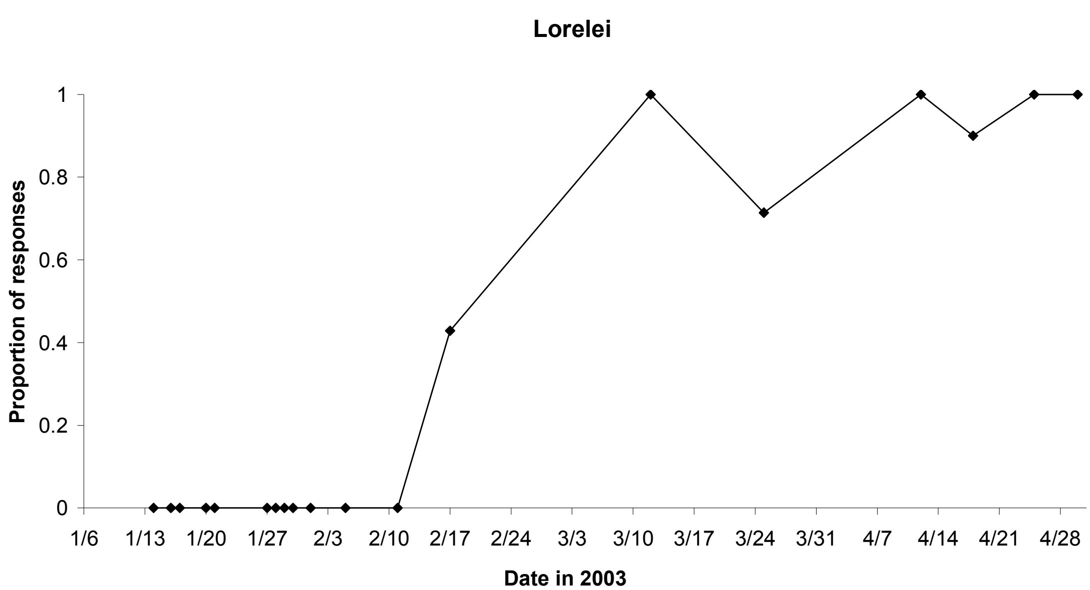


Figure 3. Proportion of accomplishment of breathing from ventral-up posture by Lorelei during husbandry training over the study period at Homosassa Springs Wildlife State Park.

Appendix E: Needle acclimation

Prior to the process of blood withdrawal, acclimatization to the needle on each pectoral flipper was required. The needle acclimatization was carried out by applying stimulation to the pectoral flipper. This was accomplished by scrubbing and cleaning the area with disinfectant (rank 1), pinching with my fingernail (rank 5), and then poking with a toothpick (rank 10), the tip of a 25-gauge needle (rank 15) and a 23 gauge needle (rank 20). Finally, a 20 1/2 gauge (rank 25) needle was inserted to a depth of 2 cm. Approximately three weeks to five months were required to complete the steps for needle acclimation per animal (Figures 4 and 5).

During the needle acclimation period, Lorelei's behavior was relatively steady. Mean duration of the needle acclimation per session on her right flipper was 77 seconds (± 15.25sec.) versus 51 seconds (± 40.23 sec.) for her left flipper (Figure 4). Willoughby, on the other hand, had a mean duration of needle acclimation per session of only 42 seconds (± 88.67sec.) on her right flipper and 37 seconds (± 84.59 sec.) on her left flipper (Figure 5).

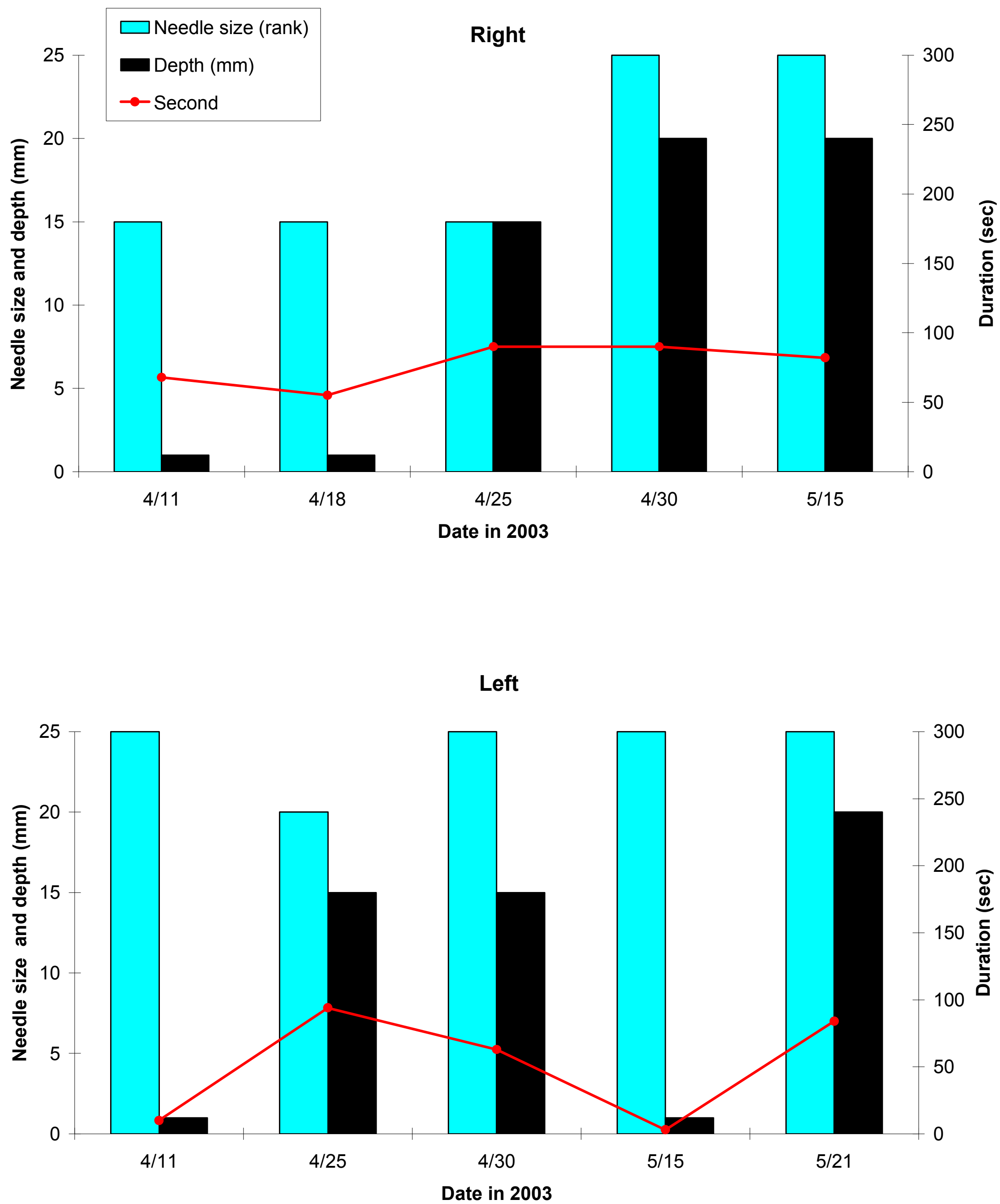


Figure 4. Progression of needle acclimation by Lorelei initiating from date of flipper stimulation until the first blood withdrawal. Rank of needle sizes were: 0 = no touch, 1 = clean up, 5 = finger nail, 10 = tooth pick, 15 = 25 gauge needle, 20 = 23 gauge needle, and 25 = 20 ½ gauge needle.

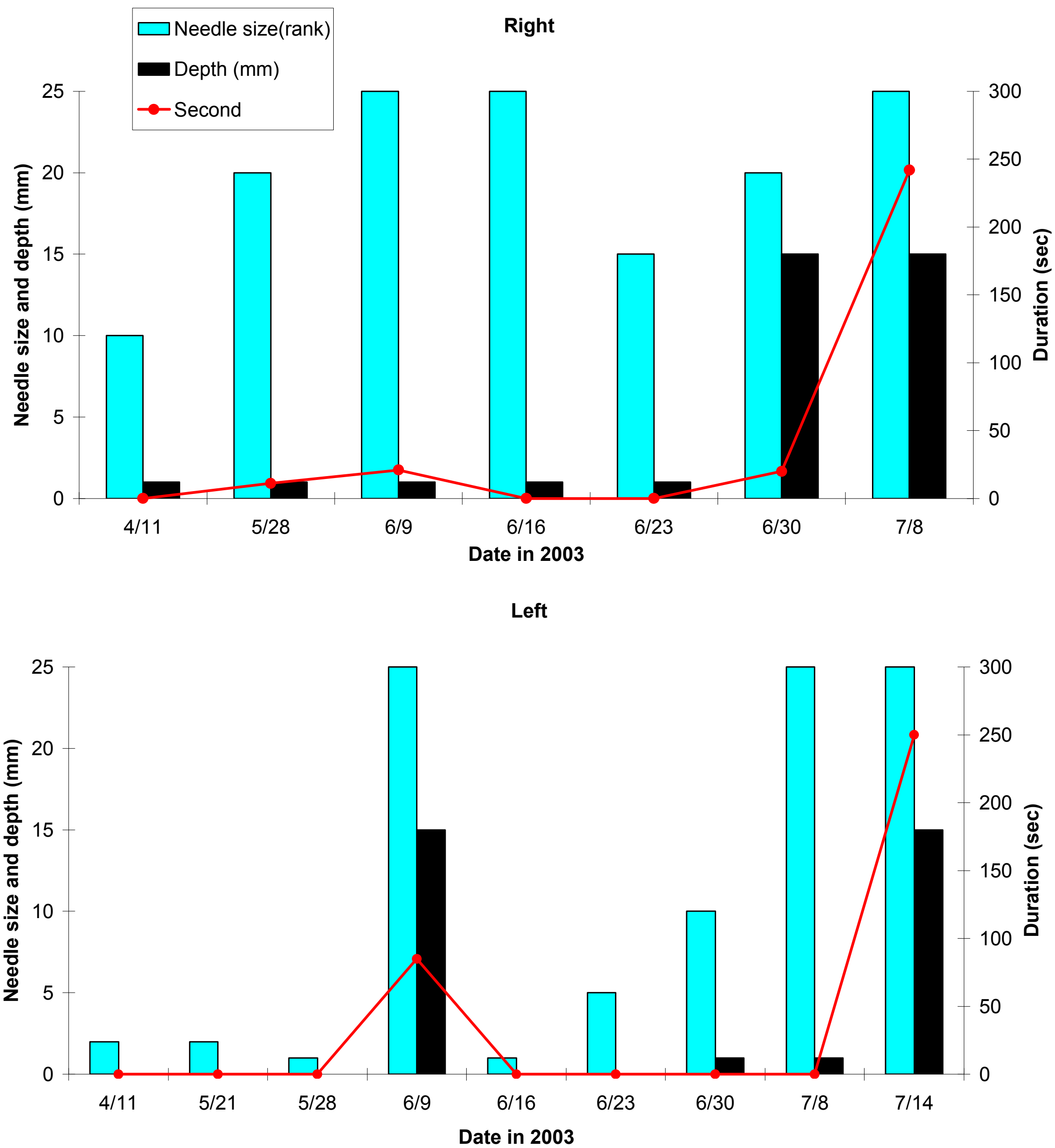


Figure 5. Progression of needle acclimation by Willoughby initiating from date of flipper stimulation until the first blood withdrawal. Rank of needle sizes were: 0 = no touch, 1 = clean up, 5 = finger nail, 10 = tooth pick, 15 = 25 gauge needle, 20 = 23 gauge needle, and 25 = 20 ½ gauge needle.

Appendix F: Blood sample collections

Blood samples were obtained once a week beginning on May 18, 2003 from Lorelei and on July 8, 2003 from Willoughby. I located myself on the steps in the water and positioned the animal ventral-up with the targeted flipper (for blood withdrawal) closest to me. I placed one leg under the animal's shoulder to support the chest above the water. I grasped the side of the flipper where the fingernails were located. The flipper was extended fully in the up-right posture and was kept above the water's surface. An area between the manatee's wrist and elbow was targeted to disinfect. I scrubbed with a chlorhexidine soap prepped gauze followed by an alcohol prepped gauze. This procedure was repeated three times before insertion of a needle. The needle was gradually inserted while monitoring the animal's body movement with my body by holding its flipper and touching its body. As I inserted the needle gradually deeper, the intensity of food and whistle reinforcement was increased. When the animal became agitated, the procedure and the reinforcement were paused until the animal relaxed. When the animal rejected the procedure, I terminated it and released the animal from the station. I emphasized reinforcement with the whistle during the blood withdrawal procedure when the animal was continuously relaxed. The rate of whistle reinforcement also indicated to the assistant what rate of food was to be provided (i.e., more whistling meant more food). Immediately after the needle was withdrawn, I applied pressure to the puncture site using alcohol-prepped gauze while holding the animal's flipper in the same fashion. When the procedure was completed, the manatee was given the end-task signal of multiple short whistles and voice alternatively while rubbing any part of the animal's body and providing a handful of sweet potato. At least two assistants were needed to complete this procedure. One assistant was responsible for providing reinforcement and for supporting the animal ventral-up posture by holding the pectoral flipper on the opposing side of the flipper used for blood collection. After the needle was inserted into the pectoral flipper, the second assistant handed me the required vials and provided me with alcohol-prepped gauze before the needle was removed. The second assistant also was responsible for handling the samples once collected, labeling vials, storing them on ice and cleaning used needles and gauze. Flipper alternation, bleeding from the right or left every other week of blood withdrawal, was required in order to minimize tissue damage.

Blood withdraw was initiated on May 18 from Lorelei's right flipper and on May 25 from her left flipper and conducted every once a week. I obtained blood from Lorelei for 7 days between the first date of blood withdrawal (May 18) and the last day of husbandry training (August 10). Blood withdrawal from Lorelei was not persistent because she sometimes rejected flipper stimulations. On the other hand, Willoughby required a longer period for the needle acclimation process. The date blood was successfully withdrawn from Willoughby was July 7 from her left flipper and July 8 from her right flipper. However, once she allowed me to withdraw the blood, she became very stable. I obtained blood from Willoughby for 8 days between July 7 and August 10.

A strong positive correlation was found between values of urinary and plasma progestin concentration of Lorelei (R = 0.96) (Figure 4). Yet, more frequent plasma sample values might substantiate a clear correlation with urine sample values.

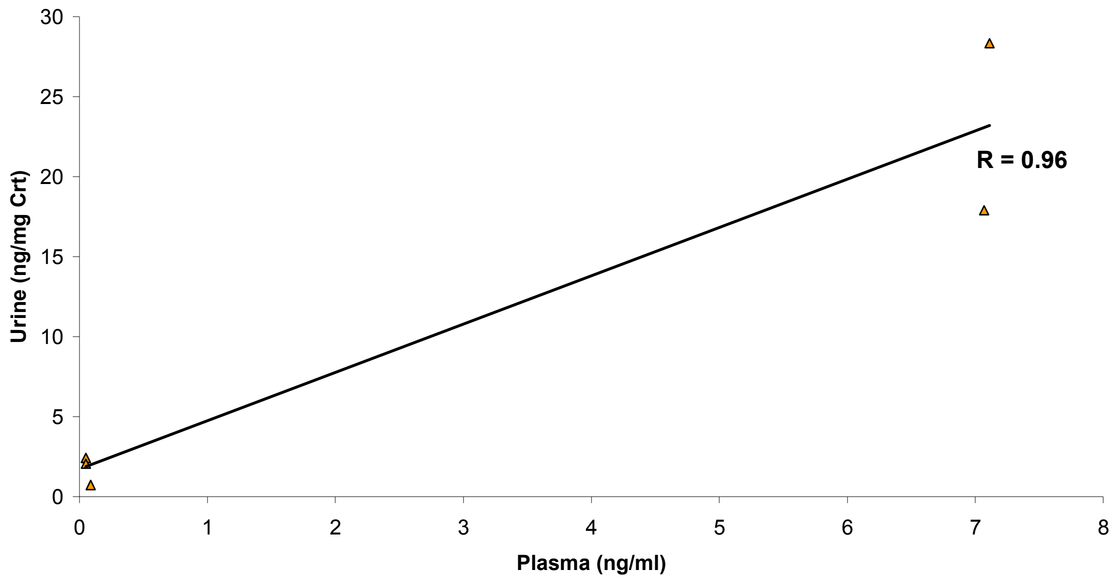


Figure 6. Positive correlation of urinary (ng/mg Crt) and plasma (ng/ml) progestins of Lorelei from May 28 to August 8, 2003 at Homosassa Springs Wildlife State Park.

Husbandry training from HSWSP manatees succeeded in a relatively short time. The regular handling of the manatees by park personnel and the relatively long time in captivity may have facilitated the learning process. Husbandry training reduced the risk of injury and stressful condition on both the animals and the handlers. A well-trained animal that voluntarily participates in such procedures increases the likelihood of regular physiological sample collection with a minimum effort, although not an inconsequential effort.

Appendix G: Individual spatial use of the facility

Individual spatial use of the facility was compared using daily proportion that differed by greater than two standard deviations from the group means. This measure was used to identify extreme outliers in the spatial use of the facility.

Habitat use by individuals seems to show similarity across time of day and across seasons. Only Lorelei and Rosie exhibited spatial patterns that showed any marked differences from the other manatees (Tables 1-3). Lorelei persistently occupied the medical pool during the day much more than the group average, accounting for 27% (mean of 5.11% ± 8.49, 1 SD), 47% (7.33% ± 14.93), and 58% (15.11% ± 16.91) of all scans during the period of noon, mid afternoon, and late afternoon, respectively. Rosie used A-2 only 15%, which was less than the group average (36.33% ± 11.01) during the noon period (Table 1). She used the enclosure differently from the other manatees during the afternoon period, occupying C-1 (6%) and B-2 (35%) much greater than the group average (2.89 %± 1.45 and 19% ± 7.87, respectively) (Table 2).

Table 1. Percentage of habitat usage per manatee in the noon period (1115-1350) during the study period from January 6 to August 10, 2003 at the Homosassa Spring Wildlife State Park.

Noon	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**	**MP**	**# time > 2 SD**
Amanda	38	44	2	11	1	2	2	0
Arial	55	37	4	2	0	1	1	0
Betsy	50	36	1	7	1	4	1	0
Electra	26	43	5	20	3	3	0	0
Holly	27	41	6	14	3	3	6	0
Lorelei	24	27	2	11	4	5	***27***	1
Oakley	29	46	2	13	2	8	0	0
Rosie	42	***15***	5	24	3	6	5	1
Willoughby	36	40	4	11	1	4	4	0
average	36.33	36.56	3.44	12.56	2.00	4.00	5.11	
SD	11.01	9.84	1.74	6.50	1.32	2.12	8.49	
Manatee > 2SD		Rosie					Lorelei	

Table 2. Percentage of habitat usage per manatee in the mid afternoon period (1350-1505) during the study period from January 6 to August 10, 2003 at the Homosassa Spring Wildlife State Park.

Mid Afternoon	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**	**MP**	**# time > 2 SD**
Amanda	38	41	3	22	2	15	3	0
Arial	22	39	4	17	2	12	4	0
Betsy	7	58	1	13	3	17	1	0
Electra	6	47	4	28	4	9	2	0
Holly	14	51	3	15	3	11	3	0
Lorelei	7	26	2	12	1	5	***47***	1
Oakley	29	46	2	13	2	8	0	0
Rosie	24	26	2	***35***	***6***	5	2	2
Willoughby	9	54	3	16	3	11	4	0
average	17.33	43.11	2.67	19.00	2.89	10.33	7.33	
SD	11.47	11.36	1.00	7.87	1.45	4.09	14.93	
Manatee > 2SD				Rosie	Rosie		Lorelei	

Table 3. Percentage of habitat usage per manatee in the late afternoon period (1610-1700) during the study period from January 6 to August 10, 2003 at the Homosassa Spring Wildlife State Park.

Late Afternoon	A1	A2	B1	B2	C1	C2	MP	# time > 2 SD
Amanda	17	8	2	10	24	33	7	0
Arial	25	6	4	11	8	28	18	0
Betsy	21	4	2	9	15	44	5	0
Electra	9	10	5	8	14	43	11	0
Holly	30	20	1	10	7	24	8	0
Lorelei	10	1	1	2	12	18	***58***	1
Oakley	22	11	0	21	16	26	4	0
Rosie	19	17	1	13	17	23	10	0
Willoughby	12	3	0	11	16	43	15	0
average	18.33	8.89	1.78	10.56	14.33	31.33	15.11	
SD	7.07	6.37	1.72	4.98	5.07	9.85	16.71	
Manatee > 2SD							Lorelei	

The different spatial use of the facility by Lorelei and Rosie might be explained by their particular histories. Lorelei, a 28 year-old captive born manatee, was regularly found in the medical pool throughout a day. Her habitual behavior in the medical pool was swimming in a counterclockwise direction (lap swim) and occasionally rolling over. Lorelei's behavior may be elucidated by her childhood environment. Lorelei was one of the two captive born manatees at this facility. She was born in Miami Seaquarium and stayed at Living Seas in Epcot for seven years until she was brought into the HSWSP in

1994. She was already 19 years old at that time. Unlike the natural riverbed at HSWSP, the other two facilities have man-made concrete tanks that are similar to the medical pool at HSWSP. Therefore, she might be acclimated to such an artificial concrete environment. In fact, one of the trainers at the Living Seas in Epcot observed Lorelei swimming circle in a pool almost every single day while Lorelei was housed there (C. Gooden, Animal trainer, Living Seas in Epcot, pers. comm.). Except for the noon period, Lorelei used A-2 area the least. Just before a manatee educational program started, I frequently recorded Lorelei swimming in the medical pool while the other eight manatees were found around A-2 or B-2 regions waiting for the feeding program to start. Lorelei often swam from the medical pool either right before or just after carrots (for the program) were thrown in the water.

Rosie, 42 years old and 36 years in captivity, was frequently recorded a certain spot in areas of B-2 or C-1 during the noon and mid afternoon period. Rosie was also regularly located by a volunteer park ranger, George Schulz, when she was at the spot in either of the areas (personal communication). This retiring behavior may be caused by her old age. In addition, the length of captivity and the absence of physical complexity such as environmental stimuli may result in such stereotyped behavior. Future research is required on this subject.

Taking into account the spatial limitation of the facility and the daily schedule by the park, the individual resemblance of spatial use and activity pattern by the nine manatees may be a result of their environmental restriction rather than their social relationships.

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