# SURVIVAL RATE ESTIMATES OF FLORIDA MANATEES (*Trichechus manatus latirostris*) USING CARCASS RECOVERY DATA

by

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# APPROVAL

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This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

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# ABSTRACT

The Florida manatee (*Trichechus manatus latirostris*) is classified as an endangered species and is also protected under the Marine Mammal Protection Act. The manatees' coastal distribution coincides with areas of high human density, making manatees particularly vulnerable to human impacts. Important management decisions on both the state and federal level rely heavily on extinction models that require estimates of survival and reproductive rates. Current mark-recapture methods are unable to estimate survival rates for younger age classes because many young manatees lack the unique scarring used to identify individuals. Using an age-ratio technique, this research utilizes carcass recovery data to provide estimates of survival rates of Florida manatees < 4.5 years old while quantifying and incorporating all sources of uncertainty. The age-ratio technique requires counts of carcasses in each age class, carcass detection probabilities, and the proportion of animals alive in each age class. In order to determine the count of carcasses in each age class, several models were designed to estimate carcass age at death. First, using physiological criteria, models were developed to determine the probability a carcass died within two weeks of birth (perinatal) based on carcass length and month of carcass recovery. Next, mathematical models were created to estimate the error in ear-bone aging techniques. Lastly, models were developed to estimate age from length for carcasses dying at ages older than two weeks old. With these models, age at death could be estimated for 98% of all collected carcasses. Using data from a region where all survival and reproductive rates are known (Upper St. Johns), carcass relative detection ratios by age class were calculated. Assuming relative detection ratios are the same for all regions, young survival rate estimates were then calculated for all other regions. Results show uncertainty in young survival rates is higher than what was previously assumed.

# INTRODUCTION

One of the primary sources for data on Florida manatee biology has been the Florida Manatee Carcass Recovery and Necropsy Program. The U. S. Fish and Wildlife Service and the University of Miami began and ran the program from 1974 to 1985. Since then, the state of Florida has been responsible for the program, currently operating under the Florida Fish and Wildlife Conservation Commission. The Marine Mammal Pathobiology Laboratory collects and necropsies every reported Florida manatee carcass, around 300 per year. The data have always been collected with the primary objective of "quantifying the causes, extent, and patterns of manatee mortality" (Rathbun *et al.* 1995). Collection of basic information on anatomy and physiology has also been a priority (Wright *et al.* 1995).

Although the carcass recovery program has answered many interesting questions about manatee physiology and biology and provided tallies of reported deaths by various causes, the data have only been minimally used to infer population status (Craig & Reynolds 2004). The analysis process has been hampered by limitations in the data collection design. The following list describes the weaknesses of the data, including design issues, protocol changes, simple failures of database organization, and real scientific complications which can only be addressed with calibration studies.

1. The primary weakness of the data is that carcasses are collected opportunistically, with researchers reliant on the public to report carcasses. Because of this potential

non-random sampling of carcasses, comparisons among regions, sexes, age classes, time periods, and even causes of death are not straightforward.

2. Another drawback relates to cause-of-death categories. When a new category was created (cold stress in 1984), older carcasses were not reclassified according to the new category. Moreover, a new category for red tide mortalities has never been introduced, even though researchers can now identify that cause of death. Also, cause of death cannot be determined for roughly 30% of all collected carcasses, largely due to decomposition (Schwarz 2004a).
3. The quality of necropsy records has improved greatly over the years. However, researchers have sometimes failed to record vital information for data analysis, and some important data has never been transferred to a database (Schwarz 2004a).
4. In order to analyze spatial and temporal trends in mortality data, carcass drift needs to be understood. Furthermore, manatees may not die in the same area as the source of the mortality. For example, animals that die from boat collisions may swim considerable distances away from the collision location before dying.
5. The carcass data have not been consistently cross-referenced with the mark-recapture (i.e. photo identification), captivity, and PIT (Passive Integrated Transponder) tag databases.

The primary purpose of my research was to obtain information about Florida manatee mortality from the necropsy data for management purposes. In the process, I was given direct access to the original paper necropsy reports. Because of the inadequacies of the mortality database for manatee population status analysis, a considerable amount of time and effort was spent on a major review of necropsy reports and a restructuring of the mortality database. The review and restructuring attempted to solve some of the limitations mentioned above. New database fields were created to cross reference carcass data with other databases, reassess some causes of mortality, describe the physiological characteristics that identify early-age mortality, and record female reproductive status. Almost 60% of all 5,865 available necropsy reports from 1978 through 2005 were reviewed. Specifically, necropsy reports for all females and carcasses ≤ 175 cm long were reviewed. Results of the database restructuring were summarized elsewhere (Schwarz 2004a).

The research presented here focuses on utilizing the carcass recovery data to estimate survival rates for manatees under 4.5 years old while quantifying and incorporating all known sources of uncertainty into those estimates. The equation for estimating survival rates of young animals is based on ratios involving counts of carcasses in each age class, detection probabilities, and the proportion of animals alive in each age class. Estimates of each carcass' age at death are required to determine counts of carcasses in each age class. After providing a general overview of Florida manatee biology and human interactions with manatees (Chapter 2), two chapters of this thesis are dedicated to describing the quantification of age estimates and error in those estimates for the carcass sample. Chapter 3 is devoted to estimating very early age at death (within about two weeks of birth vs. later in life) using physiological indicators and models. Chapter 4 describes uncertainty in age estimation using ear-bone growth layers and an age-length model. Chapter 5 combines carcass counts by age class with detection probabilities and the proportion alive in each age class. Relative detection ratios are estimated for the Upper St. Johns region, a region where all survival and reproductive rates for a small subpopulation have been estimated using mark-recapture techniques. Those relative detection ratios are used to estimate survival rates for three other regions. Uncertainty in the proportion of animals alive in each age class is quantified from knowledge of reproductive rates and the proportion of first-year calves in the population. Finally, all parameter estimates are combined with estimated adult survival rates to estimate survival rates of young animals. Chapters 3 through 5 are intended to be stand-alone research publications. The final chapter describes the steps needed to enhance the necropsy data as a management tool.

Although the review process added a considerable amount of important information to the mortality database, not all of that information has been used in the analyses reported here. However, the data are now more readily accessible for future research. Enough information on female reproductive status is now available to reevaluate female age at maturity, first described by Marmontel (1995). New information can aid in evaluating the seasonality of births. Analysis of PIT tag data cross-referenced with the mortality data is already underway. Finally, if my assessment of cause-of-death categories is confirmed by physiologists and if detection probabilities are estimated, spatial and temporal trends in mortality could be analyzed.

# THE FLORIDA MANATEE

Manatee research began in earnest in the mid-1960s with Hartman's astute observations of Florida manatee biology and behavior (Hartman 1979). Multifaceted manatee research involving the University of Miami and the U. S. Fish and Wildlife Service began in 1974, culminating in a manatee population biology workshop in 1978 (Brownell & Ralls 1981, Reynolds 1995). Additional workshops were held in 1992 and 2002, leading to various research publications on Florida manatee population biology (O'Shea *et al.* 1995, Craig & Reynolds 2004, Goodman 2004, Kendall *et al.* 2004, Langtimm *et al.* 2004, Reynolds *et al.* 2004, Runge *et al.* 2004). Today, around twenty government agencies and private organizations participate in various manatee studies (Reynolds 1999). Primary sources of data come from carcass recovery and necropsies, mark-recapture studies, aerial surveys, and radio and satellite telemetry studies. Several organizations are also involved in public education and an extensive rescue and rehabilitation program. There are many good reviews about Florida manatee research, management, population biology, behavior, and physiology (Hartman 1979, Reynolds & Odell 1991, Reynolds 1999, Reynolds & Rommel 1999, U. S. Fish and Wildlife Service 2001, Glaser & Reynolds 2003, McDonald & Flamm 2006, Reep & Bonde 2006). This chapter focuses on some of the research results pertaining to Florida manatee management and conservation, including most of the findings from the publications listed above. Some aspects of prior research are directly related to this study, and I refer you to later chapters for more information about certain research topics.

# Natural History

## Species, Subspecies, and Subgroups

Based on morphological and genetic studies, three manatee species have been identified (Domning & Hayek 1986, Reynolds 1999, Lefebvre *et al.* 2001). The West African manatee (*Trichechus senegalensis*) probably lives mostly within the rivers of the west coast of central Africa. The Amazonian manatee (*Trichechus inunguis*) is restricted to the Amazonian river basin. The West Indian manatee (*Trichechus manatus*) occupies the coastal regions of the Atlantic and Gulf of Mexico from northeastern South America to Florida, including the coastal waters of some Caribbean islands.

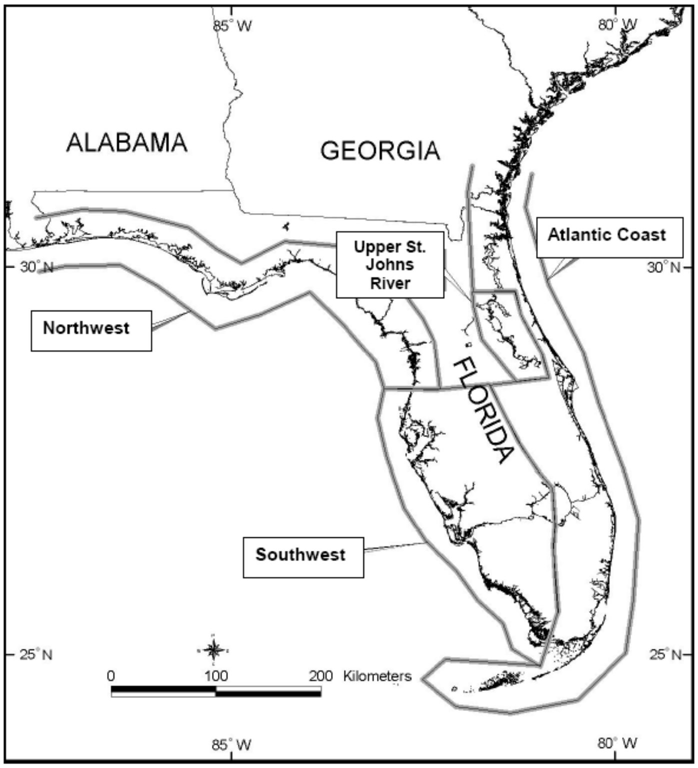
The West Indian species is further divided into two subspecies: the Florida manatee (*Trichechus manatus latirostris*) and the Antillean manatee (*Trichechus manatus manatus*). The Antillean manatee is found in the coastal waters of the Atlantic in South America north through the coastal waters of the Gulf of Mexico, sometimes into Texas (Domning & Hayek 1986). The Florida manatee represents the northern extreme for any manatee species, mainly occupying the coastal and riverine systems of Florida, sometimes moving as far northwest as Louisiana in the Gulf of Mexico and as far north as Rhode Island on the Atlantic coast during warm months (Powell & Rathbun 1984, Domning & Hayek 1986, Deutsch *et al.* 2003, Fertl *et al.* 2005). Florida manatees are more closely related to manatees from Mexico than manatees in the Dominican Republic, Puerto Rico, and northeastern South America, suggesting the islands of the Caribbean do not provide a genetic stepping stone between Florida and northeastern South America (Domning & Hayek 1986, Garcia-Rodriguez *et al.* 1998, Vianna *et al.* 2006).

Within the Florida subspecies, genetic diversity is very low, suggesting perhaps a genetic bottleneck or, more probably, a founder effect from colonization (McClenaghan & O'Shea 1988, Bradley *et al.* 1993, Garcia-Rodriguez *et al.* 1998, Garcia-Rodriguez *et al.* 2000, Vianna *et al.* 2006). However, there is some evidence for genetic separation between Atlantic and Gulf coast manatees in Florida (Garcia-Rodriguez 2000). Based on individual manatee movements and site fidelity to winter grounds, the Florida manatee subspecies has been partitioned into four subgroups or management units (U. S. Fish and Wildlife Service 2001, Haubold *et al.* 2006, U. S. Fish and Wildlife Service 2007). The Upper St. Johns region is defined as the upstream waters of the Upper St. Johns River, south of about 30º Latitude. The Atlantic region consists of the entire east coast of Florida, including the Florida Keys and the eastern half of Lake Okeechobee. The Southwest region spans from southern Monroe County to northern Pasco County and extends east to include the western half of Lake Okeechobee. The Northwest region is located north of Pasco County (Figure 2.1).

## Physiology

Through the Florida manatee carcass recovery program, much has been learned about manatee physiology, morphology, and anatomy. Manatees have many unique physiological characteristics, and only a few of those features will be reviewed here. At birth, Florida manatees are 80 – 160 cm long and weigh about 30 kg. The longest Florida manatee ever recorded was 411 cm long, and individuals can weigh up to 1,600 kg

Figure 2.1. Regions as defined by the Florida manatee recovery plan (U.S. Fish and Wildlife Service 2001).



(Reeves *et al.* 2002). As an adaptation to their completely aquatic lifestyle, their bodies are fusiform with a large spatula-like tail fluke (Berta *et al.* 2006). Their flexible pectoral limbs can aid in feeding or be used for locomotion, including “walking” on the ocean bottom and steering (Hartman 1979).

Florida manatee physiology is particularly adapted for an herbivorous diet. They are hind-gut fermentors with a digestive system similar to a horse and a long large intestine (Reynolds & Rommel 1996). Their digestive systems are highly efficient, removing 83 – 91% of the nutrients and energy from their food (Ledder 1986). Manatees have prehensile lips and prehensile vibrissae around their mouths to aid in grasping plants while feeding (Hartman 1979, Reep *et al.* 2001, Marshall *et al.* 2003). They also have continuous tooth replacement to counteract tooth wear from plant mastication (Domning & Hayek 1984).

Manatees have physiological adaptations that help them control buoyancy in their shallow-water habitat. Pachyosteosclerotic bones, little body fat compared to cetaceans, and dense, thick skin make them negatively buoyant (Taylor 2000, Kipps *et al.* 2002). The dorsal placement of the lungs and horizontal diaphragm help them maintain a horizontal position in the water column (Domning & Debuffrenil 1991). Contraction of the diaphragm also reduces gas concentrations, aiding in buoyancy control (Rommel & Reynolds 2000). The large amount of digestive matter in the intestines and the methane gas produced by digestion may also play a role in buoyancy control. In general, manatees become neutrally buoyant at relatively shallow depths in order to reduce the energy costs associated with buoyancy control during feeding (for a review, see Reep and Bonde (2006)).

Except for their tactile abilities, manatee sensory systems are not functionally exceptional. They can not echolocate. Manatee hearing is not particularly acute, nor is it very poor, with ranges and sensitivities similar to some pinniped species (Gerstein *et al.* 1999). Their eyes may have adapted to low light conditions, leading to a reduction in acuity, and their sense of smell is probably only rudimentary (Wartzok & Ketten 1999). However, their tongues have taste buds, and they avoid consuming plants with toxins or high tannin levels, indicating they do have some chemo-receptivity (Ledder 1986, Levin & Pfeiffer 2002). Manatees are very tactile with each other and their surroundings, and their body hairs may function as a highly sensitive tactile system to receive environmental cues (Hartman 1979, Reep *et al.* 2002). For a review, see Glaser and Reynolds (2003).

Unlike many other marine mammals, sirenians (manatees and dugongs) cannot tolerate cold temperatures. Compared with other marine mammals, manatees have similar vascular structures to prevent heat loss (Rommel *et al.* 2001, Rommel & Caplan 2003). However, manatees have a singularly low metabolic rate (Scholander & Irving 1941, Gallivan & Best 1980, Irvine 1983). Their low metabolic rate combined with a relatively thin blubber layer may make them vulnerable to cold temperatures (Fawcett 1942, Bryden *et al.* 1978, Gallivan *et al.* 1983, Irvine 1983). Smaller manatees are particularly sensitive to cold stress perhaps due to a large surface-to-volume ratio, a lower percent fat by body mass, or even inexperience at finding warm-water sources (Kleiber 1961, Hartman 1979, Buergelt *et al.* 1984, O'Shea *et al.* 1985, Reynolds & Wilcox 1986, Ackerman *et al.* 1995, Ortiz & Worthy 2004). Florida manatees rely on warm-water sources to survive when water temperatures drop below about 20ºC, and manatees reach their lower limit of thermal neutrality at water temperatures of about 24ºC (Allsop 1961, Irvine 1983, Deutsch *et al.* 2003).

Osmoregulation in manatees is not completely understood. They are euryhaline. In both marine and freshwater, internal water and salt levels are probably maintained via food consumption. Their food sources are 90 – 95 % water but still probably contain enough salts to sustain manatees in fresh water (Ortiz *et al.* 1998, Wells *et al.* 1999). Studies of their kidneys suggest manatees have the ability to concentrate their urine above salt water concentration levels, and osmoregulation is probably maintained via hormonal control (Hill & Reynolds 1989, Maluf 1989, Ortiz *et al.* 1998). Manatees probably do not intentionally ingest salt water, instead producing metabolic water to maintain healthy internal water levels when needed in marine environments (Ortiz *et al.* 1999). Apparently, the amount of fresh water in their diet and the manatees' osmoregulatory abilities are not enough to maintain long-term homeostasis in purely marine environments (Ortiz *et al.* 1998).

Florida manatees have relatively few parasites. Intestinal parasites include nematodes, trematodes, roundworms, and flukes (Husar 1977, Hartman 1979). Although manatees can be covered with marine algae, diatoms, barnacles, and even remoras, such epiflora and epifauna do not usually persist because they can not tolerate the dramatic salinity changes associated with manatee movements in and out of marine and fresh water environments (Hartman 1979, Williams *et al.* 2003).

So far, Florida manatees exhibit few signs of disease, potentially due to an “efficient and responsive” immune system (O'Shea *et al.* 1985, Bossart 1995, Bossart 1999). Morbillivirus has been documented, but manatees appear to be a dead-end host (Duignan *et al.* 1995). Papilloma virus may have been first described by Hartman (1979) in the Crystal River as “occasional pus-filled tumors.” The species-specific virus was officially identified in 1997 and appears in both captive and wild manatees in that area. There is some concern that papilloma virus may be expressed when manatee immune systems are suppressed due to exposure to cold, red tides, and/or pollutants (Bossart *et al.* 2002a, Bossart *et al.* 2002b, Walsh *et al.* 2005, Woodruff *et al.* 2005).

Five key physiological features are described here that have wide-ranging implications for Florida manatee management. First, they are highly adapted to an herbivorous diet. Second, although none of their senses are especially impaired, their visual and tactile abilities may be adaptations to low-light environments. Third, some manatees can not tolerate waters below 20ºC for extended periods of time. Fourth, manatees may be dependent on sources of fresh water to some extent. Last, as with all other species, depressed immune systems in manatees can open the door to disease and potential parasite proliferation. For more information on manatee physiology and the connection between physiology and manatee conservation, see Reynolds and Rommel (1999), Berta *et al.* (2006), and Reynolds and Marshall (In press).

### Life History Traits

Manatees are K-strategists with low reproductive rates, long life spans, and later ages at maturity. Twins are rare, probably representing between 2 and 4% of births (Marmontel 1995, O'Shea & Hartley 1995, Odell *et al.* 1995, Rathbun *et al.* 1995, Reid *et al.* 1995), and calves remain with their mothers for one to two years (Hartman 1979, O'Shea & Hartley 1995, Rathbun *et al.* 1995, Reid *et al.* 1995, Koelsch 2001). A mature female's inter-birth interval is one to three years with an interval less than two only when a dependent calf does not survive the first year (Hartman 1979, O'Shea & Hartley 1995, Odell *et al.* 1995, Rathbun *et al.* 1995, Reid *et al.* 1995, Koelsch 2001). Life expectancy is unknown, but one captive manatee is 58 years old. From counts of periotic growth layers, one wild manatee was estimated to have died at 60 years of age (Marmontel *et al.*

1996). There have been only three anecdotal cases of reproductive senescence in females (O'Shea & Hartley 1995, Rathbun *et al.* 1995).

Although females may become pregnant as early as three years old and around 250 cm long, only females over the age of four years and > 270 cm long have been seen with live calves in the wild (Hartman 1979, Marmontel 1995, O'Shea & Hartley 1995, Odell *et al.* 1995, Rathbun *et al.* 1995, Reid *et al.* 1995). There seems to be high individual variability in the age at maturity, with some females maturing as late as seven years old (Marmontel 1995, O'Shea & Hartley 1995, Rathbun *et al.* 1995). Such variability may be due to differing individual fitness levels (Marmontel 1995). Males may become spermatogenic as early as two to three years old. Given the mating system with intense competition for females, males probably do not sire a calf until they are older and larger (Hernandez *et al.* 1995, Reynolds *et al.* 2004).

The Florida manatee mating strategy has been labeled as “scramble promiscuity,” with groups of up to 20 males pushing and shoving each other to gain access to a single female (Hartman 1979, Rathbun *et al.* 1995, Anderson 2002). An individual female can be followed for up to a month, but the composition of the male group is fluid (Hartman 1979, Rathbun *et al.* 1995). Mating herds do not necessarily guarantee a pregnancy, and there is some evidence from captive observations that pregnant females mate throughout pregnancy (O'Shea & Hartley 1995, Odell *et al.* 1995, Rathbun *et al.* 1995). However, based on the time between observed mating herds with individual females and the first sightings of those females with calves, gestation period for the subspecies has been estimated at 11 – 14 months (O'Shea & Hartley 1995, Odell *et al.* 1995, Rathbun *et al.*

1995, Reid *et al.* 1995). Although no specific studies have investigated the annual timing of mating and birthing, evidence from aerial surveys, mark-recapture studies, radio-tagged animals, and studies investigating seasonal spermatogenesis and fetus size indicate both mating and birthing occur throughout the spring and summer (Hartman 1979, Irvine & Campbell 1979, Rathbun *et al.* 1990, Hernandez *et al.* 1995, Marmontel 1995, O'Shea & Hartley 1995, Odell *et al.* 1995, Rathbun *et al.* 1995, Reid *et al.* 1995, Weigle *et al.* 2001, Reynolds *et al.* 2004).

Apart from the cow-calf bond, social affiliations do not appear to be strong. Cows and calves maintain constant vocal contact, and vocalizations may be heard during non-cow-calf group interactions (Bengtson & Fitzgerald 1985, Reynolds *et al.* 2004, O'Shea & Poche 2006). Alarm calls are not common outside of cow-calf pairs, and with the exception of mothers with calves, individuals do not come to the aid of other manatees (Hartman 1979, Reynolds 1981a). Males may occasionally “cavort” in herds as a way to practice for mating herds or potentially to establish dominance (Hartman 1979, Wells *et al.* 1999). Although manatees may be quite tactile with each other, interactions between non-cow-calf pairs tend to be fluid and ephemeral, lasting up to a few hours with no particular social structure. In addition, herding is weak, and manatees are not behaviorally or ecologically territorial (Hartman 1979, Reynolds 1981a). Florida manatees lack natural predators, and apart from the occasional startle response, do not appear to react much to the presence of other non-human species (Hartman 1979).

Manatees are mainly herbivorous, although they probably incidentally ingest invertebrates while eating their primary sources of food (Hartman 1979). They are opportunistic consumers, feeding on a wide range of plant species (Hartman 1979, Ledder 1986). Intestinal content analysis found manatees consume at least 60 vascular plants and 61 algae species. However, 86% of the carcasses examined had recently consumed only between three and seven plant species (Ledder 1986). Algae probably does not significantly contribute to their diet, and they avoid blue-green algae (Reynolds 1981b, Ledder 1986).Both stable isotope and intestinal content studies showed manatees consume plants from fresh, brackish, and marine environments (Ledder 1986, Ames *et al.* 1996, Reich & Worthy 2006). The most common plant species consumed are widgeon grass (*Ruppia maritima*), shoal grass (*Halodule wrightii*), hydrilla (*Hydrilla verticillata*), manatee grass (*Syringodium filiforme*), and turtle grass (*Thalassia testudinum*) (Ledder 1986, Reynolds & Odell 1991, Ames *et al.* 1996, Lefebvre *et al.* 2000). The suite of consumed plant species may be correlated with their availability in different regions, and adults may show seasonal patterns in food type and amount consumed (Hartman 1979, Bengtson 1983, Powell & Rathbun 1984, Silverberg & Morris 1987, Baugh *et al.* 1989, Fertl *et al.* 2005, Reich & Worthy 2006). They will on rare occasions intentionally consume invertebrates, and encountering non-plant matter in the intestinal tracts of dead manatees is not uncommon (Ledder 1986, Beck & Barros 1991, O'Shea *et al.* 1991, Courbis & Worthy 2003). Captive manatee calves may start grazing as early as a few weeks old (Moore 1956, Phillips 1964). The youngest documented wild manatee seen consuming plants was no more than three months old (Hartman 1979).

Adult manatees may eat up to 9% of their body weight per day, and their influence on their environment is not trivial. They graze like other herbivores, leaving the root systems of plants intact (Baugh *et al.* 1989). However, a substantial amount of biomass is removed in grazed areas (Packard 1984, Lefebvre *et al.* 2000). By creating bare patches for early successional plants to grow, manatee grazing may increase or maintain species diversity in seagrass communities (Packard 1984). Manatees also tend to return annually to the same feeding areas, suggesting their "harvesting" may produce more nutrient-rich food the following year (Lefebvre *et al.* 2000).

### Distribution and Movements

Manatee distribution is largely connected to four basic biological necessities: reproduction and access to fresh water, food, and warm water. A significant proportion of the population is reliant on sources of warm water to prevent hypothermia and even death in winter. Such sources are limited to effluents from power plants, deep, unmixed water layers in southern Florida, and several small natural springs. The severity of the winter cold probably dictates just how many manatees are found at warm-water sites at any given time (Irvine & Campbell 1978, Hartman 1979, Reid *et al.* 1991, Reynolds & Wilcox 1994, Laist & Reynolds 2005b, Laist & Reynolds 2005a). At least at one warm-water source in King's Bay, manatees may avoid the warm-water spring in the summer when surrounding water is actually warmer than water from the spring (Kochman *et al.* 1985, Rathbun *et al.* 1990).

Tracking, aerial, and behavioral studies indicate Florida manatees are found more often in fresh or brackish than in pure sea water when they are not restricted to warm water sources (Irvine & Campbell 1978, Hartman 1979, Shane 1983a, Powell & Rathbun 1984, Miller *et al.* 1998, Weigle *et al.* 2001, Flamm *et al.* 2005). It is not known how long manatees can survive without fresh water. However, they are seen at warm water sites with mats of marine algae and barnacles, indicating they can (and some do) spend extended periods of time in the marine environment (Hartman 1979).

Given their herbivorous diet and constant need for food, manatees are generally restricted to the photosynthetic zone and in Florida are rarely seen in waters > 10 m deep (Hartman 1979). However, since their food source is abundant, distribution of food is probably secondary in importance to distribution of fresh water and warm-water sites. In other words, overall food patch selection is probably correlated with a patch's proximity to fresh water or warm water sources (Shane 1983a, Fertl *et al.* 2005, Flamm *et al.* 2005).

Prior to tracking research, several studies suggested at least some manatees made seasonal migrations south in the winter to avoid cold waters (Hartman 1979, Shane 1983b, Reynolds & Wilcox 1986, Craig *et al.* 1997). In general, large movements and migrations tend to be highly variable among individuals (Weigle *et al.* 2001, Deutsch *et al.* 2003). Satellite telemetry studies on the Atlantic coast have shown 12% of tagged manatees remain within a 50 km range of their winter habitat. The rest make some sort of seasonal migration north in the spring and south in the fall, with 14% of the migrating group traveling over 400 km from their winter range (Deutsch *et al.* 2003). It is unclear why some manatees make such dramatic migrations. Also in the Atlantic, temperatures below about 21ºC seem to trigger rapid movements south in fall, with all individuals, regardless of sex, age, body size, or reproductive status, responding the same way to the same environmental cue (Reid *et al.* 1991, Deutsch *et al.* 2003).

Movements along the Gulf coast of Florida are not as well understood. There is evidence that some manatees in the Northwest region move north in the spring (Powell & Rathbun 1984, Rathbun *et al.* 1990, Reid *et al.* 1991, Fertl *et al.* 2005). Seasonal movements of satellite-tagged animals in Tampa Bay were highly variable. Some females stayed in the general area throughout the year, whereas others traveled both north and south during the spring and summer (Weigle *et al.* 2001). Two females even traveled south and crossed over to the Atlantic, the only manatees ever known to do so prior to 2001 (Reid *et al.* 1991, Weigle *et al.* 2001, Deutsch *et al.* 2003). Some researchers have suggested there may be mixing between the northern and southern regions along the Gulf coast, and the southern subgroup may have repopulated the northern region when hunting ceased in the late 1800s (Powell & Rathbun 1984, Laist & Reynolds 2005b).

Manatees exhibit some general movement patterns on both coasts. Some animals visit more than one warm-water site during a year (Reid *et al.* 1991, O'Shea & Langtimm 1995). Individuals show strong fidelity to both winter and non-winter sites and movement patterns, and individuals may continue to occupy the same seasonal ranges as their mothers (Shane 1983a, Shane 1983b, Deutsch *et al.* 2003). Males move over longer distances per day, perhaps as a reproductive strategy to find mates (Hartman 1979, Rathbun *et al.* 1990, Weigle *et al.* 2001, Deutsch *et al.* 2003, Flamm *et al.* 2005). Females tend to give birth in quiet waterways and secluded locations (Hartman 1979, Rathbun *et al.* 1990, O'Shea & Hartley 1995, Reid *et al.* 1995, Weigle *et al.* 2001, McDonald & Flamm 2006).

Daily movement patterns are not well documented. However, aerial surveys have shown some individual manatees may not move from a particular 10m area for days at a time during winter (Packard *et al.* 1985). Other manatees display a diel pattern, leaving warm-water sites during particular times of day to feed (Rathbun *et al.* 1990). When not confined to warm-water sites, adults can spend six to eight hours per day feeding, so distribution of vegetation may play a role in daily movement and distribution patterns (Hartman 1979). Hurricanes, heavy rains, turbidity, and winds appear to have little-to-no effect on manatee movement patterns (Hartman 1979, Langtimm *et al.* 2006).

## Human Interactions

Overall, humans have had a profound effect on manatee movements, distribution, and survival. Humans affect manatees through changes in fresh water distribution, warm water habitat, and food. Vessel strikes kill an alarming number of manatees each year, and human activity may be increasing the intensity and strength of red tides, another source of manatee mortality. Compounding effects from exposure to several environmental stressors could also reduce a manatee's fitness.

### Warm-Water Sites

The manatees' inability to survive in cold water temperatures leads to significant challenges for Florida manatee management. An estimated 60% of the Florida manatee population depends on warm water produced by ten electric power plants during the winter (Reynolds & Wilcox 1994, Laist & Reynolds 2005b, Laist & Reynolds 2005a). After hunting ceased, manatees may have been isolated in the southern-most regions of

Florida, particularly during winter (Laist & Reynolds 2005b). The introduction of power plants and other industrial facilities in the mid-1950s extended the Florida manatee's northern range, dramatically altering their winter distribution, and perhaps providing stepping stones to allow repopulation of northern, natural, warm-water springs (Irvine & Campbell 1978, Shane 1983a, Powell & Rathbun 1984, Deutsch *et al.* 1998, Laist & Reynolds 2005b, Laist & Reynolds 2005a). Since power plants increase water temperatures as much as 11ºC above ambient water temperatures, power plant effluent does not increase water temperatures enough north of Citrus County on the west coast and probably north of the Upper St. Johns River on the east coast to sustain large numbers of manatees in winter (Shane 1983a, Powell & Rathbun 1984).

### Food and Fresh Water

Significant losses of seagrass beds in Florida due to human-induced reduction in water clarity, nutrient loading, and changes in salinity are well documented (Robblee *et al.* 1991, Fletcher & Fletcher 1995, Kurz *et al.* 2000, Provancha & Scheidt 2000). Manatee distribution on both a large and small scale has probably changed in response to such losses (Husar 1977, Deutsch *et al.* 2003, McDonald & Flamm 2006). However, no studies, to my knowledge, have documented such changes. Seagrass beds may have been rebounding in the late 1990s, at least in some areas (Kurz *et al.* 2000). Introduction of exotic hydrilla, a macrophyte manatees often consume, may have offset any seagrass bed losses in their southern range (Powell & Rathbun 1984). Manatees are such efficient consumers, they have been tested as potential weed and mosquito control agents in other countries (Reynolds & Odell 1991). In Florida, aquatic weeds are too prolific for the current manatee population to effectively control them (Reynolds & Odell 1991).

As the human population increased in Florida, access to fresh water was probably reduced for manatees, mostly due to human consumption, agriculture, and control of fresh-water flows. No studies have investigated how reduced fresh-water access has affected manatee behavior or distribution. Agriculture and consumption of fresh water by humans also may have reduced spring flows, causing some loss of natural warm water habitat (Laist & Reynolds 2005a).

## Vessels

Vessels reduce manatee survival through direct mortalities. Between 25 and 40% of all annual reported manatee mortalities are caused by vessel strikes (Ackerman *et al.* 1995, Bossart *et al.* 2005). In addition, harassment, noise, reduced habitat quality, and non-lethal strikes may reduce a manatee's fitness (ability to survive and reproduce) when time and energy must be spent adjusting to such "external stressors." The tourism industry aimed at viewing and interacting with manatees may actually reduce water quality and increase harassment in some areas (Sorice *et al.* 2006). Vessel noise can mask communication signals, particularly between cows and calves, and may even cause hearing damage (Bengtson & Fitzgerald 1985, O'Shea 1995, Gannon *et al.* 2007). Finally, the energetics of trying to constantly avoid vessels may impact a manatee's fitness (Reynolds 1999).

Not all vessel strikes are fatal, and many animals have non-lethal encounters with vessels. Ninety-seven percent of the manatees recorded in the MIPS database have more than one scar pattern (O'Shea *et al.* 2001). A small sample of animals of known age in the Upper St. Johns River ($n = 8$) indicated scar acquisition rate is over two per year (O'Shea *et al.* 2001). Even during early photo-ID studies, the catalog of individual manatees had to be constantly updated to account for new scars (Powell & Rathbun 1984). Rathbun *et al.* (1990) estimated roughly 80% of all weaned individuals in the Homosassa and Crystal Rivers were identifiable from unique scar patterns.

Manatees exhibit short- and long-term behavioral responses to vessels and industrial activities. Manatees have responded to the presence of boats and SCUBA divers by moving out of primary warm water sources in winter and away from seagrass beds when boat concentrations were high (Hartman 1979, Miksis-Olds *et al.* 2007a). In summer, animals in the Banana River tend to move away from industrial operations (Provancha & Provancha 1988). Deutsch *et al.* (2003) even suggest the reason for large-scale seasonal migrations may be to avoid boat traffic. Overall, manatees tend to increase use of sanctuaries when vessel traffic is high and select foraging patches with lower ambient noise levels (Curran & Morris 1988, O'Shea 1995, Buckingham *et al.* 1999, Miksis-Olds *et al.* 2007a). Cow-calf pairs show an increased sensitivity compared to independent individuals, perhaps due to their need for lower ambient noise levels for communication (Gannon *et al.* 2007).

Short-term responses to approaching vessels generally include an increase in swim speed and a move toward deeper water, regardless of vessel speed, direction, or habitat type (Hartman 1979, Nowacek *et al.* 2004, Miksis-Olds *et al.* 2007b). Faster vessel approaches elicit faster swim speeds (Nowacek *et al.* 2004, Miksis-Olds *et al.*

2007b). There is some speculation that the acoustic properties of the manatees' shallow water habitat make it difficult for manatees to detect boats or boat direction (Gerstein *et al.* 1999, Miksis-Olds & Miller 2006, Miksis-Olds *et al.* 2007b). However, given enough warning, manatees do maneuver successfully to avoid boats (Nowacek *et al.* 2004).

Apart from actually being killed by a boat, it is unclear how vessel activities affect manatee survival and reproduction. In general, non-lethal vessel encounters may interrupt biologically important behaviors, and animals can be displaced from preferred or critical habitat (Provancha & Provancha 1988, O'Shea 1995, Buckingham *et al.* 1999, Gorzelany 2004). All such topics warrant further investigation.

### Other

Several other human activities may affect manatees. Manatees are entangled in and ingest molofilament line and debris (Ledder 1986, Beck & Barros 1991). Reports of harmful algal blooms, or red tides, have increased globally over the years due in part to an increase in human activities, such as nutrient loading and introduction of exotic species (Landsberg *et al.* 2005). Although high levels of organochlorines have not been found in manatees, high copper concentrations have been measured in manatee livers in areas where copper is used as an herbicide against aquatic plants (O'Shea *et al.* 1984). In addition, cadmium levels in manatee kidneys may increase with manatee age (O'Shea *et al.* 1984). Finally, manatees die in floodgates and locks every year, although considerable effort has gone into reducing this source of mortality (Odell & Reynolds 1979, O'Shea *et al.* 1985).

## Causes of Mortality

### Carcass Recovery Data

Cause-of-death data come largely from the Florida manatee carcass recovery and necropsy program. As mentioned in Chapter 1, the carcass recovery data have several serious limitations, particularly with regards to cause of death. Primarily, no studies have investigated carcass recovery rates or probabilities, so all previous analyses on patterns and trends in manatee mortality have assumed no bias in detection. In addition, as researchers have improved their abilities to determine cause of death, cause-of-death categories have changed, and no reviews have attempted to reassess cause of death for carcasses collected in earlier years.

The following information describes what we have learned about causes and trends in mortality given the limitations of the carcass recovery data. Each carcass is categorized in to one of the following nine classes depending on cause of death, carcass length, and/or level of decomposition: watercraft, floodgate/lock, human (other), perinatal, natural (cold), natural (other), verified but not recovered, undetermined (too decomposed), and undetermined (other). Instead of reviewing what little analyses have been performed using the perinatal cause-of-death category, I merely discuss the problems associated with the perinatal classification. Even though red tide is not an official cause-of-death category, red tides are implicated in several large mortality events and warrant discussion here.

Trends and Patterns

Two studies have investigated the trends and patterns in Florida manatee mortality. O’Shea *et al.* (1985) calculated mortality frequency distributions using 406 carcasses collected from April 1976 to March 1981. They determined that cause of death was correlated with season and location. More “undetermined” deaths were prevalent during winter. The late juvenile/subadult age class (delimited by carcass lengths) represented a higher proportion of dead animals in the winter compared to other seasons. Geographically, southwestern Florida had a high number of undetermined deaths, and southeastern Florida had a high number of gate entrapment deaths. Boat collision mortality in northeastern Florida and calf mortality in northwestern Florida were also high. Finally, the highest number of deaths shifted from northeastern to southwestern Florida between 1976 and 1981. During that time period, collisions with boats represented 24% of all mortalities. Prior to 1986, cold stress was not officially recorded as a cause of death, so the number of undetermined subadult deaths in winter is probably inflated in O’Shea’s dataset relative to data collected in later years.

Ackerman *et al.* (1995) expanded on the work of O’Shea *et al.* (1985). With 2,074 carcasses collected from 1974 to 1992, the number of recovered carcasses had increased steadily at an average 5.9% per year. The increase in the number of recovered carcasses was significantly correlated with an increase in the size of the human population and the number of registered watercraft. However, the increase in the number of recovered carcasses may also have been due to increased recovery effort through the years and/or an increasing manatee population (O'Shea & Ackerman 1995). Watercraft related deaths increased at an average 9.3% per year, and “perinatal and unknown” deaths increased at an average of 11.9% per year.

Log-linear multiple regression analysis was performed to assess patterns in the number of deaths by cause of death, body length, sex, season, region, degree of decomposition, and three time periods (1976-1981, 1981-1986, and 1986-1992). Several three-way interactions were significant, making interpretation of results difficult. Sex was the only non-significant variable. Watercraft-related mortality was highest in eastern Florida, but the rate of increase in watercraft-related mortality (mentioned above) was greatest in southwestern Florida. Proportion of deaths in spring and summer increased over time due to a relative increase in the number of perinatal and watercraft-related deaths during the two seasons. Human-related mortality was greatest on the east coast and significantly affected adults more than other age classes. More males (58%) than females were recovered, suggesting something other than a 1:1 sex ratio in the population or higher mortality rates for males. Other results from the two studies are mentioned throughout this chapter.

## Vessels

As mentioned previously, mortalities from boat strikes represent a large fraction of all reported manatee deaths, and vessel collision is probably the primary cause of death for adults (O'Shea *et al.* 1985, Ackerman *et al.* 1995). Between 30 and 60 % of reported vessel mortalities are caused by propeller wounds. The rest are caused by impact trauma or both propeller and impact trauma (Beck *et al.* 1982, Beck & Reid 1995, Wright *et al.* 1995, Lightsey *et al.* 2006). Propeller mortalities are usually caused by larger watercraft

(> 7m long) (Beck *et al.* 1982, Rommel *et al.* 2007). Despite regulated speed zones, counts of vessel mortalities continue to increase. Although an increase in vessel mortalities could be correlated with an overall increase in the manatee population or the proportion of carcasses reported, vessel-mortality counts are also correlated with the number of registered vessels in Florida (Ackerman *et al.* 1995, Wright *et al.* 1995, Laist & Shaw 2006).

## Cold Stress

Hartman (1979) may have unknowingly first described one of the symptoms of cold exposure as “encrusted patches” on the skin. We now know death from cold stress can be acute or chronic. The symptoms of prolonged cold stress begin with lethargic behavior and reduced food intake, leading to dehydration and constipation. Eventually the nutritional and metabolic imbalances lead to immune suppression which in turn opens the door to infection, often manifested in part as skin lesions (Buergelt *et al.* 1984, O'Shea *et al.* 1985, Bossart *et al.* 2002b).

Cold stress only became an officially documented cause of mortality in 1984, so it is difficult to determine how many of the reported manatee mortalities were due to cold stress prior to that year (Ackerman *et al.* 1995). However, potential manatee cold mortalities have been reported as far back as 1895 (for a review, see Husar (1977)). The number of reported winter mortalities has consistently been higher than the number of reported summer mortalities, although mortalities in summer may be underrepresented because summer carcasses are probably more likely to decompose before they are reported (O'Shea *et al.* 1985, Ackerman *et al.* 1995). Manatee mortality may have increased during particularly cold winters in 1977-1978, 1981-1982, and 1989-1990 (O'Shea *et al.* 1985, Ackerman *et al.* 1995).

### Red Tide

Red tides in Florida are caused by the dinoflagellate *Karenia brevis*, previously named *Gymnodinium breve*. Manatees can be exposed to the brevetoxin created by red tides through ingestion of tunicates, seagrasses, and seawater (Bossart *et al.* 1998, Landsberg & Steidinger 1998, Flewelling *et al.* 2005, Landsberg *et al.* 2005). When cell lysis occurs, *K. brevis* concentrates brevetoxin at the surface, releasing it as aerosols (Pierce *et al.* 1990). Thus, manatees can also inhale the toxin (Bossart *et al.* 1998, Landsberg & Steidinger 1998, Landsberg *et al.* 2005). Oceanographic conditions make for very successful *K. brevis* blooms in southwestern Florida between Clearwater and Sanibel Island, and blooms originating in the southwest region of Florida occasionally drift into the Atlantic (Tester & Steidinger 1997).

Several factors determine the extent to which a red tide bloom affects manatees. A red tide bloom’s concentration, distribution, salinity, and persistence in relation to manatee distribution and length of exposure all play a role. In general, manatees in the Southwest nearshore waters are most likely at risk when a bloom occurs between February and April, coinciding with manatee dispersal away from warm water sites (Landsberg & Steidinger 1998). Since *K. brevis* is a marine species, manatees in low salinity areas are not exposed (Landsberg & Steidinger 1998). In addition to direct mortality, blooms may influence overall species abundance and diversity in an area, potentially reducing habitat quality. They sometimes harbor and transmit pathogens and could create long-term changes in overall manatee fitness and behavior through chronic exposure (Landsberg *et al.* 2005). More investigation into such secondary factors is needed.

The first reported Florida manatee deaths from red tide occurred after a bloom in 1963 when seven manatees may have died from red tide exposure (Layne 1965). Other red tide blooms potentially responsible for manatee mortalities took place in the springs of 1982, 1996, and 2003 (Buergelt *et al.* 1984, O'Shea *et al.* 1991, Bossart *et al.* 1998, FWRI 2003). It is difficult to determine just how many reported manatee deaths were caused by historic red tide blooms because a method to identify red tide mortality has only recently been developed (Suzik 1997). In addition, red tide is not a formally recognized cause of mortality in Florida manatee necropsy protocol, and not all carcasses are tested for red tide exposure. Furthermore, some evidence suggests manatees in the Southwest may be continuously exposed to some level of red tide toxins, and red tide deaths may occur long after a bloom has dissipated due to accumulation in seagrasses and tunicates (Bossart *et al.* 1998, Flewelling *et al.* 2005).

### Perinatal

Florida manatee researchers created a “perinatal” category based on expert opinion and an easily measured variable: carcass body length. Carcasses less than 150 cm in length in which cause of death was not diagnosed as directly human-related were classified in a special category: perinatal (natural or undetermined). The category was designed to separate out carcasses that probably died of natural causes related to pregnancy or birth or early separation from their mothers (Bonde *et al.* 1983).

However, the perinatal cause-of-death category presents several problems. First, no other cause-of-death category is based on length of the carcass, and another categorical variable, age class, has been traditionally determined by length (O'Shea *et al.* 1985, Ledder 1986, Ackerman *et al.* 1995, McDonald & Flamm 2006). Thus, describing different causes of death by age class becomes awkward. Second, managers have reported misinterpretation of the perinatal cause of death, with some people misconstruing it as death from "perinatal disease." Third, grouping all small carcasses together in one category does not allow for the distinction between natural death related to birth and death due to other causes. Fourth, the grouping assumes all animals < 150 cm long died of natural causes when a human factor could not be established. Since many carcasses of this length are too decomposed to determine cause of death, such an assumption may not be suitable.

In addition, the deterministic, static cut-off of 150 cm overlooks the uncertainty associated with the length vs. age-at-death relationship, although other data suggest such variation. Unborn fetus lengths have been recorded in the upper 140 cm range, and one unborn fetus was 152 cm long (Marmontel 1995). Counts of ear-bone growth layers have determined animals as small as 124 cm long lived at least one year, and some animals longer than 200 cm in length may not have survived a year, assuming no error in ear-bone age estimates (Marmontel *et al.* 1996).

In general, the Florida manatee perinatal cause-of-death category makes potentially inappropriate assumptions about the relationships between three variables: length, age, and cause of death. Therefore, the minimal analyses that have explored the perinatal category are suspect and not considered further here. One of the objectives of this research is to present a new definition of perinatal mortality that focuses on physiological indicators to determine age at death, without making assumptions about cause of death (see Chapter 3).

### Other Sources

Roughly 15% of collected carcasses had ingested some sort of foreign object, usually monofilament line. Of that 15%, about six percent died due to debris ingestion (Ledder 1986, Beck & Barros 1991). At least 11 animals died due to debris entanglement between 1978 and 1986, and roughly two percent of carcasses collected from 1974 to 1985 showed evidence of non-lethal entanglement (Beck & Barros 1991). Although reported carcass counts do not increase with increased storm activity, mark-recapture studies indicate hurricanes may reduce adult manatee survival (Langtimm & Beck 2003). Other natural causes of death include infection, digestive problems, and parasites.

From 1974 to 1978, floodgate/lock deaths made up 25% of reported manatee mortalities. That fraction has gone down considerably over the years, indicating a potential reduction in this source of mortality (Odell & Reynolds 1979, Ackerman *et al.* 1995). Floodgate/lock deaths are more prevalent in southeastern Florida, particularly in Dade County where floodgates and locks are common. Necropsies determined the animals dying from floodgates and locks probably died in one of two ways. First, the strong current caused by opening the dam pulled in the animals. The current then pinned the animals against the mouth opening (underwater) where they drowned and/or were crushed when the dam closed. Second, calves were more easily pulled through the dam opening. Subsequently, the mother may have died while trying to reach her calf. Even if the mother survived, calves may have died if they were separated from their mothers for too long (Odell & Reynolds 1979).

Lastly, recent research has suggested exposure to one or more environmental stressors could reduce a manatee's fitness. Both red tides and cold stress may suppress immunity, making manatees more susceptible to parasites and bacterial and viral infections (Bossart *et al.* 1998, Bossart *et al.* 2002b, Landsberg *et al.* 2005). In addition, nutritionally stressed animals may have a reduced ability to produce heat, making them more vulnerable to cold stress (Buergelt *et al.* 1984). Current methods in necropsy data collection do not allow for analysis of such interactions.

## Current Concerns

The two most immediate manatee conservation issues are the loss of warm water habitat and high vessel mortality (Runge *et al.* 2007b). Loss of fresh water and continued or increased red tide and hurricane activity should also be of concern.

Closure of some aging power plants and reduced spring run-off over the next 40 years are expected to reduce the number and change the distribution of warm water sites available to manatees. It is unclear how manatees will respond to such a loss. Manatees currently relying on power plants that will inevitably be shut down may have the ability to explore and find new sources of warm water. However, some opportunistic studies have shown they may not (see Laist and Reynolds (2005a) for a review). Even if the manatees move south to warmer waters, there may not be enough suitable habitat to support them, especially since habitat quality in southern regions has degraded considerably since manatees started to shift their distribution north in the mid-1950s (Laist & Reynolds 2005b, Laist & Reynolds 2005a). The most likely scenario is a shift in manatee distribution of unknown scale and an increased number of cold stress mortalities, leading to a reduced number of manatees in all regions except the northwest (Packard *et al.* 1989, Deutsch *et al.* 2003, Laist & Reynolds 2005a). It is unclear how increased exposure to cold waters will affect reproductive rates.

Watercraft collisions continue to be a serious threat to the persistence of the Florida manatee. Since Hartman (1979) first documented clear changes in manatee behavior and distribution in response to boats and divers, a complex network of sanctuaries and vessel slow-speed zones has been established throughout the state. The actual effectiveness of these management decisions has been difficult to measure. Although slow-speed zones probably reduce vessel mortalities in a given area, boaters may shift their movement patterns to avoid such zones, resulting in no net change in the number of vessel mortalities (O'Shea *et al.* 1985, Laist & Shaw 2006). In addition, changes in vessel design over the years may have increased the number and speed of boats entering shallow-water manatee habitat (Wright *et al.* 1995). Manatees do utilize no-boat sanctuaries, but it is uncertain how effective sanctuaries are at increasing manatee survival and reproductive rates (O'Shea 1995, Buckingham *et al.* 1999).

Boater compliance in slow-speed zones may also be an issue. Although an overwhelming majority of boaters support sanctuaries and slow-speed zones, one study has determined boater compliance in slow-speed zones in two counties is around 60%, and surveys estimate boaters generally violate speed zones over 50% of the time (O'Shea 1995, Aipanjiguly *et al.* 2003, Gorzelany 2004). Larger vessels tend to be more compliant, and presence of law enforcement appears to be the most effective method of increasing compliance (Gorzelany 2004, Sorice *et al.* 2007). Even when compliance rates are high, the number of non-compliant vessels is more important in areas where vessel traffic and manatee densities are high (Gorzelany 2004, McDonald & Flamm 2006).

The carcass recovery data clearly tell us at least two things: the number of vessel mortalities continues to increase, and the proportion of vessel mortalities seen in the carcass data continues to be high (Schwarz 2004a). The increasing number of vessel mortalities could be an artifact of increasing carcass detection probabilities or an increasing manatee population size. However, if the goal of sanctuaries and slow-speed zones is to reduce vessel mortality to zero, the carcass data indicate that current management strategies are clearly not effective enough (Wallace 1994).

The effects of global warming on manatee abundance are completely unknown. Increased temperatures may increase the severity and frequency of hurricanes in the area, which are known to reduce adult manatee survival rates in the Northwest (Langtimm & Beck 2003, Jiang & Perrie 2007). In addition, the severity of red tides may increase when hurricane activity causes increased nutrient runoff (Landsberg *et al.* 2005, Hu *et al.* 2006). More studies need to determine the extent to which human activities cause or increase the severity of harmful algal blooms in Florida and how such blooms may be correlated with global warming. Other effects of global warming on manatee habitat, such as changes in food and fresh water availability and warm-water habitat needs, should be researched.

As the human population continues to increase and human consumption of fresh water and agriculture continue to increase, access to fresh water will most likely continue to decline for manatees. In the south, manatee distribution may change in response to shifts in fresh water flow from Everglades restoration. In addition, subsequent changes in water control could either increase or decrease floodgate mortalities (Browder *et al.* 2005).

In general, there is a high level of uncertainty in how management actions and human-induced changes in the environment will affect the Florida manatee's long-term persistence. Current data collection methods in the carcass recovery program limit our ability to use the data to answer some important management questions. Suggested changes in necropsy data collection protocol are discussed in Chapter 6.

## Population Assessment

### Population Size and Trends

Aerial surveys have been the standard method to estimate manatee abundance, and many different methods have been used and critiqued over the years (Ackerman 1995, Lefebvre *et al.* 1995). Some aerial surveys are conducted immediately after winter cold fronts when the daily low air temperature drops below 4.5ºC, and surveys focus on counts of manatees at warm water sites (Reynolds & Wilcox 1994, Craig & Reynolds 2004).

The aerial survey technique has several limitations to overcome before aerial counts can be used to estimate actual population size. Mainly, several biases need to be quantified (Garrott *et al.* 1994). Sampling bias, the number or proportion of manatees not within the sampled area, needs to be estimated. Visibility bias, either due to observers missing manatees that are visible (perception bias) or observers missing animals that are in the study area but not visible (availability bias), also needs to be measured. In particular, those biases need to be estimated in relation to highly diverse manatee behavior, group size, and environment (Irvine & Campbell 1978, Provancha & Provancha 1988, Ackerman 1995, Lefebvre *et al.* 1995).

Several studies have tried to measure or adjust for such biases. Some studies have used water temperature as a variable to correct for "sighting probability," which is mostly sampling bias (Garrott *et al.* 1994, Garrott *et al.* 1995, Craig *et al.* 1997, Eberhardt *et al.* 1999, Craig & Reynolds 2000, Craig & Reynolds 2004). Other studies have attempted to measure both sampling and visibility bias by comparing aerial counts with ground counts, determining the detection probability of radio-tagged manatees in known locations, comparing counts between two tandem observers, performing replicate counts, or counting animals marked with special aerial survey flags (Packard *et al.* 1985, Packard *et al.* 1986, Lefebvre & Kochman 1991, Miller *et al.* 1998, Edwards *et al.* 2007). Visibility bias has even been estimated for dugongs using a dummy dugong placed at different depths and in different environmental conditions (Pollock *et al.* 2006). There have been many suggestions to reduce or measure biases, but so far no consistent method has been incorporated into survey techniques (Lefebvre & Kochman 1991, Ackerman 1995, Lefebvre *et al.* 1995, Edwards *et al.* 2007).

In general, aerial survey studies have shown an increasing trend in manatee counts at warm-water sites through the 1980s. Counts may have leveled off in the early 90s in some areas (Provancha & Provancha 1988, Garrott *et al.* 1994, Ackerman 1995, Garrott *et al.* 1995, Craig *et al.* 1997, Eberhardt *et al.* 1999, Craig & Reynolds 2000, Wright *et al.* 2002, Craig & Reynolds 2004). Numbers of manatees at warm-water sites have continued to increase along the Atlantic coast, but no studies have looked at count trends in other areas since the mid-1990s (Craig & Reynolds 2004). Such trends could be a result of increased population size, increased use of warm-water sites, improved survey methods, or any combination of those factors (Garrott *et al.* 1994, Ackerman 1995, O'Shea & Ackerman 1995).

## Population Dynamics and Population Viability Analyses

Reproductive rates have been estimated from both carcass recovery and mark-recapture data. Marmontel (1995) estimated between 22 and 39% of all mature female manatees were pregnant, whereas between 16 and 18 % were lactating based on examination of female carcasses. However, carcass reproductive status may not be a representative sample of reproductive rates in the live population since carcasses may consist of a subsample of females with overall lower fitness. In addition, pregnancies and lactation might be missed during necropsy (Schwarz 2004a). Mark-recapture studies estimated 83% of identified females along the Atlantic coast were seen at least one winter with a newborn calf between 1982 and 1992 (Reid *et al.* 1995). However, the proportion may be biased high since association with a calf is one of the ways to identify adult females. Data from captive and radio-tagged manatees suggest some variability in reproductive rates among individuals (Odell *et al.* 1995, Reid *et al.* 1995). Using mark-recapture data, Kendall *et al.* (2004) and Runge *et al.* (2004) estimated conditional reproductive rates for females over 4.5 years old. The rates estimate the probability a female will produce a calf given that she did not reproduce the previous year. Rates accounted for sighting probabilities of both calves and adult females with and without calves. Depending on the region, roughly 30 – 40% of adult females in any given year produce calves that survive to their first winter. Those percentages concur with previous studies in Crystal River, Sarasota Bay, Blue Spring, and the Atlantic (Hartman 1979, O'Shea & Hartley 1995, Rathbun *et al.* 1995, Reid *et al.* 1995, Koelsch 2001). Runge *et al.* (2004) suggest breeding rates may be biased high since the females sampled with mark-recapture techniques inherently have high winter site fidelity which may be correlated with increased breeding success.

Several studies have estimated adult survival rates using several mark-recapture techniques (O'Shea & Langtimm 1995, Langtimm *et al.* 1998, Langtimm & Beck 2003, Kendall *et al.* 2004, Langtimm *et al.* 2004, Runge *et al.* 2007b). With the exception of the Southwest region, adult survival rates are high (< 95%). See Table 5.2 for the most recent adult survival and reproductive rate estimates. Mortality rates of radio-tagged animals in the Atlantic agree with survival rate estimates from mark-recapture data (Reid *et al.* 1995). In general, adult survival rate does not change with age and is not significantly different by sex (O'Shea & Langtimm 1995, Langtimm *et al.* 1998). Although there is inter-annual variability in adult survival rates, annual changes only appear to be statistically significant in the Northwest region where years with hurricanes show reduced adult survival (Langtimm *et al.* 1998, Langtimm & Beck 2003, Langtimm *et al.* 2004). So far, reductions in annual adult survival rates have not been statistically detected during years with high numbers of reported red tide or cold stress mortalities (Langtimm *et al.* 2004, McDonald & Flamm 2006). Emigration is not included in current mark-recapture analyses, so adult survival rates may be biased low (Runge *et al.* 2004).

Survival rates for animals younger than about 4.5 years old are difficult or impossible to measure using current mark-recapture methods. Since animals are generally born in the spring or summer and mark-recapture data are collected in the winter, survival rate estimates for animals younger than about six months old can only be estimated from observation of noticeably pregnant females in one winter in connection with presence/absence of calves with those females the following winter. In both the Upper St. Johns and the Northwest regions, survival rate of a calf from late pregnancy to the following winter is about 0.6 (O'Shea & Hartley 1995, Rathbun *et al.* 1995). Many animals do not acquire unique scarring until after weaning, so mark-recapture methods are only able to follow unscarred calves during the pre-weaning period when they are highly associated with their mothers. Weaned animals only re-enter mark-recapture analysis when they have unique scars and have been seen for at least a four year period, making them adults. The only region where survival rate estimates are available for animals younger than 4.5 years old is the Upper St. Johns region (O'Shea & Hartley

1995, Langtimm *et al.* 2004). Runge *et al.* (2004) assumed the ratios of survival rates by age class were the same for all regions and used those ratios from the Upper St. Johns region to estimate young survival rates in other regions. Overall, we can expect juvenile survival rates to be more sensitive to environmental variability and to have high annual fluctuations compared to adult survival rates (Langtimm *et al.* 2004).

With few exceptions, there seems to be a slightly higher proportion of males than females identified in the wild and seen in the carcass recovery data (Hartman 1979, Rathbun *et al.* 1990, Ackerman *et al.* 1995, Marmontel 1995, O'Shea & Hartley 1995, O'Shea & Langtimm 1995, Rathbun *et al.* 1995, Reid *et al.* 1995). However, differences have never been statistically strong, so current analyses assume a 1:1 sex ratio and equal survival rates by sex.

Several population dynamics models and population viability analyses have been published for the Florida manatee. Assuming exponential growth, Eberhardt and O'Shea (1995) developed the first model and estimated population growth rates ($\lambda$) in three regions using deterministic values of adult survival and reproductive rates. They assumed the juvenile survival rate was the same for all animals under 4.5 years old. "Juvenile" survival rate was estimated from mark-recapture data in the Upper St. Johns region. The Upper St. Johns  juvenile survival rate was used for the Northwest region, and the juvenile survival rate in the Atlantic was estimated using the same ratio technique as Runge *et al.* (2004). Estimates of $\lambda$ varied between 1.01 and 1.07.

Marmontel *et al.* (1997) performed a population viability analysis for Florida manatees using survival and reproductive rates estimated from carcass recovery data.

Although they did incorporate annual variability in rates and catastrophes in their simulations, several concerns arise when reviewing their analyses. First, carcass age at death was determined using counts of ear-bone growth layers (Marmontel *et al.* 1996). Uncertainty in those age estimates was not incorporated into survival rate estimates. Second, their analyses assume the carcass sample reflects the age structure of the living population. Such an assumption does not account for differing carcass detection probabilities by age, and review of Marmontel's work reveals that the subsample of ear-bone aged carcasses is skewed toward older animals (Schwarz 2004b). As pointed out by Runge *et al.* (2004), several other assumptions and aspects of their methods make the analysis suspect. Instead of incorporating uncertainty through distributions on parameter estimates, they tested many different model scenarios. Finally, they assumed the population was in stable age distribution and assumed a population growth rate of 1.0.

Runge *et al.* (2004) developed the most recent stage-based population dynamics model focusing on female Florida manatees. To determine $\lambda$, they used adult survival and reproductive rates estimated from mark-recapture data for all four regions (Kendall *et al.* 2004, Langtimm *et al.* 2004, Runge *et al.* 2004). Non-adult survival rates for the Upper St. Johns region were also estimated from mark-recapture data (Langtimm *et al.* 2004). Non-adult survival rates were estimated using the ratio technique described above. Without incorporating environmental or demographic stochasticity, population growth rate estimates were above 1.0 for the Northwest and Upper St. Johns regions. The Southwest and Atlantic regions, the regions containing the highest proportions of the population, exhibited population growth rates at or below 1.0. Since then, significantly higher adult survival rate estimates for the Southwest region have been calculated, but no new population growth rates have been estimated (Runge *et al.* 2007b).

Using the model developed in Runge *et al.* (2004), Runge *et al.* (2007a) created a core stochastic model, expanding the previous model to include males. See Chapter 5 for more details about this model. New analyses with the model incorporated demographic and environmental stochasticity, catastrophes, density dependence, and changes in carrying capacity. The model was first used to assess population reduction risks over different time frames (Haubold *et al.* 2006). They found a 12.1% probability of a 50% decline in the next three generations and 55.5% probability of a 20% decline in the next two generations (Haubold *et al.* 2006). Results, combined with changes in state listing criteria, ultimately led to the downlisting of the Florida manatee on June 7, 2007, from endangered to threatened on the state level. The model was then used to estimate probabilities of quasi-extinction (defined as 100, 150, or 200 adult animals) for the east and west coasts over different time periods (Runge *et al.* 2007b). They quantified, through expert opinion, the impacts of loss of warm-water sites (Runge *et al.* 2007b). Assuming carcass detection is not biased by cause of death and identification of cause of death is not biased by decomposition, Runge *et al.* (2007b) used a Bayesian method to estimate the proportion of different sources of mortality affecting the population: watercraft, floodgates/locks, entanglement, and “other.” Red tides and disease were also incorporated as catastrophic events. Overall, the probability of quasi-extinction was low enough (8.6% probability of fewer than 250 adult animals on either coast in 100 years) for the U. S. Fish and Wildlife Service to recently recommend federal downlisting of the Florida manatee from endangered to threatened (U. S. Fish and Wildlife Service 2007).

Regardless of the model or type of viability analysis, several consistent conclusions have emerged. First, estimates of population growth rate are most sensitive to uncertainty in adult survival rates, and increasing the adult survival rate is the most important factor in ensuring the persistence of the species (Goodman 1981, Eberhardt 1985, Eberhardt & O'Shea 1995, Marmontel *et al.* 1997, Eberhardt 2002, Runge *et al.* 2004, Haubold *et al.* 2006, Runge *et al.* 2007a). Second, reduction of watercraft mortalities and protection of areas in and around current warm-water sources are the most effective ways to increase adult survival (Ackerman *et al.* 1995, Haubold *et al.* 2006, Runge *et al.* 2007b).

## Management

Hunting manatees was banned by Florida state law in 1893, making manatees one of the first protected wildlife species in the United States (Marine Mammal Commission 2001). Over the years, state laws have generally continued to strengthen, culminating in the Florida Manatee Sanctuary Act of 1978. Although the spirit of the law is similar to federal laws, the primary function of the Act has been to allow the state of Florida to create boat-free manatee sanctuaries and designate boat slow-speed zones. However, state laws appear to have less impact on Florida manatee protection than federal laws (Rizzardi 1997).

On the federal level, Florida manatees are currently listed as endangered under the Endangered Species Act of 1973. They are also afforded protection under the Marine

Mammal Protection Act of 1972. Federal laws carry heavier penalties for non-compliance than state laws. In addition, the federal permit review process for “taking” of manatees or loss of manatee habitat either through development or vessel activities has resulted in more rulings in favor of manatees than all other federally endangered species combined, at least through the 1990s (O'Shea & Langtimm 1995, Reynolds 1999).

Several recent developments in policy may have long-lasting effects on Florida manatee management and research. In 2005, the state of Florida adjusted the state policies for endangered species listing criteria. The state definition of endangered became equivalent to the IUCN listing definition of critically endangered. Likewise, the state definition of threatened was adjusted to fit the criteria of an IUCN listing of endangered (Haubold *et al.* 2006). As mentioned above, that change in policy, along with results from a new population dynamics model, led to the state recommendation to downlist the Florida manatee from endangered to threatened in June 2007 (Haubold *et al.* 2006). In addition, the U. S. Fish and Wildlife Service is currently considering federal downlisting (U. S. Fish and Wildlife Service 2007).

# BAYESIAN METHODS TO ESTIMATE PERINATAL MORTALITY

## Introduction

Perinatal mortalities, deaths occurring around the time of birth, can provide managers and scientists with important information on the overall condition of populations. Perinatal data has played a central role in, or pointed to changes in, overall population dynamics and growth of several species (Jarnemo & Liberg 2005, Beier *et al.* 2006, Schwartz *et al.* 2006). Studies of perinatal survival can lead to an understanding of the physiological and developmental requirements needed for successful reproduction and early survival (Linklater *et al.* 2004, Jenkins & Barten 2005, Olson *et al.* 2005). Perinatal mortality can differ or change with differing or changing environmental conditions (Courtenay & Santow 1989, Escos & Alados 1991, Lent & Davis 1993) or with exposure to organochlorines or disease (Beckmen *et al.* 2003, Gutierrez *et al.* 2005, Sepulveda *et al.* 2006). In some situations perinatal mortality is the product of a complex relationship between environmental conditions and behavior (Ono *et al.* 1987, Rothe *et al.* 1992, Frank 1997), and understanding such relationships can lead to conservation measures that increase perinatal survival (Pojar & Bowden 2004, Olson *et al.* 2005, Duncan & Holland 2006).

The Florida manatee is protected under the federal Marine Mammal Protection Act (MMPA) and the Endangered Species Act (ESA) as well as the state Florida Manatee Sanctuary Act (FMSA). Threats associated with increased boat traffic, lowered water tables, coastal development, and loss of winter warm-water habitat raise concerns for the future (U. S. Fish and Wildlife Service 2001). Systematic manatee research to aid in management decisions began in the mid-1970s. Three major sources of population data have come from aerial surveys, mark-resight studies, and collection and necropsy of carcasses.

Since its inception, the Florida Manatee Carcass Recovery and Necropsy Program has collected and necropsied almost every reported manatee carcass (with the small exception of carcasses that were reported but lost before recovery). Extensive physiological data have been recorded for over 5 800 carcasses from 1974 through 2005 in the form of necropsy reports. The reports include physiological data to identify perinatal status for only a fraction of the carcasses small enough to have possibly been perinatal. Where status is not determined, either data were not recorded or decomposition had removed any indicators of perinatal status (Schwarz 2004a).

This research focuses on two objectives: identify perinatal status for as many manatee carcasses as possible and utilize the Florida manatee carcass recovery data to explore perinatal mortality. First, I define the physiological indicators used to identify near-birth mortality based on features described in necropsy protocol documentation (Bonde *et al.* 1983). Second, after review of necropsy data to identify perinatal and non-perinatal carcasses, assumptions of perinatal status are made based on reported minimum non-perinatal and maximum perinatal lengths. Next, Bayesian models are fit to the fraction of perinatal carcasses as a function of length and month of carcass recovery. When necropsy reports lack the physiological data needed to identify perinatal status, the models and assumptions allow me to estimate the probability a given carcass is perinatal based on length and recovery month. Lastly, the models are used to give a first glance at the counts and binomial proportions of perinatal carcasses by month, region, and year by region.

## Materials and Methods

### Data and Perinatal Criteria

In the past, carcasses less than 150 cm in length for which cause of death was not human-related were given a special cause-of-death category in the carcass database: “perinatal (natural or undetermined)”. The category was intended to identify carcasses that probably died of natural causes related to pregnancy or birth or early separation from their mothers (Bonde *et al.* 1983). However, that “perinatal” cause-of-death category makes potentially incorrect assumptions about the relationships among three variables: length, age, and cause of death. In addition, that category does not address variability in the age-length relationship. The perinatal definition used in this present work focuses instead on physical indicators that can determine age at death without making assumptions about cause of death.

Physiological evidence recorded reasonably consistently in the necropsy reports allows many small carcasses to be assigned to one of two categories: died within about two weeks of birth, including stillborns (perinatal) vs. died later in life (not perinatal). A carcass was classified as “perinatal” if at least one of the following indicators was present in the necropsy report:

1. consolidated or partially expanded lungs

2. presence of fetal folds
3. umbilical cord or placenta attached
4. meconium in the digestive system
5. open or partially healed umbilicus
6. comments in the report state the animal was stillborn, newborn, premature, died at or shortly after birth, or was a fetus

A carcass was classified as "not perinatal" if at least one of the following indicators was present in the necropsy report:

1. vegetation/ingesta/digesta in the lower intestine
2. epibiota such as barnacles, algae, warts or fungus
3. permanent scars indicating completely healed wounds
4. healed broken bones
5. internal parasites such as nematodes or trematodes
6. comments in the report state the animal was not stillborn or did not die at or shortly after birth
7. at least two of the following indicators were reported: closed ductus arteriosis, healed umbilicus, milk or debris in the lower intestine, infection, closed urachus

To determine perinatal status of collected carcasses, 1,464 necropsy reports for animals ≤ 175 cm long for years 1978 – 2005 were reviewed, and presence/absence of all indicators were tallied in a database for each carcass. Reports prior to 1978 were excluded because the level of detail needed to identify perinatal status was not available from those data. When perinatal status could not be determined from physiological data, the models developed below were used to estimate the probability a carcass was a perinatal mortality based on carcass length and month of carcass recovery.

## Models

After review of necropsy reports, two justifiable assumptions could be made about the relationship between length and age at death: carcasses shorter than the minimum reported non-perinatal length were assumed perinatal, and carcasses longer than the maximum reported perinatal length were considered non-perinatal. The models investigate the probability a carcass was a perinatal mortality within the limited length window between those minimum and maximum lengths. I assumed all length measurements were accurate (did not account for uncertainty of length estimates), and I assumed the probability of necropsy reports containing physiological information sufficient for determining perinatal status was independent of perinatal status. Finally, the probability of a carcass being detected and reported was assumed the same for a given length regardless of perinatal status.

Carcasses for which perinatal status could be determined from data in necropsy reports were first stratified by month of carcass recovery. To determine the relationship between length, recovery month, and perinatal status, only carcasses within the given length window were used. For some recovery months, length distributions of perinatal carcasses were not different from those of non-perinatal carcasses. In other words, length, beyond knowledge that it was in the length window for possible perinatal death, could not provide additional information about perinatal status in those months. For such cases, a simple Bayesian analysis of the binomial proportion of perinatal carcasses in a given month was performed. The likelihood is a binomial:

$$L = \frac{T!}{(T-s)!s!} p^s (1-p)^{T-s} \tag{3.1}$$

where

$T$ = total number of carcasses of known status in a given month

$s$ = number of perinatal carcasses in a given month

$p$ = binomial proportion of perinatal carcasses in a given month

Choosing a broad conjugate beta prior distribution of $\beta eta(1,1)$ results in a beta posterior distribution for $p$ (Robert 2001):

$$p \sim \beta eta(s+1, T-s+1) \tag{3.2}$$

When overall lengths of perinatal carcasses were different than overall lengths of non-perinatal carcasses in a given month, a Bayesian logistic regression was used to model the fraction of perinatal carcasses with respect to length:

$$\Pr(Y=1) = \frac{1}{1+e^{-(\beta_0 + \beta_1 length)}} \tag{3.3}$$

Where Y=1 if an animal is perinatal, and Y=0 if the animal is not perinatal. Using animals of known perinatal status and length, Pr(Y=1) is defined as the binomial proportion of perinatal carcasses of a given length. Parameters $\beta_0$ and $\beta_1$ are unknown. Since the data are binomial, the likelihood function is a multiple of the binomial pdf:

$$L = \prod_{i=1}^{k} \frac{N_i!}{(N_i - m_i)!m_i!} \alpha^{m_i} \cdot (1-\alpha)^{N_i - m_i} \tag{3.4}$$

where

$i$ = length group

$k$ = total number of length groups

$\alpha = \Pr(Y=1)$

$N_i$ = total number of carcasses of known status within a length group

$m_i$ = number of perinatal carcasses within a length group

The variables of interest, $\beta_0$ and $\beta_1$, were given uniform priors.

Distributions of the unknown model variables ($\beta 0$ and $\beta 1$) were determined using the program MTG (Metropolis within Gibbs, developed by Dr. Daniel Goodman, Montana State University). MTG is a semi-random walk program that samples combinations of variables depending on the value of the posterior probability. If a "sample" of a combination of variables is "accepted" by the program, that combination may be used to determine posterior correlation between variables and may be stored in an output file. To prevent auto-correlation within the Markov chain samples, MTG can be set to sample at a particular interval. The output file can later be used in other analyses as a representative sample of the posterior distribution of the unknown variables. This simulation was set to output a sample of 20,000 parameter combinations at an interval of 5,000 trials.

### Regional and Temporal Comparisons

Using the carcass recovery data from 1978 - 2005, the temporal and regional trends in counts and binomial proportions of perinatal carcasses were calculated. Regions were defined from the four subpopulations described in the ESA recovery plan for

Florida Manatees: Northwest, Southwest, Atlantic, and Upper St. Johns River (U. S. Fish and Wildlife Service 2001). Although the annual timing of births for Florida manatees has not been quantified, informal compilations of evidence and studies on the reproductive biology of Florida manatees suggest most births occur from March through November (Hernandez *et al.* 1995, Marmontel 1995, O'Shea & Hartley 1995, Odell *et al.* 1995). Therefore, a year was defined as March 1st to the last day of February. Because of small sample sizes in the Upper St. Johns River and Northwest regions, annual counts and binomial proportions were only calculated for the Atlantic and Southwest regions. The models were used to estimate perinatal status for carcasses between the minimum reported non-perinatal length and maximum reported perinatal length when status was unknown.

An inference on number of perinatal carcasses by region and year was performed derived from the inference on perinatal status based on report indicators, length, and recovery month. First, all carcasses of known status were tallied accordingly. Each carcass for which perinatal status was unknown was assigned a probability of being perinatal according to the model, and then the probabilities of aggregate numbers of perinatals were calculated by numerical integration, assuming the uncertainties were independent between individuals.

Specifically, each carcass for which perinatal status was unknown was tallied as a perinatal or non-perinatal carcass based on two random numbers. One number was drawn from a uniform distribution between zero and one. The second number depended on month of carcass recovery. If the carcass was recovered during months when length could provide additional information about perinatal status, the second number was calculated from a random posterior sample of the logistic regression parameters $\beta_0$ and $\beta_1$ (depending on recovery month) combined with the carcass' length. If the carcass was collected during months when length could not provide additional information about perinatal status, the second number was drawn from a beta distribution representing the probability a carcass is perinatal based solely on carcass recovery month (Equation 3.2). If the first number was less than the second number, the carcass was tallied as a perinatal carcass. The process was repeated for all carcasses to calculate the total number of perinatal carcasses by month, region, and region-year. The entire simulation was repeated 100,000 times.

To estimate the binomial proportion of perinatal carcasses by region and year, Bayesian analysis with a binomial likelihood and a broad beta prior was once again used (Equation 3.1). However, with uncertainty in the number of perinatal carcasses ($s$ from Equation 3.1), the likelihood function for the inference on the binomial proportion of $s$ was multiplied by the probability of $s$ from the inference described above.

## Results

### Applying Criteria to the Data

Of the 1,464 necropsy reports reviewed from 1978 - 2005, perinatal status of 877 carcasses could be classified based directly on the defined criteria. Five hundred fifty-eight were classified as perinatal, and 319 were categorized as not perinatal. More than one physiological indicator was recorded in the necropsy reports for 62% of the carcasses defined as perinatal, whereas 45% of non-perinatal carcasses could be classified from more than one indicator. Of the carcasses with only one reported indicator, meconium was reported in 43% of the perinatal carcasses, and vegetation in the lower intestine was reported in 67% of the non-perinatal carcasses.

Twenty-seven, around 3%, of the necropsy reports containing information on perinatal status provided contradictory data. No particular contradictory indicators were consistently recorded together, meaning the contradictions were probably due to misidentification of indicators rather than inappropriate criteria to define perinatal status. Given the small percentage of inconsistent cases, misidentification of indicators is probably infrequent and would affect the counts of perinatal and non-perinatal carcasses equally. Inconsistent cases were not used in the model analyses. They were instead grouped with other carcasses of unknown perinatal status.

### Models

The longest reported perinatal carcass was 160 cm long, and the shortest reported non-perinatal carcass was 82 cm long. Based on the assumption that carcasses < 82 cm long were perinatal, and carcasses > 160 cm long were non-perinatal, perinatal status for another 4,141 carcasses could be determined. All but six of those carcasses were classified non-perinatal.

Figure 3.1 shows the binomial proportion of perinatal carcasses within each month for carcasses 82-160 cm long, limited to carcasses of known perinatal status. Perinatal binomial proportions are highly seasonal, with a peak in April of 0.93 and a low of 0.15 in December. Figure 3.2 compares lengths of perinatal and non-perinatal

Figure 3.1. Binomial proportion perinatal within each month for carcasses 82-160 cm long using all data for which perinatal status is known. Points are means, and bars represent one standard deviation.

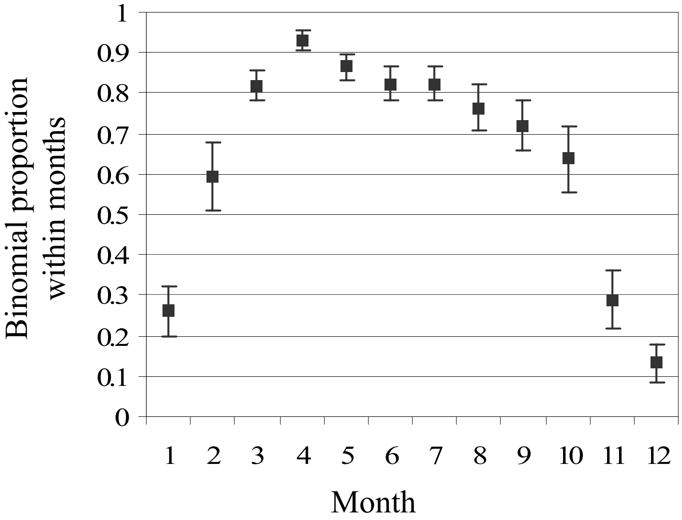
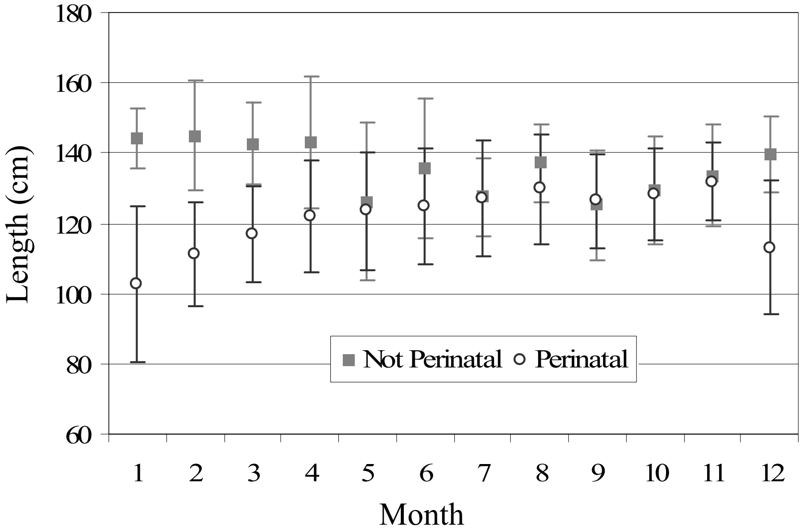


Figure 3.2. Length of perinatal and non-perinatal carcasses by recovery month using all data for which perinatal status is known. Carcasses were limited to the range 82 – 160 cm. Points are means, and bars represent one standard deviation.



carcasses in each month for carcasses of known perinatal status within the 82-160 cm window. For the months of May, July, September, October, and November, lengths do not differ between perinatal and non-perinatal carcasses. Non-perinatal carcass lengths in June and August are slightly longer than non-perinatal lengths in surrounding months. However, the lengths of non-perinatal carcasses are not different enough from perinatal carcasses within those months to provide any information about perinatal status. Therefore, the probability an animal is perinatal when it was recovered May through November can be simply estimated as the binomial proportion of perinatal carcasses 82-160 cm long in that month (Figure 3.1, Table 3.1, and Equation 3.2). Carcass length does not provide any additional information about perinatal status for those months.

Table 3.1. Values of parameters of the beta distributions used to describe the posterior probability a carcass 82 to 160 cm long is perinatal by month when length is not a parameter.

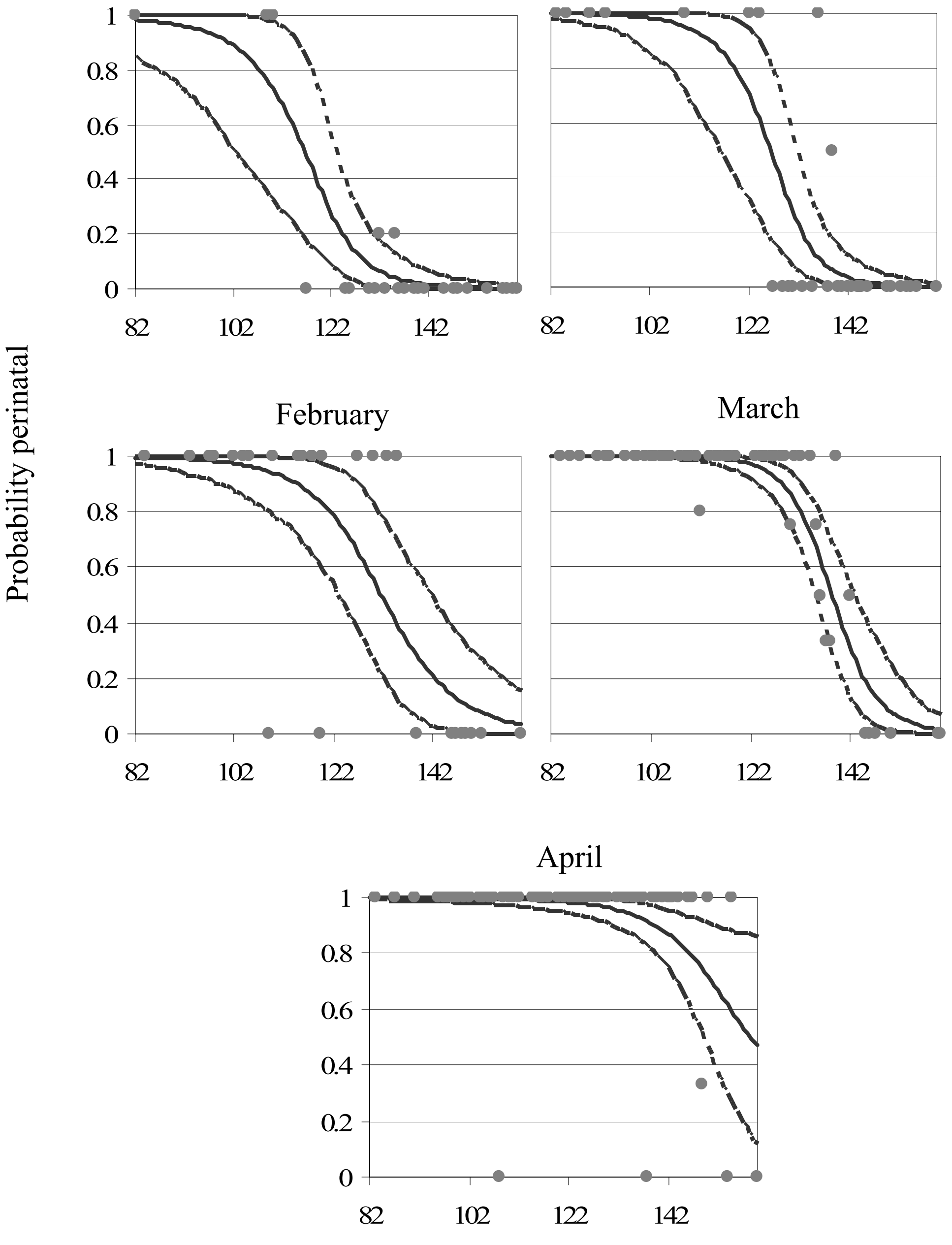
| Month | $s+1$ | $T-s+1$ |
|---|---|---|
| May | 15 | 101 |
| June | 14 | 66 |
| July | 14 | 65 |
| August | 12 | 39 |
| September | 14 | 36 |
| October | 12 | 21 |
| November | 27 | 11 |

For the months of December through April, length does provide additional information about perinatal status, so the Bayesian logistic regression analysis was performed. Final posterior distributions of $\beta_0$ and $\beta_1$ were relatively normal and strongly negatively correlated (Table 3.2). Figure 3.3 shows the posterior distribution of the resulting monthly logistic regression curves. In general, the binomial proportion perinatal increases for any given length as we move from December to April. The models provided

Table 3.2. Posterior distribution statistics for logistic regression parameters $\beta_0$ and $\beta_1$ modeling length vs. probability a carcass 82 to 160 cm long is perinatal by month.

| | $\beta_0$ | | | $\beta_1$ | | | |
|---|---|---|---|---|---|---|---|
| Month | Mode | Mean | StDev | Mode | Mean | StDev | Correlation |
| December | 19.1 | 22.4 | 8.0 | -0.16 | -0.19 | 0.07 | -0.996 |
| January | 26.4 | 30.0 | 9.3 | -0.21 | -0.24 | 0.07 | -0.997 |
| February | 16.4 | 19.5 | 6.3 | -0.12 | -0.15 | 0.05 | -0.995 |
| March | 30.7 | 31.9 | 6.4 | -0.22 | -0.23 | 0.05 | -0.998 |
| April | 17.1 | 19.0 | 6.1 | -0.11 | -0.12 | 0.04 | -0.997 |

Figure 3.3. Logistic regression analysis on length vs. binomial proportion perinatal by month for months December through April. Points are fraction perinatal by length using carcasses of known perinatal status. Solid lines represent means of logistic regression curves using the posterior samples of $\beta_0$ and $\beta_1$. Dashed lines are lower and upper 95% posterior intervals.



estimates of perinatal status for an additional 525 carcasses 82-160 cm long. The number represents 40% of all carcasses < 160 cm long.

## Regional and Temporal Comparisons

Figures 3.4 and 3.5 show the resulting counts and binomial proportions of perinatal carcasses by month and region using the models to estimate perinatal status when it is unknown. The number and binomial proportion of collected perinatal carcasses jumps dramatically between February and March. Counts and binomial proportions peak in May and then gradually decline. Although the count of recovered perinatal carcasses is low in the Northwest region compared to other regions, the binomial proportion of perinatal carcasses within the Northwest region is comparatively high.

Figure 3.4. Counts and binomial proportions of perinatal carcasses by month using the models to estimate perinatal status of animals 82 – 160 cm when status is unknown. Points are means. Bars represent minimum and maximum values for counts and standard deviations for binomial proportions.

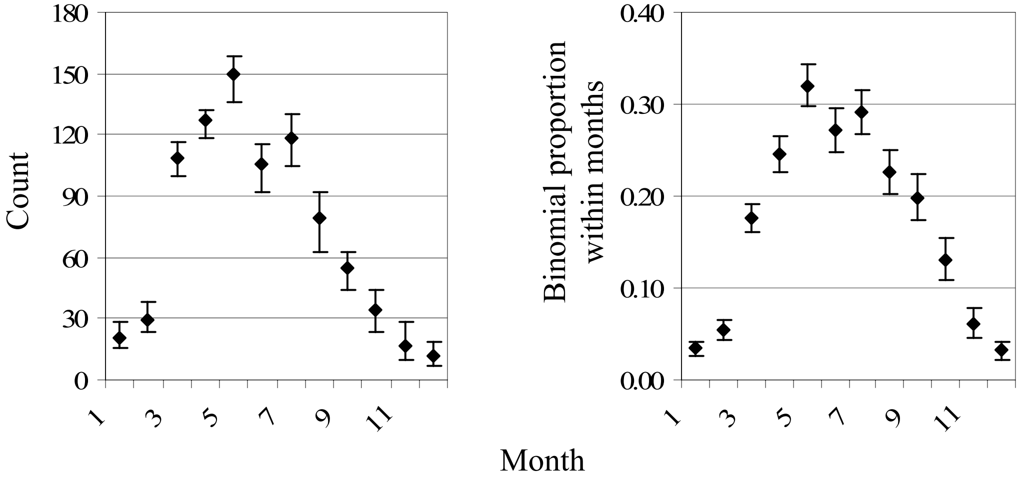
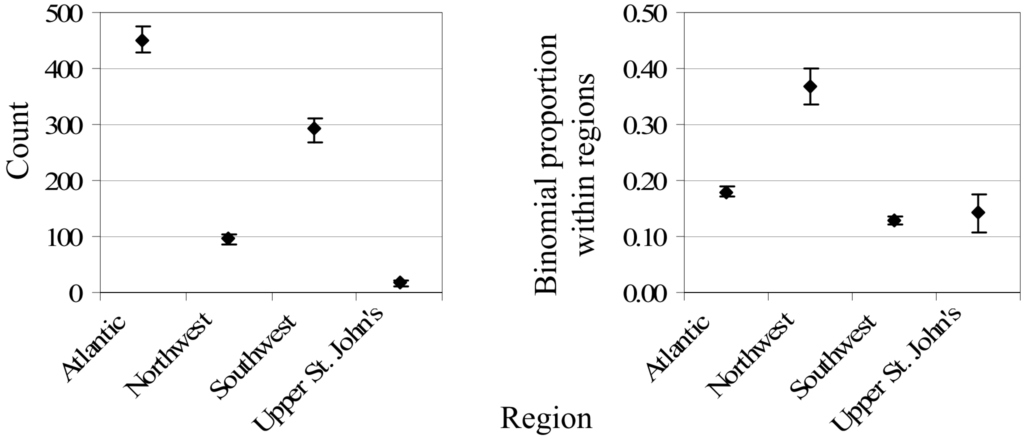


Figure 3.5. Counts and binomial proportions of perinatal carcasses by region using the models to estimate perinatal status of animals 82 – 160 cm when status is unknown. Points are means, and bars represent minimum and maximum values for counts and standard deviations for binomial proportions.



There were sufficient data to separate the Atlantic and Southwest regions by year and provide a first look at temporal trends. Counts in both regions have generally increased over the years (Figure 3.6). In general, the binomial proportion of perinatal carcasses collected in the Atlantic appears to have increased over the years while binomial proportions in the Southwest show little evidence of a temporal trend (Figure 3.7). Comparing binomial proportions with counts by year, the binomial proportion of perinatal carcasses has increased with increased counts in the Atlantic, whereas binomial proportions and counts in the Southwest do not appear to be correlated (Figure 3.8).

## Discussion

### Models

Manatee biology most likely limits the carcass lengths at which perinatal mortalities occur. Animals less than 82 cm long are probably not developed enough to

Figure 3.6. Counts of perinatal carcasses within the Atlantic and Southwest regions by year using the models to estimate perinatal status of animals 82 – 160 cm when status is unknown. Points are means, and bars represent minimum and maximum values. A year runs from March 1st to March 1st.

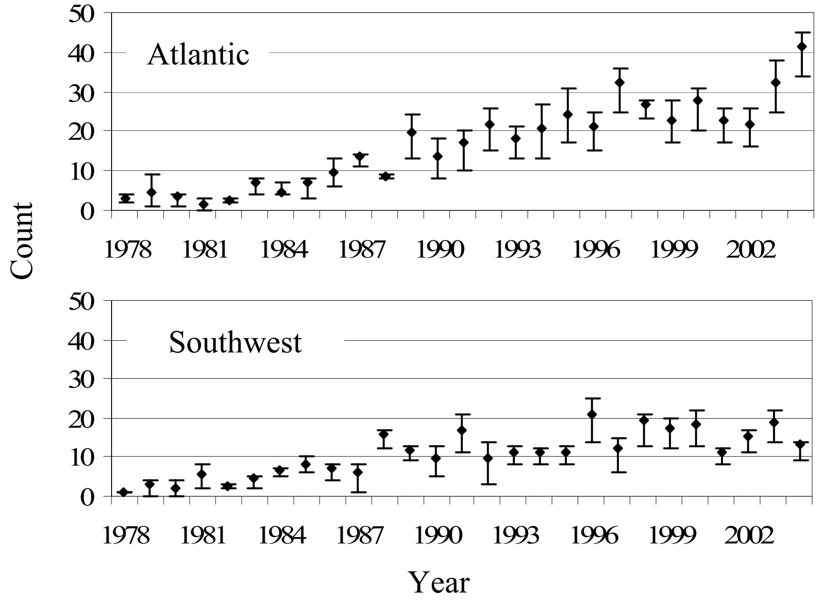


Figure 3.7. Binomial proportion perinatal carcasses within the (a) Atlantic and (b) Southwest regions by year using the models to estimate perinatal status of animals 82 – 160 cm when status is unknown. Points are means, and bars represent standard deviations. A year runs from March 1st to March 1st.

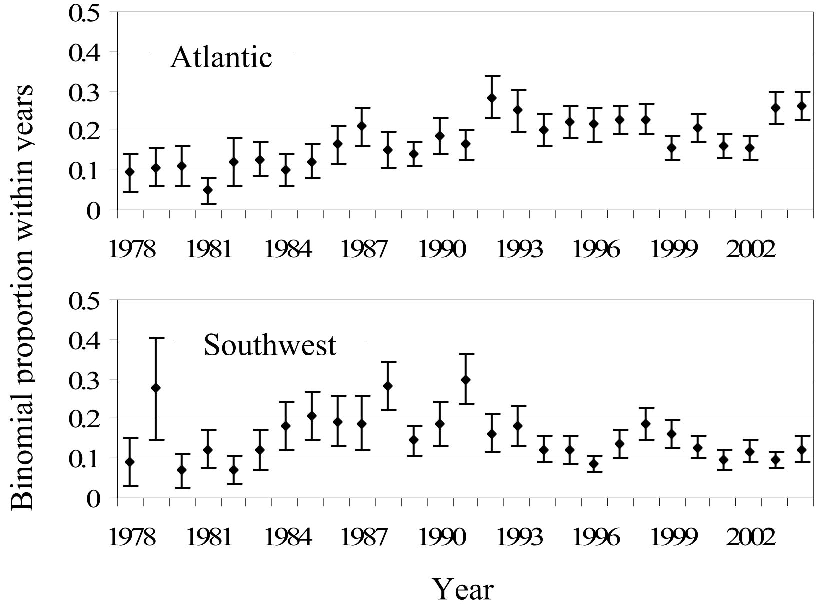
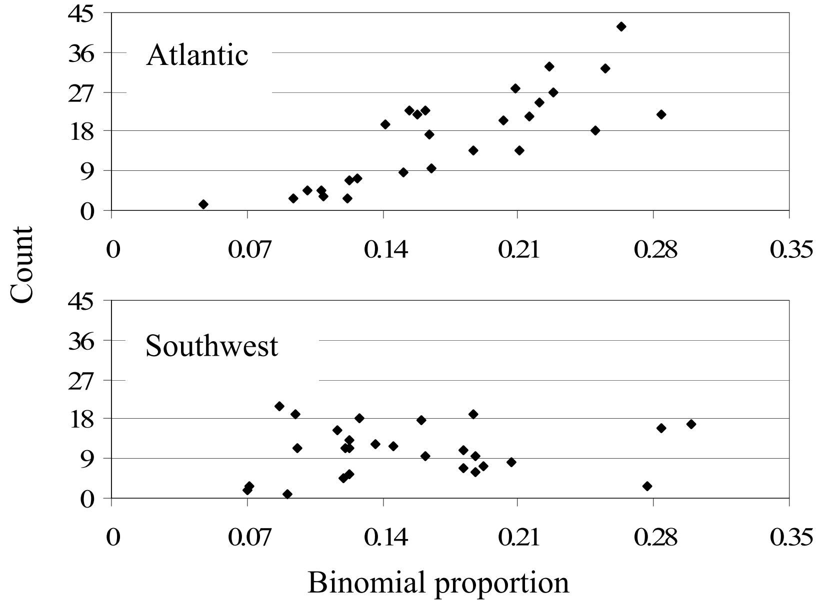


Figure 3.8. Relationship between binomial proportions and counts of perinatal carcasses within the Atlantic and Southwest regions by year using the models to estimate perinatal status of animals 82 – 160 cm when status is unknown. Points are means. Error bars have been removed to show general relationship.



survive long outside the womb (Marmontel 1995). Adult female size almost certainly limits the maximum length of newborns to near 160 cm. Therefore, the assumption that perinatal status is only uncertain from 82 to 160 cm long is almost certainly biologically based.

Results of the models show a seasonal effect on both the binomial proportion of perinatal carcasses recovered and the relationship between length and binomial proportion perinatal within the length group 81 to 160 cm. The definition of perinatal includes stillborns and fetuses, so the overall shorter lengths of perinatal carcasses in winter (December through February) are probably a reflection of premature births. In fact, with the exception of very small animals representing premature births, small animals dying in winter may be “runts” that were born the previous summer and then experience increased mortality due to cold stress compared to larger individuals of the same age. Length is a poor indicator of perinatal death for seven of the nine months when animals are most likely born, probably because of normal variability in length at birth and growth rates.

## Regional and Temporal Comparisons

Without formal analyses, one can only speculate at this point as to the cause of the regional and temporal patterns seen in this study. Comparisons of both counts and binomial proportions indicate regional differences and trends in newborn mortality rates, pregnancy rates, population sizes, non-newborn mortality rates, and detection probabilities by age. Since differences in detection probabilities by age are probably a result of differences in decomposition rates, one can assume comparisons of binomial proportions between years and regions are relatively consistent. Since smaller animals are likely to decompose more quickly, the overall binomial proportion of perinatal carcasses is probably underestimated, particularly during warm months when decomposition is most rapid. Nevertheless, the obvious changes in counts and binomial proportions of perinatal carcasses by month can tell us something about seasonal differences. For example, if other studies determine the cause and location of perinatal mortalities, potential mitigation measures to reduce those mortalities would be most effective March through July.

Regional counts roughly follow the counts one would expect given the fraction of the population found in each region. Although exact population sizes are not known, roughly four percent of the population resides in the Upper St. Johns River. Twelve percent is found in the Northwest region. The Southwest region probably contains 37% of the population, and the largest subpopulation is found in the Atlantic, about 47% (U. S. Fish and Wildlife Service 2001). However, we find a high binomial proportion of perinatal mortalities in the Northwest compared to other regions. With a much smaller dataset, O'Shea *et al.* (1985) also noticed a larger proportion of carcasses < 150 cm long in the Northwest. Adult survival rates are high, and conditional reproductive rates (which measure the probability a female produces a calf and the calf survives to winter given the female did not reproduce the previous year) are low for this region (Kendall *et al.* 2004, Langtimm *et al.* 2004). If pregnancy rates are the same by region, a low conditional reproductive rate could indicate high newborn mortality in the Northwest. High newborn mortality in addition to low adult mortality could lead to a higher binomial proportion of perinatal carcasses.

Seasonal movements may also be a factor with regional differences. Although individual movement patterns are highly variable, on the east coast manatees generally move south during winter months to avoid cold. They shift north as the water warms in spring (Deutsch *et al.* 2003). West coast migration patterns are less understood. Some females living in the Southwest region during winter may migrate north in the spring and give birth there (Moore 1951, Reid *et al.* 1991, Laist & Reynolds 2005b). However, radio-tagged female manatees in Tampa Bay in the northerly part of the Southwest region actually stayed in the area or moved south to give birth, and the four regions described in the Florida manatee recovery plan are based on known manatee movements and seasonal ranges (U. S. Fish and Wildlife Service 2001, Weigle *et al.* 2001). If a northward seasonal shift in distribution is occurring, it could explain the higher binomial proportion of perinatal carcasses in the Northwest, particularly when coupled with the low binomial proportion of perinatal carcasses in the Southwest. Conversely, the Southwest experiences lower adult survival and a higher conditional reproductive rate (Kendall *et al.* 2004, Langtimm *et al.* 2004), which could also account for the low perinatal binomial proportion in that region.

The cause of the apparent connection between counts and binomial proportions of perinatal carcasses by year in the Atlantic is also complex. Increased counts of perinatal carcasses over time could indicate an increase in the population size, newborn mortality rates, or pregnancy rates. Increased binomial proportions of perinatal carcasses over time could indicate a decrease in non-newborn mortality, or an increase in newborn mortality rates or pregnancy rates. Mark-recapture studies have shown no significant change in adult survival rates (and thus mortality rates) in the Atlantic between 1985 and 2001 (USGS 2007). Likewise, conditional reproductive rates in the Atlantic did not change significantly between 1982 and 1999 (Kendall *et al.* 2004). Further review of necropsy data show pregnancy rates (among dead animals) have not changed with time (Schwarz 2004, Schwarz unpublished data). Additional analysis of necropsy data show counts and binomial proportions of perinatal carcasses are not correlated with decomposition levels by age class (Schwarz unpublished data). If carcass detection probability by age class is related to decomposition level, relative detection probabilities by age class have not changed with time. By process of elimination, the likely factors causing the relationship between counts and binomial proportions of perinatal carcasses in the Atlantic region are limited to an increasing population size coupled with a decrease in juvenile and older-calf mortality rates over time. Although my findings are supported by aerial survey counts in the Atlantic region (Craig & Reynolds 2004), a more formal analysis of such trends is warranted.

Counts and binomial proportions of perinatal deaths show a somewhat weak pattern associated with major mortality events in the Atlantic and Southwest regions. In the winter of 1989-1990, severe cold weather killed many manatees in the Atlantic region (Ackerman *et al.* 1995). The counts of perinatal carcasses were higher for that year, but binomial proportions were the same or lower than the previous two or following two years. The same pattern holds for 1996 and 2003 in the Southwest when prolonged and extensive red tides were linked to a large number of manatee mortalities (Bossart *et al.* 1998, Flewelling *et al.* 2005). In 1982 another prolonged red tide event took place (O'Shea *et al.* 1991). However, perinatal carcass counts and binomial proportions were comparatively low that year. Given the changes in binomial proportions during the events, cold and red tide seem to affect older age classes more than the perinatal age class. More in-depth analyses should explore the role of timing and severity of such events on manatee survival.

### Possibilities for Future Research

The criteria employed to define perinatal and non-perinatal in this analysis were based on data consistently collected during Florida manatee necropsies over a 17 year period. However, other physiological indicators could be used or added to the list as long as those indicators can provide conclusive clues as to age at death. Some other indicators to consider would be

1. size of ductus arteriosis vs. size of pulmonary artery
2. open/closed foramen ovale
3. presence/absence of fetal hair
4. flexibility of skeleton
5. fussion of skull or other bone sutures
6. phase of healing of urachus: open, closed but probe patent, closed, median umbilical ligament
7. phase of healing of umbilical region:
    a. external umbilicus: open, scabbed, completely healed
    b. umbilical vessels: open, closed but probe patent, closed, medial umbilical ligament

The age at which such physiological changes occur would have to be established, and of course such physiological indicators would need to be recorded consistently in necropsy reports.

## Conclusions

This research presents a method to identify very-early-age mortality based on clear definitions, quantifiable data, and statistical models. After defining perinatal mortality and reviewing necropsy reports, 15.5% of carcasses collected between 1978 and 2005 were classified as perinatal or not-perinatal based on physiological data alone. The review resulted in some reasonable simplifications: carcasses < 82 cm long are assumed perinatal, and carcasses > 160 cm are assumed not perinatal. Perinatal status was determined for an additional 73.3% of the carcasses based on the simplifications. The Bayesian models allowed me to estimate the perinatal status of 9.3%. Although the Bayesian models were used to determine perinatal status for a relatively small percentage of the total carcass sample, those carcasses represent 40% of the sample that could possibly be perinatal (< 160 cm long). Perinatal status could not be determined for 1.9% of the carcasses, representing the carcasses with no length or physiological data. The parameters chosen to predict perinatal status emphasize manatee physiology and biology. Length by month is an indicator of physiological growth, and month itself is connected to seasonal timing of births. Having established early age-at-death for all carcasses, comparisons between regions, months, and years by region could be explored. Results show some interesting connections among regions and years that warrant further investigation.

# BAYESIAN ESTIMATION OF AGE AND AGE ERROR

## Introduction

Age estimation of individuals is often an integral part of population dynamics research, and many techniques exist to estimate age. However, overlooking error in age measurements can create inaccuracies in growth, recruitment, and mortality analyses, leading to significant consequences for species management (Beamish & McFarlane 1983, Summerfelt & Hall 1987, Leberg *et al.* 1989, Campana 2001). Therefore, when error is present in age measurements, analyses utilizing such measurements should include quantification and incorporation of error, particularly in management models (Taylor *et al.* 2000, Campana 2001, Cope & Punt 2007).

Error in age measurements is a real phenomenon when studying wild animals, where age is often approximated and sometimes is measured only after death. Age is frequently estimated using secondary physiological characteristics, such as sizes of bones or counts of bone growth layers. Such methods give rise to error, either through inconsistencies or biases in measurements or via inherent variability in the process (Gilbert & Stolt 1970, Beamish & McFarlane 1983, Hohn 1990, Kimura & Lyons 1991, Campana 2001, Stewart *et al.* 2006). In addition, limitations in resolution, and interactions between resolution and age, can produce inaccuracies in age measurements (Leberg *et al.* 1989).

The Florida Manatee Carcass Recovery and Necropsy Program has the potential to be one of the primary sources of population dynamics data for researchers and managers working to protect this endangered species. Almost all manatee carcasses reported since 1974 have been collected and necropsied, leading to a dataset of over 5,800 specimens through 2005. Marmontel *et al.* (1996) established a technique to estimate ages of Florida manatee carcasses via counts of bone layers in ear bones, calibrated using carcasses of animals previously tetracycline-marked and carcasses of known age. Although the calibration sample size was small (n = 17), there was strong evidence for one growth layer group per year, making the scale of age measurement equal to one year. Since the introduction of this technique, age has been estimated from ear-bone counts for 36% of all collected Florida manatee carcasses. The age determination method is complex and time-consuming, and sampling has been sporadic and non-random over the years (Schwarz 2004b).

The ear-bone aging method may include several sources of error. Marmontel *et al.* (1996) suggested differences in the number of ear-bone growth layers (process error) may be large due to individual variations in the formation of growth layers. Differences in lactation, pregnancy, malnutrition, mineral or dietary imbalance, enforced immobility, hibernation, or even hypothermia could all contribute to process variation. In addition, measurement error increases with age as bone is resorbed and becomes more difficult to "read." Within- and between-reader variation may also be present. Lastly, although Florida manatee births occur seasonally, with most births occurring in the spring, some fraction of births probably occur through the summer and even into the fall (Marmontel 1995). Since ear-bone growth layer counts measure age on an annual scale, they provide a relatively less accurate estimate of age for younger animals when birth month is not known.

To more-fully utilize the carcass recovery data, the goal of this research is to provide methods to estimate age, with quantified error, for the majority of collected Florida manatee carcasses. Chapter 3 describes the methods used to determine the probability a carcass died within about two weeks of birth vs. later in life, so this analysis focuses on providing age estimates for carcasses more than two weeks old. First, error is quantified for the ear-bone aging technique. Second, to estimate ages of almost all the remaining 64% of carcasses that have not been ear-bone aged, this study introduces a new method to estimate age from length. A Bayesian model estimates the Schnute growth curve parameters for Florida manatees. The method uses age-length data of carcasses that have been ear-bone aged, and the uncertainty in ear-bone age estimates calculated from the first step is incorporated into the second step. The joint posterior distribution of the Schnute parameters are then used to estimate age from length for animals that were not ear-bone aged and were subsequently not part of the calibration.

## Methods

### Data

Within the manatee carcass dataset, Marmontel *et al.* (1996) determined seven animals were of known age, either born in captivity or first identified alive as calves. My review of the manatee necropsy database revealed one other carcass of known age that had also been ear-bone aged. Data from those eight animals were used to estimate error in the ear-bone aging technique by comparing true ages with counts of growth layer groups.

In addition to the eight carcasses of known age, ages of 2,063 Florida manatee carcasses have been estimated from ear bones for the period of 1978 to 2005. During the same time period, almost all carcasses were measured for length (5,717 of 5,865 carcasses). Carcasses of known length and measured ear-bone age were used to estimate Schnute growth parameters.

To ensure the resulting Schnute curve applied only to animals greater than two weeks old, carcass data were filtered based on perinatal criteria developed in Chapter 3. Only carcasses known to have lived at least two weeks were included in the analysis. The data subset consisted of carcasses ≤ 160 cm long classified as non-perinatal based on physiological data plus all carcasses > 160 cm long.

The carcass sub-sample used to estimate the Schnute growth curve parameters does not provide a uniform distribution of animals across all age classes. Also, as mentioned before, the ear-bone aging method has not been used to estimate ages of a random sub-sample of the carcasses. For this particular analysis, the sub-sample of ear-bone aged carcasses represents a higher proportion of longer (and older) animals compared to the entire carcass sample. However, other studies have shown non-uniform and non-random sampling by age does not strongly affect estimation of growth curve parameters, particularly when sample size is large (Leberg *et al.* 1989, Cope & Punt 2007).

Data used for estimating the Schnute curves were stratified into four categories. Since growth curves often vary by sex, Schnute curve parameters were estimated separately for males and females. In addition, a seasonal pattern presented itself in the data (Figure 4.1), indicating animals collected November-March are somewhat smaller than animals collected April-October with the same ear-bone age estimate. Therefore, data were also separated into winter (Nov-Mar) and non-winter (Apr-Oct) periods. Table 4.1 shows the sample sizes used to estimate Schnute growth curve parameters, stratified by sex and season.

Figure 4.1. Measured ear-bone age vs. length for females and males separated in to winter and non-winter periods. Points are means, and bars represent standard deviations. The zero age class only includes non-perinatal animals.

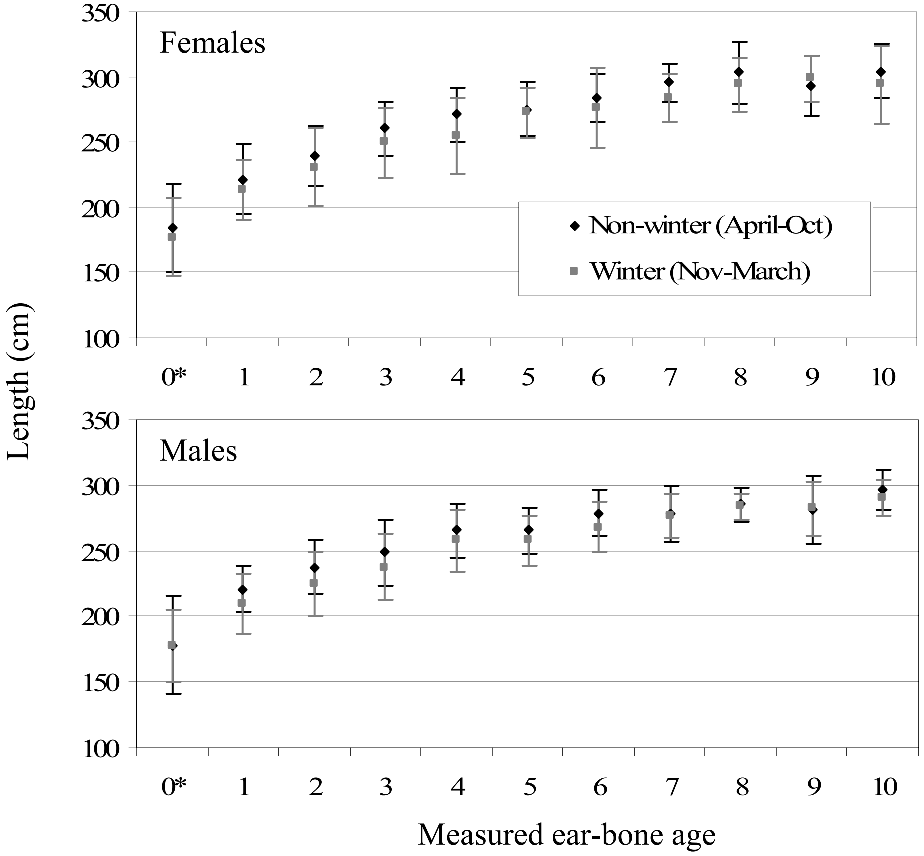


Table 4.1. Sample size for estimation of Schnute growth curve parameters.

| Sex | Season | Sample Size |
|---|---|---|
| Female | Non-winter | 335 |
| | Winter | 426 |
| Male | Non-winter | 414 |
| | Winter | 556 |

## Ear-Bone Error and Month-of-Birth Uncertainty

Although process and measurement error for ear-bone age estimates have not been formally measured, Marmontel *et al.* (1996) described an increase in bone resorption with age. The proportion of carcasses exhibiting bone resorption also increases with age. Consequently, process error increases with age. Those increases were also coupled with an increase in within-reader variability with age. Therefore, a multiplicative ear-bone error was assumed, combining both reading (measurement) error and process variation.

$$t_{earbone} = t_{trueEB} \cdot \varepsilon_t \tag{4.1}$$

where

$t_{earbone} =$ measured ear-bone age

$t_{trueEB} =$ true age on a yearly scale

$\varepsilon_t =$ ear-bone error

Ear-bone error was represented by a scaled beta distribution with beta parameters $\alpha$ and $\beta$, scaled between 0.5 and 2.0, meaning true ear-bone age could not be less than 0.5 times or greater than 2.0 times measured ear-bone age.

$$\frac{\varepsilon_t - 0.5}{2.0 - 0.5} \sim Beta(\alpha, \beta) \tag{4.2}$$

The calibration model for the estimation of earbone-aging error utilizes the eight known age animals, comparing true and measured ear-bone ages. Ear-bone error was calculated for each of the eight animals by dividing their measured ear-bone ages by their true ages ($\varepsilon_t^*$).

Bayesian analysis with a vague joint prior on *α* and *β* was used based on a joint uniform on the mean and a modified standard deviation (Gelman *et al.* 1995):

$$\left(\frac{\alpha}{\alpha+\beta}, (\alpha+\beta)^{-\frac{1}{2}}\right) \tag{4.3}$$

On the scale of *α* and *β*, the prior transforms to the following probability:

$$\pi(\alpha, \beta) = (\alpha+\beta)^{\frac{-5}{2}} \tag{4.4}$$

Thus, the joint posterior distribution for *α* and *β* is:

$$p\left(\alpha, \beta \mid \underline{\varepsilon_t^*}\right) \propto \left[\frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)}\right]^8 \cdot \prod_{i=1}^{8} \left(\frac{\varepsilon_{ti}^* - 0.5}{2.0 - 0.5}\right)^{\alpha-1} \left(1 - \frac{\varepsilon_{ti}^* - 0.5}{2.0 - 0.5}\right)^{\beta-1} \cdot \pi(\alpha, \beta) \tag{4.5}$$

where observed ear-bone error for each carcass ($\varepsilon_{ti}^*$) is the ratio of measured to “known” actual age, both on a year scale. In part because of the small sample size, the distribution of ear-bone error estimated from the posterior distributions of *α* and *β* had a tendency to be bimodal, which seemed implausible for the population distribution. To prevent such shapes, an additional constraint was put on the model, forcing the parameters *α* and *β* to be in the joint range producing only unimodal distributions on ear-bone error.

Specifically, the mode of the unscaled distribution, defined as a function of $\alpha$ and $\beta$, was restricted.

$$0.033 \le \frac{\alpha - 1}{\alpha + \beta - 2} \le 0.93 \tag{4.6}$$

Witnessing a Florida manatee birth is relatively rare, so the probability an animal was born at a given time of year must be estimated by other means. Evidence from aerial surveys, mark-recapture studies, radio-tagged animals, reproductive studies, and perinatal mortalities all show a fairly broad distribution of date of birth during the warm months (Hartman 1979, Irvine & Campbell 1979, Rathbun *et al.* 1990, Hernandez *et al.* 1995, Marmontel 1995, O'Shea & Hartley 1995, Odell *et al.* 1995, Rathbun *et al.* 1995, Reid *et al.* 1995, Weigle *et al.* 2001, Schwarz In review). However, no single study has produced definitive data on the timing of manatee births within the warm months. Therefore, a somewhat diffuse distribution was used to represent the probability an animal was born in a given month. The probability of month of birth was relatively uniform: equal for months May through September, decreasing monthly by 50% out to March and November (Table 4.2).

Both the joint posterior distribution on $\alpha$ and $\beta$ and the distribution of birth month were used to characterize age error. Real-valued true age ($t_{true}$) becomes a function of the integer true ear-bone age ($t_{trueEB}$), birth month ($M_B$), and month of carcass recovery ($M_R$):

$$M_R > M_B: \quad t_{true} = \left(12 \cdot t_{trueEB}\right) + \left(M_R - M_B\right) \tag{4.7}$$

$$M_R < M_B: \quad t_{true} = \left[12 \cdot \left(t_{trueEB} + 1\right)\right] - \left(M_B - M_R\right) \tag{4.8}$$

$$M_R = M_B: \quad t_{true} = \left(12 \cdot t_{trueEB}\right) + 0.5 \tag{4.9}$$

The model assumes carcasses are reported and collected in the same month the animals die. Florida manatee carcass decomposition is usually rapid. Carcasses are most likely reported within a month of death or not reported at all, as evidenced by the high proportion of carcasses that are "badly decomposed" at necropsy (0.56) compared to the proportion of carcasses labeled as "skin and bones" (0.02) (Schwarz 2004a).

Table 4.2. Month and the probability an animal that survived more than two weeks was born in that month. Probabilities were used in estimating ages of carcasses that had been measured for age using ear-bone growth layer groups.

| Month | Probability Born in Month |
|---|---|
| March | 0.038 |
| April | 0.077 |
| May | 0.154 |
| June | 0.154 |
| July | 0.154 |
| August | 0.154 |
| September | 0.154 |
| October | 0.077 |
| November | 0.038 |

### Schnute Growth Curve Model

The Schnute equation creates a very flexible growth curve (Schnute 1981):

$$Y(t) = f(t) = \left[ y_1^b + \left(y_2^b - y_1^b\right) \frac{1 - e^{-a(t-\tau_1)}}{1 - e^{-a(\tau_2-\tau_1)}} \right]^{\frac{1}{b}} \tag{4.10}$$

where

$t =$ age

$Y(t) =$ length or weight at age t

$\tau_1$ and $\tau_2$ = chosen and fixed ages (usually min and max possible values)

$y_1$ and $y_2$ = length or weight at ages $\tau_1$ and $\tau_2$, respectively

$a$ and $b$ = Schnute curve parameters

Parameters $a$ and $b$ represent the rate of the relative growth rate, assuming a linear acceleration or deceleration in growth rate with age. Special values or ranges of $a$ and $b$ represent often-used simplifications of the Schnute curve. The estimated curve is insensitive to the choice of reference ages, $\tau_1$ and $\tau_2$, although they must be less than the minimum recorded age and greater than the maximum recorded age, respectively.

Bayesian Estimation of Schnute Parameters with Age Error. Bayesian analysis allows us to incorporate information we have about age error into the posterior distribution of the Schnute curve parameters estimated from the subset of carcasses with measured length and ear-bone age. This is accomplished in a state-space model where the "hidden" state variable of true age is represented as a separate explicit parameter for each individual carcass (Cope & Punt 2007). The final model consists of a product of three probability functions.

Two functions describe measurement and process error in age estimates from ear-bone readings. One probability function corresponds to independent age error for individual carcasses and is a product of two "sub-functions" representing ear-bone error and uncertainty in birth month. Both ear-bone error and birth month uncertainty vary independently from each other and for each individual carcass, so the probability function of age error ($\varepsilon_A$) is a multiplicative function of the probability of ear-bone error (Equation 4.2) and birth month probability (Table 4.2) for all $n$ individuals:

$$p\left(\underline{\varepsilon_A} \mid \alpha, \beta, \underline{M_B}\right) = p\left(\underline{\varepsilon_t} \mid \alpha, \beta\right) \cdot p\left(\underline{M_B}\right) = \prod_{j=1}^{n} p\left(\varepsilon_{tj} \mid \alpha, \beta\right) \cdot p\left(M_{Bj}\right) \tag{4.11}$$

A second probability function, Equation 4.5, is needed since the parameters $\alpha$ and $\beta$ that define the probability of ear-bone error have inherent uncertainty.

The third and final probability is the likelihood function, which describes process error in the Schnute age-length relationship. The process error in this relationship represents variation in length calculated from the Schnute model, for the same age, and is different from the process error described for estimating age from ear-bones. Inspection of the data showed a relatively normal and stable (non-variable with age) distribution of length given age (Figure 4.1). Therefore, the process error ( $\varepsilon_Y$ ) for variation in length given age was assumed normal and additive with mean = 0 and unknown variance ($\sigma^2{}_Y$) and was incorporated into Equation 4.10:

$$Y\left(t_{true}\right) = f\left(t_{true}\right) + \varepsilon_Y \tag{4.12}$$

where

$$\varepsilon_Y \sim \mathrm{N}(0, \sigma^2{}_Y) \tag{4.13}$$

The likelihood function is therefore a product of $n$ normal distribution where $n$ equals the number of individual carcasses:

$$L = \prod_{i=1}^{n} \frac{1}{\sigma_Y \sqrt{2\pi}} \cdot \exp\left[-\frac{1}{2}\left(\frac{\varepsilon_{Yi}}{\sigma_Y}\right)^2\right] \tag{4.14}$$

Length measurement error can be separated from process error if it is known, but length measurement error has never been quantified for Florida manatees. However, manatee carcass lengths are measured to the centimeter, and variability in length at age is probably high among individuals. Therefore, measurement error is probably quite small compared to process variation in the Schnute relationship. For the present analysis, measurement error is inseparable from process error.

The Bayesian posterior probability function for Schnute parameters is a product of the likelihood function, the probability of age error, the probability of ear-bone error parameters, and the priors.

$$\begin{aligned} p\left(y_1, y_2, a, b, \sigma_Y^2, \underline{\varepsilon_A}, \alpha, \beta, \underline{t_{earbone}} \mid \underline{Y(t)}\right) \propto\ & p\left(\underline{Y(t)} \mid y_1, y_2, a, b, \sigma_Y^2, \underline{\varepsilon_A}, \underline{t_{earbone}}\right) \\ & \cdot p\left(\underline{\varepsilon_A} \mid \alpha, \beta, \underline{M_B}\right) \cdot p\left(\alpha, \beta \mid \underline{\varepsilon_t^*}\right) \\ & \cdot \pi\left(y_1, y_2\right) \cdot \pi(a) \cdot \pi(b) \cdot \pi\left(\sigma_Y^2\right) \end{aligned} \quad (4,15)$$

Priors on the Schnute parameters ($y_1$, $y_2$, $a$, $b$, and $\sigma_Y^2$) were assumed relatively vague:

$$\pi\left(y_1, y_2\right) \sim UNIF(80,420), \text{ with } y_1 < y_2 \quad (4.16)$$

$$\pi(a) \text{ and } \pi(b) \sim UNIF(-100,100) \quad (4.17)$$

$$\pi\left(\sigma_Y^2\right) \propto \sigma_Y^{-2} \quad (4.18)$$

Limits on the uniform priors of $a$ and $b$ were wide enough not to influence the posterior. Minimum length was determined from review of small carcasses. The smallest carcass known to have lived more than two weeks was 82 cm long (Chapter 3). The largest Florida manatee carcass ever reported was 411 cm long (Florida Fish and Wildlife Research Institute, Unpub. data), so lengths were limited between 80 and 420 cm. All other priors were vague or based on the assumptions or limitations of the Schnute curve

(Equation 4.10). Parameters *a* and *b* could not equal 0 and $y_1 < y_2$. Variable $\tau_1$ was set equal to zero, and $\tau_2$ was set to 1420 months, twice the maximum ear-bone age ever recorded. Posterior distributions and joint posterior samples of unknown variables were determined using the program MTG (Metropolis within Gibbs) developed by Daniel Goodman of Montana State University (Chapter 3).

Age Estimates with Uncertainty

To correspond with age classes used in mark-recapture studies, the probability a carcass died at a certain age was binned by one year increments starting at six months old. Animals over 4.5 years old were combined into one age class (Table 4.3). Since uncertainty in age is quite large for older and longer carcasses using either ear-bone ages or the Schnute model, a maximum possible true age was set at 80.5 years old. The defined age class bins focus on younger age classes, so change in the maximum possible true age did not affect the probability a carcass was in a particular age class.

Table 4.3. Age classes and their respective ages.

| Age Class | Age (y) |
|---|---|
| 0 | ≤ 0.5 |
| 1 | 0.5 to 1.5 |
| 2 | 1.5 to 2.5 |
| 3 | 2.5 to 3.5 |
| 4 | 3.5 to 4.5 |
| 5 | > 4.5 |

The distribution of true age was calculated for measured ear-bone ages based on the inference from the calibration data. Non-scaled ear-bone age error was drawn from a beta distribution with parameters $\alpha$ and $\beta$ which were in turn drawn from the joint posterior samples of $\alpha$ and $\beta$ from the inference on the ear-bone-age calibration data. Then the ear-bone error was rescaled to the interval (0.5, 2.0). Measured ear-bone age was divided by ear-bone error and rounded down to the nearest integer. A random month of birth was drawn in relation to the probability of birth month (Table 4.2). Then true age was calculated using Equations 4.7-4.9. The simulation was run 100,000 times for each ear-bone age and each carcass recovery month.

Given the process variation in the model, rearranging the Schnute equation to estimate age from length does not provide a precise estimate of age distribution (see Appendix A). Therefore, inference on age from length used random draws of the posterior sample of Schnute parameters from the inference on the ear-bone-age-length calibration data, and random draws of age between 0.5 m to 80.5 y. The results were tallied into bins of 1 cm lengths and into the respective age bins. The probability an animal of a given length was a particular age was estimated using the proportion of counts in each age bin for that length.

$$\gamma_{Li} = \frac{x_i}{\sum_{j=1}^{5} x_j} \tag{4.19}$$

where

$i$ = age class

$x_i$ = count of “hits” in age class i for a specific length

$\gamma_{Li}$ = proportion of “hits” in age class i for a specific length

The samples of age assume the proportion of carcasses in each age class is uniform. In other words, the Schnute model is the only information available to determine age at death. Uniform age sampling most likely overestimates carcass age at death, since the carcass sample is probably weighted toward younger animals. However, additional independent information about carcass age distribution can also be incorporated into the simulation. See Appendix B for more information on how such information was derived for each region.

Resulting estimates of age-at-death are presented for males and females in winter and non-winter months and by region for several lengths that have traditionally been used as cut-offs for age classes. Subadults have been defined as animals between 175 and 275 cm (O'Shea *et al.* 1985, Ackerman *et al.* 1995), although both males and females can be sexually mature around 240-250 cm long (Hernandez *et al.* 1995, Marmontel 1995).

## Results

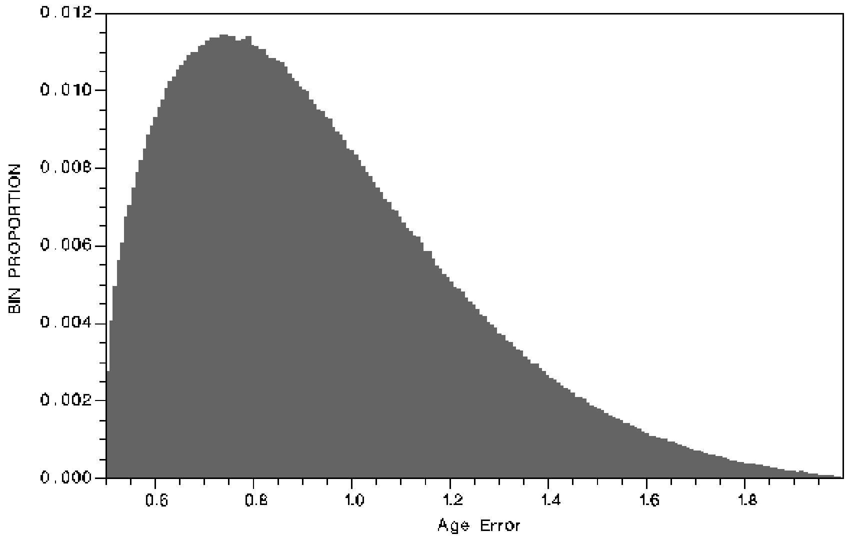
### Ear-bone Error

Using the eight known true-age carcasses to estimate ear-bone error, posterior distributions of parameters $\alpha$ and $\beta$ were left skewed and correlated (Table 4.4). Enforcing unimodality also limited posterior values of $\alpha$ and $\beta$ to > 1.0. The posterior distribution of ear-bone error resulting from joint samples of $\alpha$ and $\beta$ is wide and left-skewed with a mode of 0.73, mean of 0.93, and standard deviation of 0.28 (Figure 4.2). The results indicate measured ear-bone ages generally underestimate true ear-bone ages.

Table 4.4. Posterior statistics on parameters of the beta distribution describing error in the ear-bone age estimation technique.

| | Marginal | | | Joint | |
|---|---|---|---|---|---|
| Parameter | Mode | Mean | StDev | Mode | Correlation |
| $\alpha$ | 1.18 | 1.73 | 0.56 | 1.05 | 0.75 |
| $\beta$ | 3.5 | 4.54 | 1.99 | 2.49 | |

Figure 4.2. Posterior distribution of ear-bone error calculated from a beta distribution using joint posterior samples of $\alpha$ and $\beta$.



Schnute Growth Curve Model

Table 4.5 shows summary statistics for the marginal posterior distributions of the Schnute parameters. Correlations between $y_2$, $a$, and $b$ are particularly strong (Table 4.6). Figure 4.3 shows the resulting age-length curves produced from the joint posterior sample of Schnute parameters. The graphs do not show large differences between sexes or among seasons. However, overall length estimates are shorter in winter months compared to non-winter months for animals of the same age. Females tend to be slightly longer than males of the same age. The differences resulting from the parameter

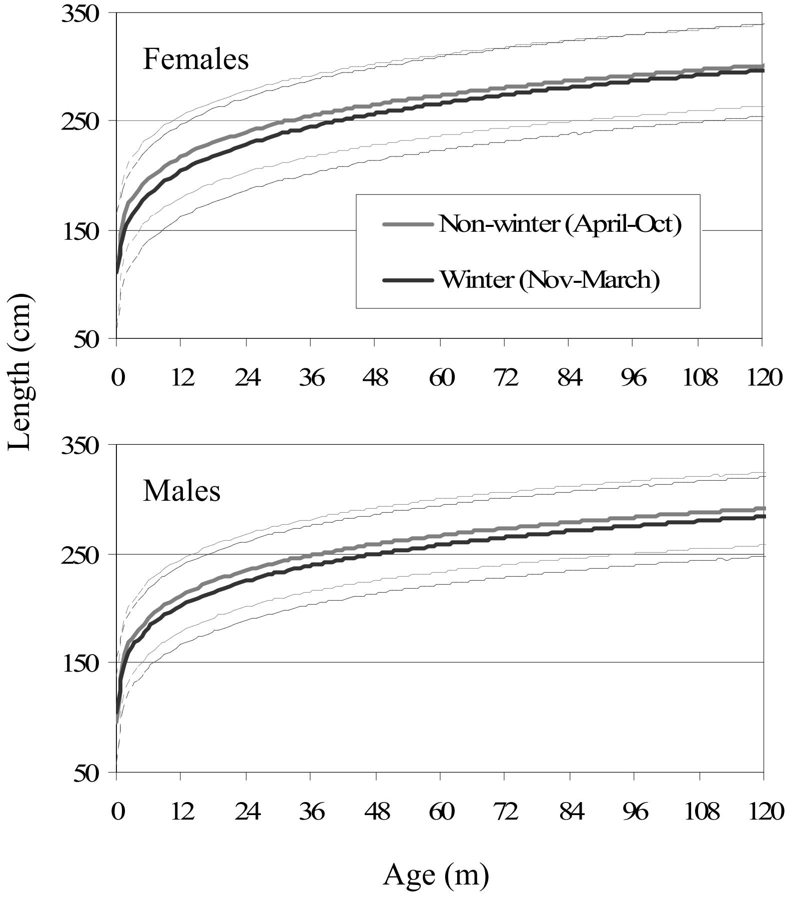
Table 4.5. Statistics on posterior distributions of Schnute curve parameters. Numbers are means with standard deviations in parentheses.

| Sex | Season | Parameter | | | | |
|---|---|---|---|---|---|---|
| | | $y_1$ | $y_2$ | $a$ | $b$ | $\sigma_Y^2$ |
| Female | Non-winter | 111.5 (18.6) | 393.7 (13.5) | 0.0012 (0.0007) | 6.81 (0.37) | 361.9 (36.4) |
| | Winter | 110.3 (18.1) | 383.7 (16.9) | 0.0022 (0.0010) | 5.74 (0.36) | 473.1 (44.3) |
| Male | Non-winter | 95.9 (11.3) | 335.7 (6.8) | 0.0042 (0.0010) | 6.56 (0.35) | 284.5 (27.5) |
| | Winter | 104.1 (15.6) | 343.3 (10.6) | 0.0030 (0.0010) | 6.30 (0.33) | 341.4 (27.6) |

Table 4.6. Posterior correlations between Schnute growth curve parameters for the four models separated by sex and season.

| Sex | Season | | Correlation | | | | |
|---|---|---|---|---|---|---|---|
| | | | $y_1$ | $y_2$ | $a$ | $b$ | $\sigma_Y^2$ |
| Female | Non-Winter | $y_1$ | 1.00 | -0.09 | 0.14 | -0.24 | 0.10 |
| | | $y_2$ | ***** | 1.00 | -0.95 | 0.63 | -0.10 |
| | | $a$ | ***** | ***** | 1.00 | -0.82 | 0.08 |
| | | $b$ | ***** | ***** | ***** | 1.00 | -0.01 |
| | | $\sigma_Y^2$ | ***** | ***** | ***** | ***** | 1.00 |
| | Winter | $y_1$ | 1.00 | -0.19 | 0.28 | -0.47 | 0.08 |
| | | $y_2$ | ***** | 1.00 | -0.95 | 0.70 | -0.09 |
| | | $a$ | ***** | ***** | 1.00 | -0.86 | 0.07 |
| | | $b$ | ***** | ***** | ***** | 1.00 | -0.03 |
| | | $\sigma_Y^2$ | ***** | ***** | ***** | ***** | 1.00 |
| Male | Non-Winter | $y_1$ | 1.00 | -0.02 | 0.06 | -0.11 | 0.05 |
| | | $y_2$ | ***** | 1.00 | -0.93 | 0.63 | -0.05 |
| | | $a$ | ***** | ***** | 1.00 | -0.83 | 0.00 |
| | | $b$ | ***** | ***** | ***** | 1.00 | 0.09 |
| | | $\sigma_Y^2$ | ***** | ***** | ***** | ***** | 1.00 |
| | Winter | $y_1$ | 1.00 | -0.09 | 0.16 | -0.33 | 0.11 |
| | | $y_2$ | ***** | 1.00 | -0.94 | 0.62 | -0.07 |
| | | $a$ | ***** | ***** | 1.00 | -0.82 | 0.05 |
| | | $b$ | ***** | ***** | ***** | 1.00 | -0.02 |
| | | $\sigma_Y^2$ | ***** | ***** | ***** | ***** | 1.00 |

Figure 4.3. Age-length curves calculated from joint posterior samples of Schnute parameters for females and males separated in to winter and non-winter periods. Thicker lines are mean values of length given age. Thinner lines represent 95% posterior intervals.

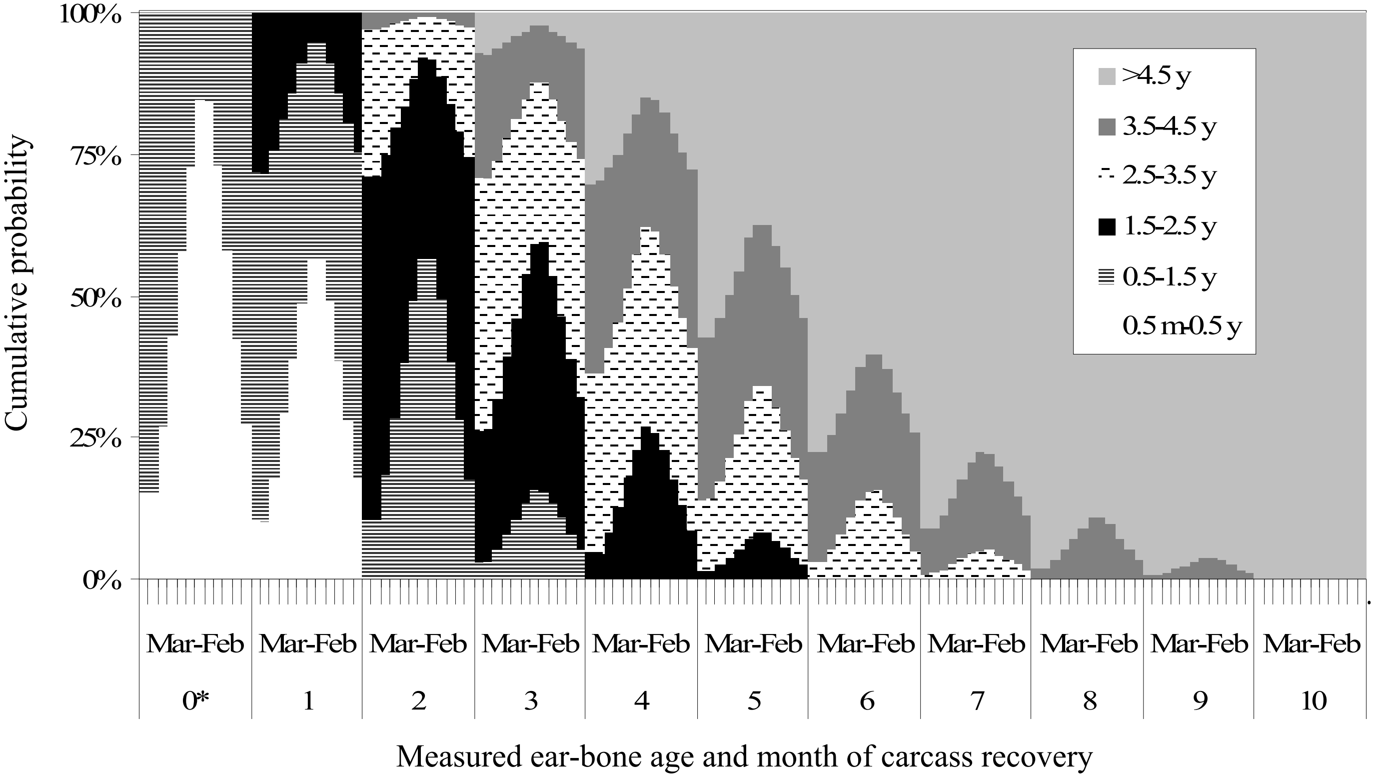


differences by sex and region become clearer when the Schnute equation is inverted to estimate age from length.

## Age Estimates with Uncertainty

Figure 4.4 displays the probability of age at death given carcass measured ear-bone age and month of carcass recovery. Values reflect the uncertainty in the ear-bone aging technique given process error and birth month. Moving from spring to summer, the

Figure 4.4. Cumulative probability of age at death given carcass measured ear-bone age and month of carcass recovery. Since births occur March through November, month values run from March to February on the x-axis. *The age class probabilities for measured ear-bone age = 0 are only valid for non-perinatal carcasses (known to have lived at least two weeks).



probability a carcass falls within a younger age class gradually increases. Carcasses with a given ear-bone age are probably the youngest when recovered in October and the oldest when recovered in March. The seasonal pattern of variability is a reflection of the variability in birth month.

Without adjusting for the carcass age distribution, age-from-length probabilities are shown in Figure 4.5. Carcasses of the same length have different age class probabilities depending on sex and season. In general, carcasses collected in winter months are older than carcasses of the same length collected in non-winter months. Males are older than females of the same length.

Incorporating carcass age distribution into the results of the Schnute model, the maximum likelihood probability for age at death given carcass length and sex, collected in a specific region during a specific time of year, is illustrated in Figure 4.6. Previous studies have used general length categories to describe manatee age. Subadults were 175 - 275 cm long, with calves < 175 cm long and adults > 275 cm long. Given what we now know about the relationship between age and length, the "subadult" length category includes carcasses of all age classes. A carcass 175 cm long still has a relatively high probability of being less than six months old, and a 275 cm long carcass has a very high probability of being more than 4.5 years old (Figure 4.6). Inspection of carcasses has shown males and females may be sexually mature when they reach a length of 240 cm (Hernandez *et al.* 1995, Marmontel 1995). The length of 240 cm represents all but the youngest age class. Regional differences in age at length are a reflection of regional differences in survival and reproductive rate estimates that determine the proportion of

Figure 4.5. Cumulative probability for age at death given carcass length, sex, and season of carcass recovery. Lengths less then 161 cm assume carcass is not perinatal (survived at least two weeks). Probabilities in this graph do not incorporate carcass age distribution.

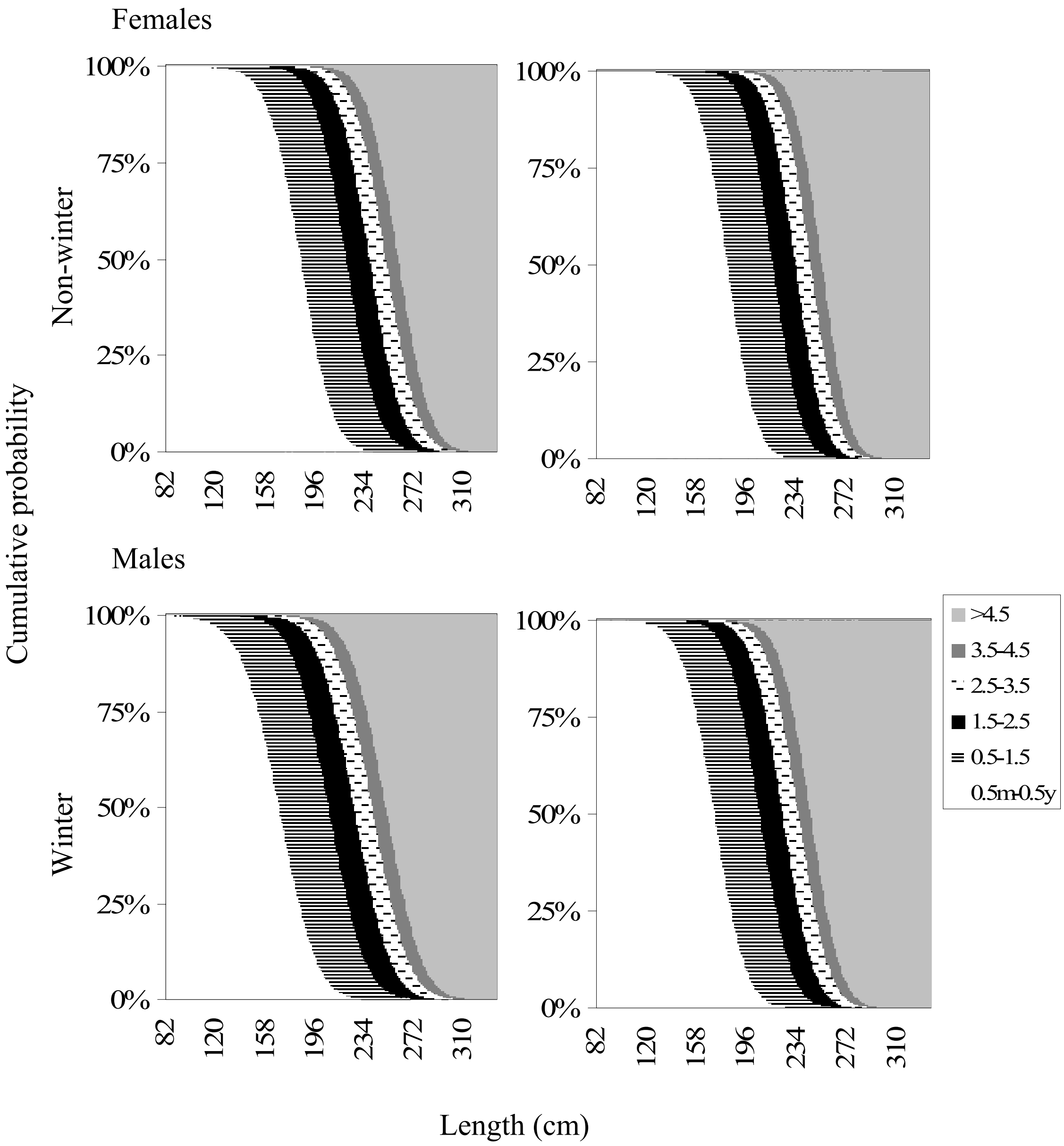
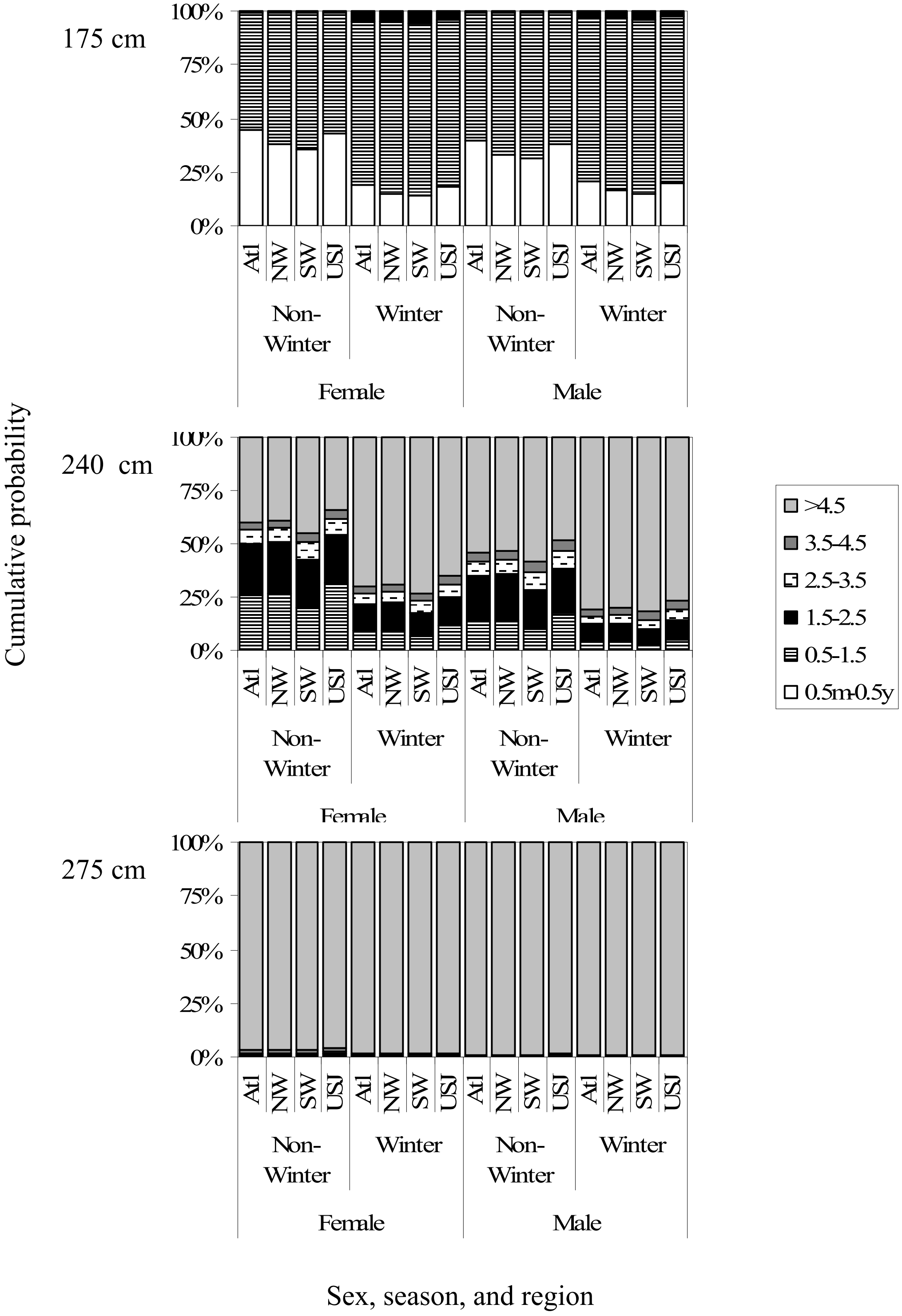


Figure 4.6. Maximum likelihood cumulative probabilities for age at death given carcass length, sex, and season of carcass recovery. The probabilities incorporate carcass age distribution by region.



carcasses in each age class (Appendix B). In general, males are older than females of the same length. Animals collected in winter are older than animals of the same length collected in non-winter.

## Discussion

Results show counts of ear-bone growth layers may underestimate carcass age. The findings are consistent with current information on bone resorption, which removes layers due to remodeling (Marmontel *et al.* 1996). The small sample size for comparisons between true age and ear-bone age limit our ability to quantify ear-bone age error for several reasons. First, it is unclear if formation of ear-bone growth layers is purely a function of age or a function of age and environmental variability. In other words, the observed striations in ear-bone layers could be due to changes in bone coloration/density from seasonal changes in water temperature and/or nutrient availability. If such information was known, along with better estimates of birth month, quantification of ear-bone age error would be more accurate. Second, the small sample size forced a constraint of unimodality on the Bayesian analysis of ear-bone age error. A larger sample size would most likely make such a constraint unnecessary (Gelman *et al.* 1995).

The goal of evaluating the Schnute growth curve was to use length and ear-bone age data of some carcasses to create a way to estimate ages of other carcasses using only length measurements. Since a sample of dead animals was used, sex and seasonal differences could represent life-history and environmental differences linked to the relationship between age, length, and cause of death, particularly cold stress in smaller animals. In other words, the resulting Schnute curves calculated for the carcass population might not be appropriate when applied to live animals. For example, shorter lengths in winter (Figure 4.1) do not represent “shrinking” animals during cold periods. In addition, animals dying in winter do not necessarily grow differently than animals dying at other times. In fact, smaller animals are probably more likely to die from cold stress perhaps due to a large surface-to-volume ratio compared to larger animals of the same age (Kleiber 1961, Hartman 1979, Buergelt *et al.* 1984, O'Shea *et al.* 1985, Reynolds & Wilcox 1986, Ackerman *et al.* 1995). Therefore, mortalities in winter are probably somewhat size-specific. Separate models by sex and season account for such differences when using the models to estimate age-at-death from length.

The Bayesian Schnute models produce a range of parameter values with their respective probabilities. In testing all possible combinations of parameters, Bayesian analysis provides the likelihood of all traditional simplifications and variations of the Schnute model without a need to statistically compare different models. Posterior distributions of $a$ and $b$ show two options for simplification or variation of the Schnute model.

Neither parameter falls below zero, suggesting a generalized von Bertalanffy equation (GVB) may be an appropriate variation (Schnute 1981). The GVB equation is just a modification of several values in the model: instead of estimating length at age = 0 ($\tau_1$), GVB estimates a hypothetical age at length = 0. Instead of estimating length at age = $\tau_2$, GVB estimates asymptotic length.

Since parameter *a* is near zero, a simplification could be considered (Equation 17 in Schnute 1981):

$$Y(t) = \left[ y_1^b + \left( y_2^b - y_1^b \right) \frac{t - \tau_1}{\tau_2 - \tau_1} \right]^{\frac{1}{b}} \tag{4.20}$$

The above growth model has no inflection point and implies manatees never reach an asymptotic length (Schnute 1981). As with other mammals of this size, continuous growth throughout life is not biologically reasonable for the manatee. Therefore, the results, once again, most likely reflect size-selective mortality, with larger animals more likely to reach an older age. The findings simply re-illustrate that the final Schnute curves do not represent true manatee growth.

## Future Research

Ideally, estimation of age error from ear-bone growth layers requires several separate analyses. To calculate process error, ear-bone growth layer counts should be compared with ages of as many individuals known since birth as possible. Also, the growth layers of each ear bone should be independently counted at least three times by the same researcher to measure within-reader variability. Third, all researchers should count the growth layers from a consistent sub-sample of ear bones, preferably the sub-sample of known-age individuals, to estimate between-reader error. Campana (2001) describes the complex, but necessary, steps needed to quantify errors in fish otolith age estimation. The same procedures apply when using any secondary physiological characteristic to estimate age, including counts of ear-bone growth layers.

When estimating age from ear bones in Florida manatees, most of the steps needed to quantify all forms of age estimation error are nearly in place. Each ear bone is read independently three to five times by the same reader. Therefore, within-reader variability could be easily quantified if all ear-bone readings were entered into a database. In addition, a more accurate estimate of process error could be calculated if more carcasses of known age were also ear-bone aged. Review of necropsy reports and the carcass recovery database revealed at least 13 new animals of known age. As of yet, ear-bone age estimates have not been determined for those carcasses. Between-reader variability, although potentially present, is assumed to be quite small in this case. Only three researchers have ear-bone aged Florida manatees, and each researcher goes through extensive training and qualitative comparison of their ear-bone readings with previous work before reading new ear bones. However, between-reader error and potential individual reader bias may also be relatively easy to quantify since all three readers are still involved in manatee research. Finally, no studies are actively attempting to determine the annual timing of births. Florida manatees are particularly difficult to study during non-winter months when they disperse from warm-water sources, so direct measurement of such a variable may not be feasible. However, compiling data from pregnant carcasses as well as secondary evidence from other studies may lead to better estimates than the estimates used in this study.

## Conclusion

This research provides methods to estimate age, with quantified error, of Florida manatee carcasses given that they survived at least two weeks. It incorporates what knowledge we have about ear-bone age estimation process error and uncertainty in month of birth. Length-to-age models were developed to estimate age from length for carcasses that have not been ear-bone aged. Those models also quantify the uncertainty involved with using length to estimate age. Results of this study will allow researchers to estimate age, with uncertainty, for 98% of the collected carcasses: 36% by ear bones, 62% by length.

# SURVIVAL RATES USING AGE RATIO TECHNIQUES

## Introduction

Management of the endangered Florida manatee has been both intense and contentious, and many important management decisions on both the state and federal levels rely almost completely on reproductive and survival rate estimates (U. S. Fish and Wildlife Service 2001). Mark-recapture studies have provided estimates of some survival and reproductive rates by region, using natural markings and scars mostly from vessel collisions to identify individual manatees (Beck & Reid 1995, Kendall *et al.* 2004, Langtimm *et al.* 2004, Runge *et al.* 2004, Langtimm In review). Apart from the small subpopulation in the Upper St. Johns River where all survival and reproductive rates have been estimated, mark-recapture techniques are unable to measure survival rates of young manatees (> 4.5 years old) because many young animals lack unique scarring. Using the known survival rates from the Upper St. Johns River and adult survival rates from the other regions, Runge *et al.* (2004) estimated survival rates for younger age classes by assuming the ratio of young survival rates to adult survival rates was the same for all regions.

The mortality data collected through the Florida Manatee Carcass Recovery and Necropsy Program is another potential source of population dynamics data. Almost every reported Florida manatee carcass has been collected and necropsied since 1974, leading to a dataset of over 5,800 carcasses through 2005. However, the recovery program relies on the public to report carcasses, and the number (or percentage) of carcasses that go unreported is unknown. In addition, the percentage of unreported carcasses may be different depending on a suite of potential factors including cause of death, region, sex, age, and year. Therefore, without detection probabilities, analysts can only make limited use of such an important and extensive dataset.

This research uses an age ratio method to first determine relative detection ratios by age for the Upper St. Johns region. Relative detection ratios are particularly appealing to many researchers because, although overall detection probabilities may change over time and space, the detection probability of one group in relation to another group should be more constant (Udevitz & Pollock 1995). Assuming relative detection ratios by age class are similar for all regions, survival rates of young animals in other regions can then be estimated from age ratios. The technique utilizes data from several sources: carcass counts by age class, live age distribution, and known survival and reproductive rates. The results of this analysis are designed to be used in pre-existing population dynamics models, and the methods incorporate uncertainty in all parameters.

## Methods

### Age Ratio

The model described here is a modification of the change-in-ratio method originally designed to estimate population sizes (Udevitz & Pollock 1991, 1992, 1995), and White *et al.* (1996) first described using the age ratio technique to quantify survival rates. Although previous studies have incorporated uncertainty in the number of live animals in the population, earlier research has assumed all dead animals have been accounted for. This work expands to include uncertainty in the number of collected carcasses and, to my knowledge, is the first attempt at using the age ratio method with known survival rates to estimate unknown survival rates.

When all dead animals in a population are recovered, the number of collected carcasses in age class $i$ ($C_i$) for a given time period is a function of the number of animals alive in that age class ($A_i$) at the beginning of that time period and the mortality rate (one minus the survival rate ($s_i$)):

$$C_i = A_i \cdot (1 - s_i) \tag{5.1}$$

If only a fraction of the carcasses are collected, the number of collected carcasses in a given age class ($C_i^*$) becomes a function of the true number of carcasses arising from mortality and an age-specific detection probability ($d_i$):

$$C_i^* = C_i \cdot d_i = A_i \cdot (1 - s_i) \cdot d_i \tag{5.2}$$

Creating a ratio to compare different age classes leads to:

$$\frac{C_i^*}{C_j^*} = \frac{A_i \cdot (1 - s_i) \cdot d_i}{A_j \cdot (1 - s_j) \cdot d_j} \tag{5.3}$$

The age ratio technique has several unique characteristics that make analysis easy and flexible. First, counts of carcasses and/or live animals in each age class are not necessarily needed; proportions in each age class can be used. In fact, proportions should be used to represent any uncertainty in the ratio of live animals (White *et al.* 1996). Second, a relative detection ratio ($d_i^*$) can be calculated as a combination of known counts (proportions) of live animals, counts (proportions) of carcasses, and survival rates. Therefore, exact detection probabilities by age class are not required.

$$d_i^* = \frac{d_i}{d_j} = \frac{A_j \cdot \left(1 - s_j\right) \cdot C_i^*}{A_i \cdot \left(1 - s_i\right) \cdot C_j^*} \tag{5.4}$$

Lastly, we can utilize the information we have about one age class to estimate variables for another age class. For example, survival rates for one age class can be determined if four parameters are known: counts (proportions) of live animals, counts (proportions) of dead animals, relative detection ratio by age class, and survival rate for one age class.

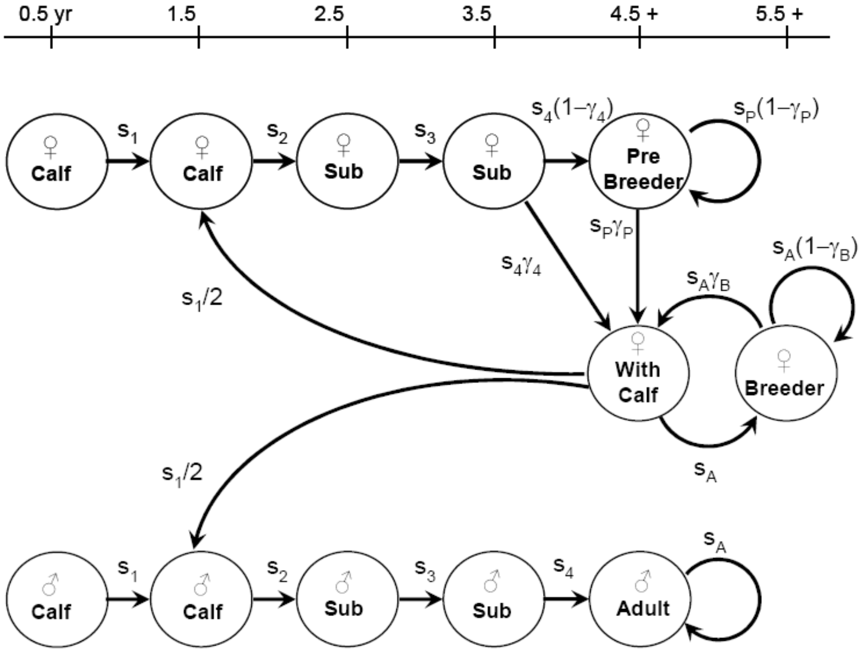
$$s_i = 1 - \frac{C_i^* \cdot A_j \cdot \left(1 - s_j\right)}{C_j^* \cdot A_i \cdot d_i^*} \tag{5.5}$$

Population Dynamics Model

This research compliments ongoing analysis of Florida manatee population dynamics. Utilizing mark-recapture data, Runge *et al.* (2007a) uses a stage-based model to predict extinction risks for the manatee (Figure 5.1). The following Leslie matrix describes the model in mathematical terms:

$$\begin{bmatrix}
0 & 0 & 0 & 0 & \frac{1}{2}s_1 & 0 & 0 & 0 & 0 & 0 \\
s_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & s_3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & s_4\left(1-\gamma_4\right) & s_P\left(1-\gamma_p\right) & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & s_4\gamma_4 & s_P\gamma_p & 0 & s_A\gamma_B & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & s_A & s_A\left(1-\gamma_B\right) & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{1}{2}s_2 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & s_2 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & s_3 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & s_4 & s_A
\end{bmatrix} \tag{5.6}$$

Figure 5.1. Life-history diagram for the stage-based model (Runge *et al.* 2007a)



The model is based on six survival rates ($s_i$) and three reproductive rates ($\gamma_i$). Mark-recapture data are collected in the winter when manatees assemble at warm water sources, and manatees are born in the spring or summer. Therefore, age classes were based on a yearly cycle of data collection starting at an assumed age of six months (Table 5.1). Survival rates of animals younger than six months are actually folded into reproductive rates. Animals older than 4.5 years are grouped together as “adults.” Sex ratio is assumed 1:1, and survival rates are assumed equal for females and males. Although the model allows for different survival rates by reproductive state, current analyses assume adult females have the same survival rate ($s_A$) regardless of reproductive state: pre-breeder (state P), with calf (state C), or resting breeder (state B). For more information on this model, see Runge *et al.* (2004) or Runge *et al.* (2007a).

Table 5.1. Age classes and the probability a carcass died within a given age class. Probabilities are combinations depending on the probability a carcass dies within two week of birth ($\theta$) and the probability a carcass died at a certain age ($i$) given it did not die within two weeks of birth ($\rho_i$). * = If a carcass was classified as $\leq 0.5$ years old, it was not used to estimate survival rates.

| Age Class | Age (yrs) | Probability of Carcass Age at Death |
|---|---|---|
| 0* | $\leq 0.5$ | $\theta+(1-\theta)\,\rho_0$ |
| 1 | 0.5 to 1.5 | $(1-\theta)\,\rho_1$ |
| 2 | 1.5 to 2.5 | $(1-\theta)\,\rho_2$ |
| 3 | 2.5 to 3.5 | $(1-\theta)\,\rho_3$ |
| 4 | 3.5 to 4.5 | $(1-\theta)\,\rho_4$ |
| Adult | > 4.5 | $(1-\theta)\,\rho_5$ |

### Data Selection and Stratification

Analyses and data were stratified by age, region, and time period to correspond with published survival rates and population dynamics models for the manatee. Analyses were performed separately for the four regions defined in the Florida manatee recovery plan: Atlantic, Northwest, Southwest, and Upper St. Johns River (U. S. Fish and Wildlife Service 2001). Since birthing occurs in the spring and summer and mark-recapture data (the source of survival and reproductive rate estimates) are collected in the winter, a year was defined as March 1st to February 28th.

In addition, analyses were restricted to years when adult survival rates had been estimated from mark-recapture data. For the Northwest and Atlantic regions, adult survival rates are available for 1986-2000 (Runge *et al.* 2007b). Analysis in the Southwest is limited to 1995-2000 (Langtimm *et al.* 2004). Survival rates for all age classes are only available for the years 1990-1999 in the Upper St. Johns region (Langtimm *et al.* 2004). However, adult survival rates in this region showed no significant annual variability from 1979-2001 (Langtimm *et al.* 2004). In addition, this subpopulation is not regularly exposed to conditions that cause mass mortality events, such as red tides and cold stress, which could dramatically change survival rates. Therefore, to increase sample size in the Upper St. Johns region, survival rates for all age classes were assumed to apply to almost all years when carcass data were collected (1978-2004).

For all regions, variables were estimated by combining years. Grouping years together assumes constant survival rates over those years, and the ratio of live animals represents the ratio of live animals over the entire time period instead of the beginning of the time period.

### Survival and Reproductive Rates from Mark-Recapture Data

Table 5.2 lists measured survival and reproductive rates for the four regions and the corresponding years of data analysis. Kendall *et al.* (2004) and Runge *et al.* (2004) estimated most reproductive rates for all four regions using mark-recapture data. Reproductive rates were used here to estimate proportion alive in each age class (see below).

Adult survival rates have been estimated for all four regions (Langtimm *et al.* 2004, Runge *et al.* 2007b). Results have shown little-to-no temporal variability in adult survival rates for the Atlantic, Southwest, and Upper St. Johns regions. In the Northwest, severe storms may lower annual adult survival rates (Langtimm & Beck 2003, Langtimm *et al.* 2006). For the small subpopulation in the Upper St. Johns region, survival rates for

Table 5.2. Regional survival and reproductive rates, estimated from mark-recapture data. Uncertainty column is roughly the 95% confidence interval.

| | Atlantic | | Northwest | | Southwest | | Upper St. John's River | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Estimate | Uncertainty | Estimate | Uncertainty | Estimate | Uncertainty | Estimate | Uncertainty |
| $s_1$ | | | | | | | 0.81[d] | (0.73, 0.87) |
| $s_2$ | | | | | | | 0.92[d] | (0.83, 0.96) |
| $s_3$ | | | | | | | 0.96[d] | (0.92, 0.98) |
| $s_4$ | | | | | | | 0.96[d] | (0.92, 0.98) |
| $s_P = s_A$ | 0.96[a] | (0.94, 0.98) | 0.96[a] | (0.94, 0.97) | 0.91[d] | (0.87, 0.94) | 0.96[d] | (0.94, 0.98) |
| $\gamma_4$ | | | 0.00[c] | (0.00, 0.29) | | | 0.21[c] | (0.07, 0.42) |
| $\gamma_P$ | | | 0.38[c] | (0.18,0.62) | 0.30[c] | (0.13, 0.53) | 0.61[c] | (0.51, 0.71) |
| $\gamma_B$ | 0.38[b] | (0.29, 0.47) | 0.43[b] | (0.22, 0.54) | 0.60[c] | (0.42, 0.75) | 0.61[c] | (0.51, 0.71) |

[a] Runge *et al.* (2007b)
[b] Kendall *et al.* (2004)
[c] Runge *et al.* (2004)
[d] Langtimm *et al.* (2004)

all age classes have been estimated (Langtimm *et al.* 2004). Estimated survival rates for all age classes from the Upper St. Johns region were used to calculate relative detection probabilities. Adult survival rates estimated in the other three regions were then used to estimate survival rates of younger age classes in those regions. When calculating the values of other parameters, survival and reproductive rates were drawn independently and randomly from logit-normal distributions as described by Runge *et al.* (2004).

### Carcass Counts by Age Class

Estimation of individual carcass age involves several different models. Models have been created that estimate the probability a carcass died within about two weeks of birth ($\theta$) vs. later in life (Chapter 3). The models use carcass length and month of carcass recovery to estimate very-early age at death. Depending on the data available, another set of models estimates the probability a carcass is a certain age given it did not die within two weeks of birth ($\rho_i$). One model estimates age from ear-bone growth layers and month of carcass recovery. Other models use carcass length and month of carcass recovery to estimate age (Chapter 3). Ultimately, the probability a carcass died in any age class is a combination of the different probabilities (Table 5.1). See Chapters 3 and 4 for more details on these models.

To determine carcass counts by age class, each carcass was randomly assigned an age based on the probability of its age of death from the models above. The carcass was then tallied in that age class. The final tally of all carcasses collected within the given time periods then provided a random estimate of $\underline{C}^*$.

Proportion Alive by Age Class

Population size has not been estimated for Florida manatees, and counts of live animals by age class have been even more difficult to determine. Therefore, proportion alive in each age class is used here. Some limited information about the different subpopulations can help us estimate the live age distribution (with large levels of uncertainty).

Samples of the stage distribution for all regions were calculated as the dominant eigenvector (ev) of the Leslie matrix in Equation 5.6. The proportion in each age class ($A_i$) is a function of those eigenvectors: grouping males and females together by age class, combining all adult females regardless of reproductive status, and accounting for the fact that first year calves are counted with their mothers (Table 5.3). As in Runge *et al.* (2007a), age class 3 survival rate was assumed equal to age class four survival rate ($s_3 = s_4$), and age class 4 reproductive rate was limited to less than pre-breeder reproductive rate ($\gamma_4 < \gamma_p$).

Table 5.3. Age classes and stable age distribution of live animals calculated as a function of the dominant eigenvector of the stage-based Leslie matrix.

| Age Class | Stable Age Distribution |
|---|---|
| 1 | $ev_5/\Sigma$ |
| 2 | $(ev_1 + ev_7)/\Sigma$ |
| 3 | $(ev_2 + ev_8)/\Sigma$ |
| 4 | $(ev_3 + ev_9)/\Sigma$ |
| Adult | $(ev_4 + ev_5 + ev_6 + ev_{10})/\Sigma$ |

$$\Sigma = \sum_{i=1}^{10} ev_i + ev_5$$

Age distribution of live animals was estimated using measured adult survival and reproductive rate estimates and wide uniform distributions for unknown survival and reproductive rates (Table 5.4). Using age-specific survival and reproductive rate estimates from Runge *et al.* (2007a), maximum and minimum values on uniform distributions for estimated survival rates were determined from 500,000 simulated draws from logit-normal distributions. When age class 4 reproductive rates ($\gamma_4$) were unknown, a uniform distribution between 0.0 and 0.5 was used. Since age class 4 represents a small fraction of potentially reproductive females, the distribution of the reproductive rate for this age class does not significantly affect age distribution results. However, reproductive rates for age class 4 are thought to be low. Pre-breeder reproductive rate ($\gamma_p$) in the Atlantic region is also unknown, so a wide uniform distribution represented that rate. As in Runge *et al.* (2007a), known survival and reproductive rates were drawn from logit-normal distributions.

## Simulations

Relative detection ratios of adults compared to non-adults were calculated by plugging random samples of $\underline{A}$, $\underline{s}$, and $\underline{C}^*$ from the Upper St. Johns region into Equation 5.4. Samples of $\underline{C}^*$ were drawn independent of $\underline{A}$ and $\underline{s}$. Samples of $\underline{A}$ and $\underline{s}$ were correlated since $\underline{A}$ is the stable age distribution at $\underline{s}$. Simulations were run to create 500,000 independent and random samples of $\underline{d}^*$ that were then used to estimate survival rates of younger age classes in the other three regions.

Table 5.4. Regional survival and reproductive rate distributions used to estimate the proportion alive in each age class. U = uniform distribution with minimum and maximum values shown. *Logit-N* = Logit-normal distribution with mean and standard deviation shown.

| Parameter | Atlantic | Northwest | Southwest | Upper St. Johns River |
|---|---|---|---|---|
| $s_1$ | *U*(0.40,0.96) | *U*(0.38,0.96) | *U*(0.33,0.95) | *logit-N*(1.45,0.24) |
| $s_2$ | *U*(0.30,0.996) | *U*(0.29,0.996) | *U*(0.20,0.99) | *logit-N*(2.38,0.42) |
| $s_3 = s_4$ | *U*(0.84,0.99) | *U*(0.90,0.98) | *U*(0.70,0.97) | *logit-N*(3.17,0.29) |
| $s_P = s_A$ | *logit-N*(3.17,0.29) | *logit-N*(3.07,0.16) | *logit-N*(2.26,0.23) | *logit-N*(3.17,0.29) |
| $\gamma_4$ | *U*(0.0,0.5) | *logit-N*(-6.91,3.06) | *U*(0.0,0.5) | *logit-N*(-1.34,0.50) |
| $\gamma_P$ | *U*(0.01,0.99) | *logit-N*(-0.49,0.45) | *logit-N*(-0.83,0.45) | *logit-N*(0.45,0.21) |
| $\gamma_B$ | *logit-N*(-0.49,0.19) | *logit-N*(-0.29,0.23) | *logit-N*(0.39,0.34) | *logit-N*(0.45,0.21) |

Random samples of $\underline{A}$, $s_A$, $\underline{C}^*$ and $\underline{d}^*$ were used in Equation 5.5 to estimate survival rates of animals < 4.5 years old in the Atlantic, Northwest, and Southwest regions. All samples of vectors and $s_A$ were independent of each other, producing 100,000 output samples of $s_i$, $i = 1,2,3,4$. Simulations were programmed in Fortran 90 using a Compaq compiler with IMSL libraries or Microsoft Visual Studio.

To compare resulting survival rate distributions with other studies, survival rate samples were converted to the logit-scale and the mean and standard deviation were calculated. Parameters of a beta distribution estimated from the mean and mode of the sample were also explored and compared with the actual sample distribution.

## Results

Figure 5.2 shows the calculated marginal proportion alive in each age class by region. The Atlantic and Northwest regions have roughly the same distributions, whereas the Southwest region has a slightly higher proportion in age classes 2, 3, and 4 and a lower proportion in the adult age class. Because all reproductive and survival rates are known for the Upper St. Johns region, uncertainty in the marginal proportion alive in each age class is lower for that region.

Table 5.5 shows the count of collected carcasses and the fraction of those carcasses that were determined to be ≤ 0.5 years old in each region for the years of analysis. Counts of collected carcasses were high in the Atlantic and Southwest regions compared to the Northwest and Upper St. Johns regions. The fraction of carcasses ≤ 0.5 years old was highest in the Northwest. The fraction of carcasses ≤ 0.5 years old was roughly the same for the Atlantic and Upper St. Johns regions, while the Southwest had the lowest fraction of carcasses in the ≤ 0.5 years old age class. Figure 5.3 shows the marginal fraction of collected carcasses in each age class by region, excluding carcasses ≤ 0.5 years old. The fraction of carcasses collected in each age class is roughly the same by region.

Figure 5.2. Marginal proportion alive in each age class by region (means ± 1 standard deviation).

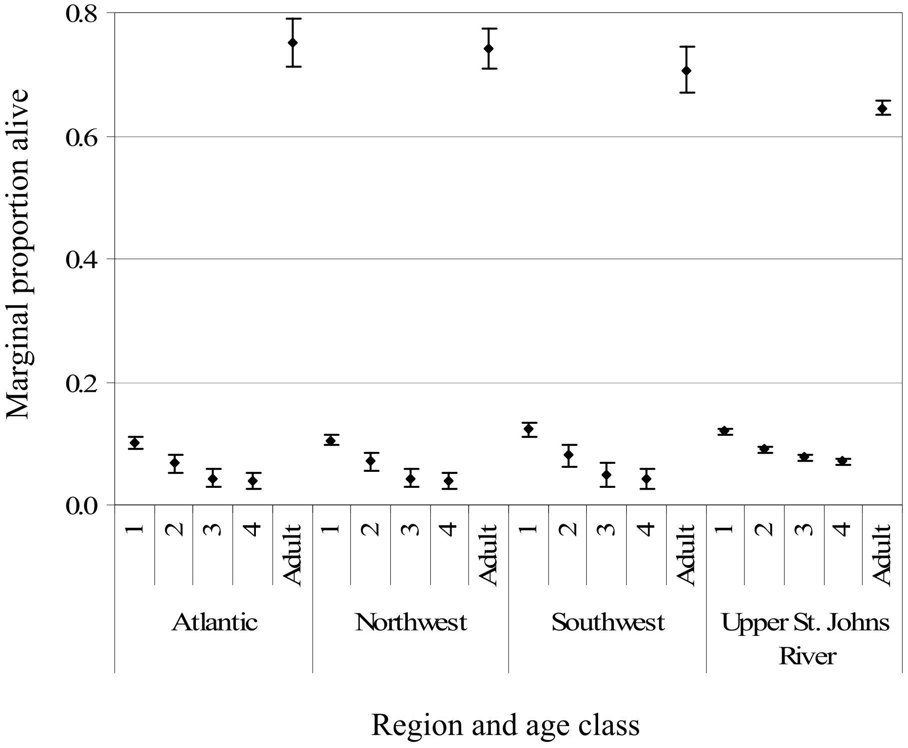


Table 5.5. Carcass count and fraction of those carcasses ≤ 0.5 years old by region for specific years. Variance in fractions is due to uncertainty in age class for each carcass.

| Region | Years (March to March) | Carcass Count | Fraction ≤ 0.5 years old Mean (1 StD) |
|---|---|---|---|
| Atlantic | 1986-2000 | 1,548 | 0.457 (0.005) |
| Northwest | 1986-2000 | 145 | 0.574 (0.014) |
| Southwest | 1995-2000 | 820 | 0.340 (0.005) |
| Upper St. Johns | 1978-2004 | 120 | 0.464 (0.015) |

Figure 5.3. Marginal fraction of collected carcasses in each age class by region (means ± 1 standard deviation).

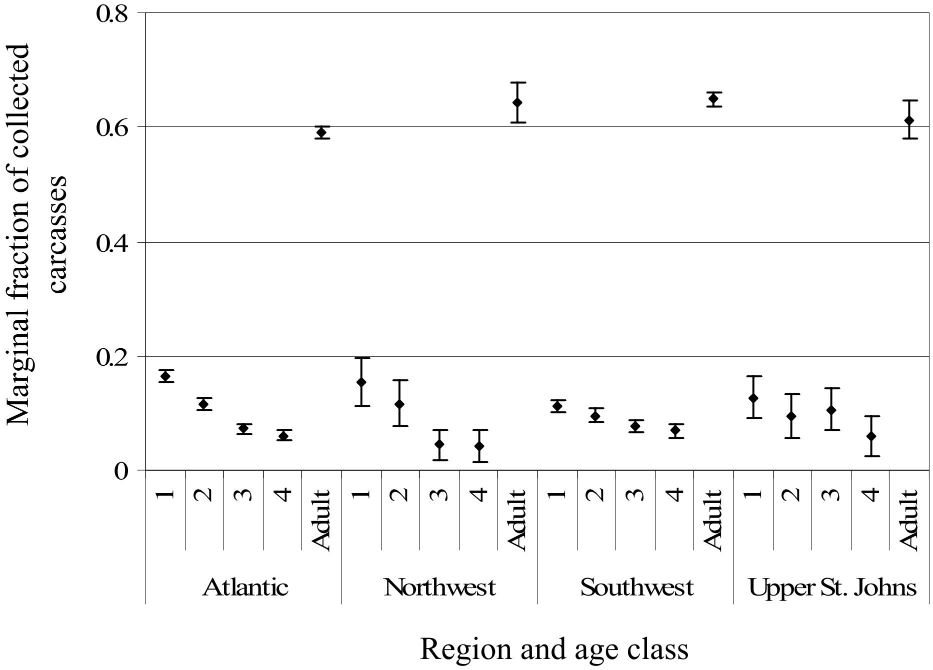


Figure 5.4 displays the marginal relative detection ratios for age classes one through four in relation to the adult age class, calculated from the Upper St. Johns region data. Because the values are ratios, they have a tendency to be positively skewed on the nominal scale. On a log scale, the distributions are almost symmetric. Overall, animals in age classes one and two are less likely to be detected than adult animals ($d^* < 1.0$). Although there is uncertainty in the relative detection ratios for age classes three and four, carcass detection is probably equal for adults and age classes three and four ($d^*$ equally distributed around 1.0 on the log scale).

Figure 5.5 shows the final distributions of survival rates by age class calculated for the Atlantic, Northwest, and Southwest regions. The Atlantic and Northwest regions have roughly the same survival rates for age classes 1 and 2. Survival rates for age

Figure 5.4. Relative detection ratio distributions on both the nominal and log scale for age classes one through four in relation to the adult age class. Boxes represent 1st and 3rd quartiles. Lines within the boxes are medians. Whiskers are minimum and maximum values, and dots are arithmetic mean values.

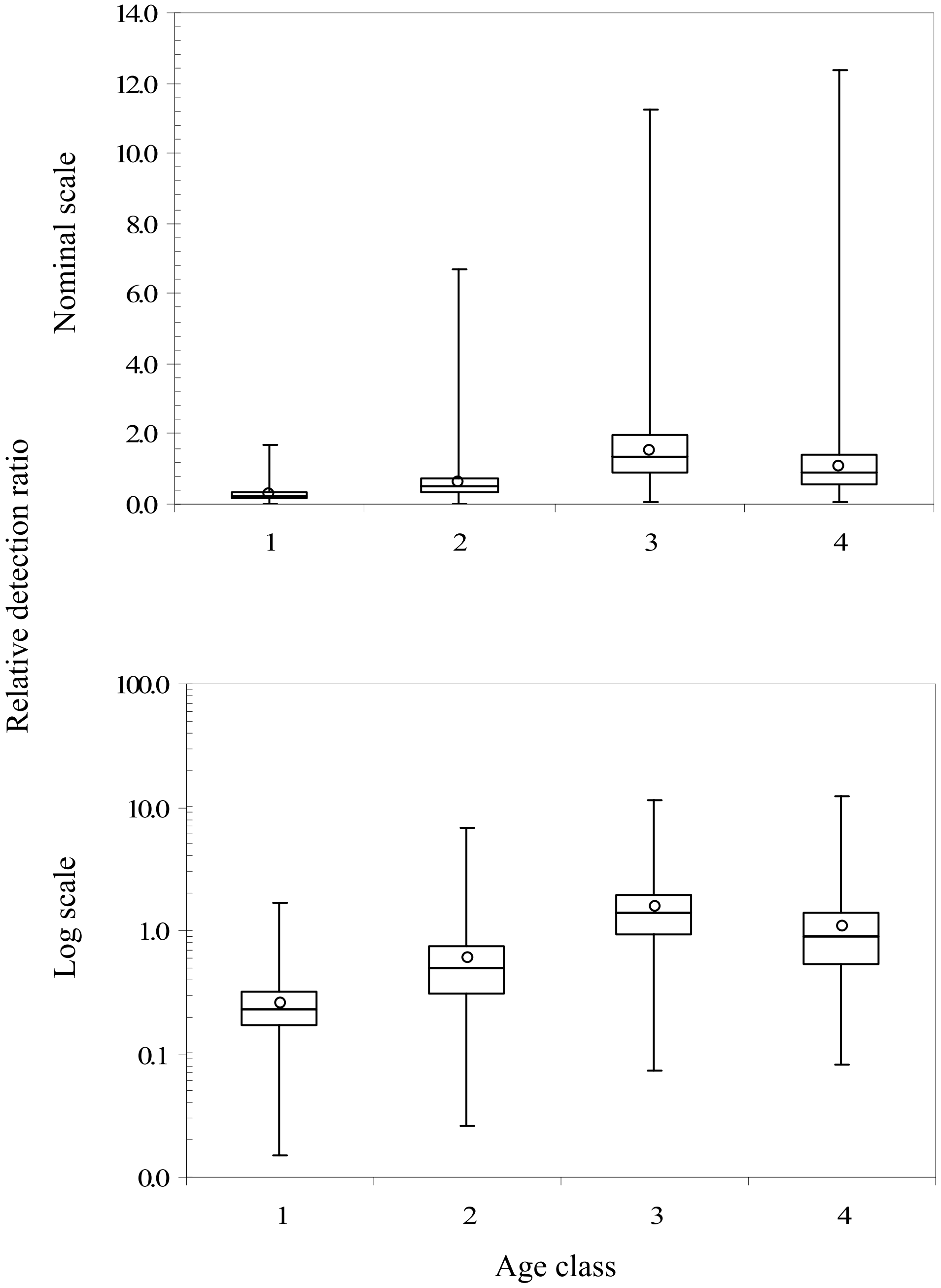
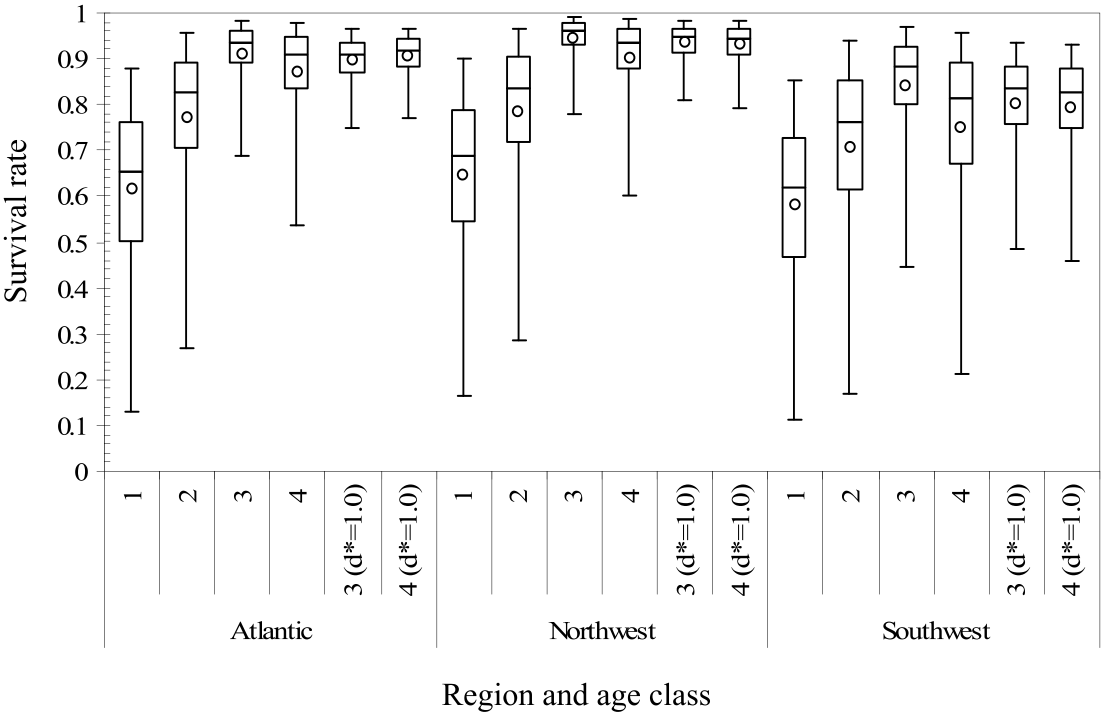


Figure 5.5. Survival rate distributions for age classes one through four by region. Survival rate distributions for age classes three and four with the assumption $d_3^* = d_4^* = 1.0$ are also given. Boxes represent 1st and 3rd quartiles. Lines within the boxes are medians. Whiskers are 2.5% and 97.5% cumulative probability values, and open circles are arithmetic mean values.



classes 3 and 4 are highest in the Northwest region. All survival rates in the Southwest are lower compared to the Atlantic and Northwest. Survival rates generally increase with age, but $s_4$ appears to be less than $s_3$ for all regions. The difference between $s_4$ and $s_3$ is reduced when relative detection ratios for those age classes are set constant at one. All values are negatively skewed.

The final distributions of survival rates are difficult to define as a particular probability curve. A logit-normal representation creates a wider distribution than the distribution constructed of random samples. Likewise, beta distributions with the same means and modes as the random sample may not be an accurate representation of the survival rates estimated here. See Figure 5.6 for an example. If the resulting survival rates are used in population dynamics models, actual survival rate samples produced from the above simulations should be used, rather than estimating the probability distributions with logit-normal or beta probability functions. However, for comparison, the means and variances estimated from the logit-transformed random samples of survival rates are provided in Table 5.6. In general, survival rate estimates from Runge *et al.* (2004) fall within the upper standard deviation of the logit-normal distribution produced in this study.

Figure 5.6. Comparisons of probability functions that might represent the sample distribution of survival rate estimates for the Northwest region, age class one. The logit-normal distribution is based on estimating the mean and standard deviation of the survival rate samples, transformed to the logit. The beta distribution is based on the mean and mode of the survival rate samples.

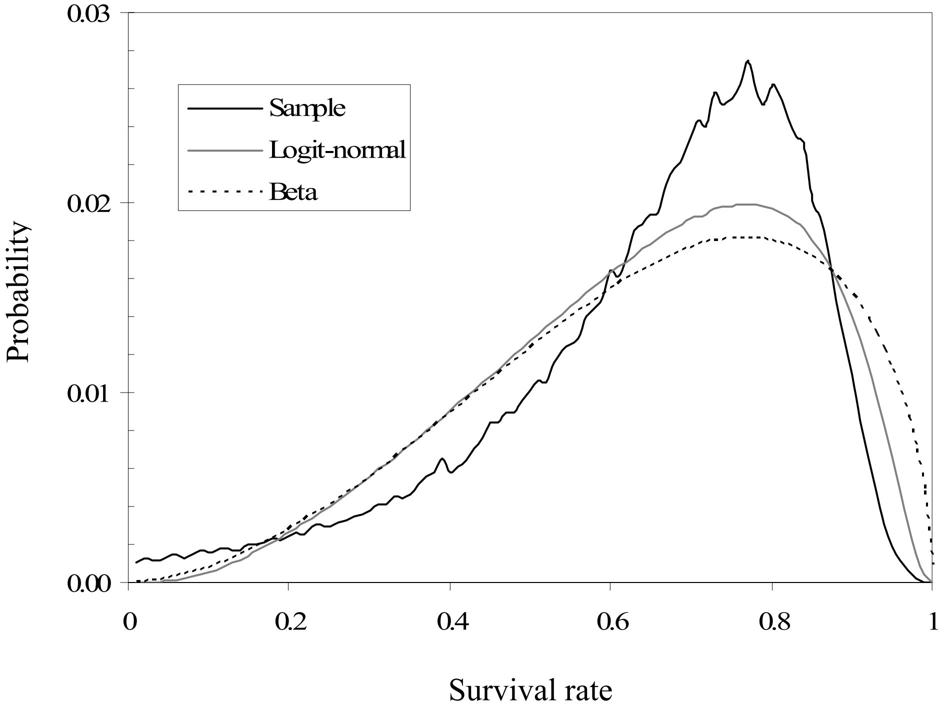


Table 5.6. Means and standard deviations of logit-normal distributions representing final survival rate estimates. [a]The Atlantic adult survival rate estimate was updated in 2007 (Runge *et al.* 2007b), so new young survival rate estimates were calculated using the same ratio technique described in Runge *et al.* (2004). * Distributions estimated when relative detection ratio is constant at 1.0.

| | Atlantic | | Northwest | | Southwest | |
|---|---|---|---|---|---|---|
| Age Class | This study | Runge *et al.* 2004[a] | This study | Runge *et al.* 2004 | This study | Runge *et al.* 2004 |
| 1 | 0.49 (0.99) | 1.45 (0.36) | 0.68 (0.97) | 1.43 (0.36) | 0.33 (0.98) | 1.18 (0.36) |
| 2 | 1.43 (1.05) | 2.38 (0.62) | 1.53 (1.08) | 2.33 (0.62) | 1.02 (1.10) | 1.85 (0.62) |
| 3 | 2.61 (0.84) | 3.17 (0.29) | 3.16 (0.92) | 3.07 (0.16) | 1.92 (0.95) | 2.26 (0.23) |
| 4 | 2.23 (0.96) | | 2.63 (1.05) | | 1.33 (1.14) | |
| 3* | 2.28 (0.57) | | 2.86 (0.70) | | 1.54 (0.70) | |
| 4* | 2.39 (0.56) | | 2.79 (0.72) | | 1.48 (0.71) | |

## Discussion

The variable counts of carcasses by region are a reflection of the number of years of data used in the analysis and the spatial distribution of the population. Although exact population sizes are not known, roughly four percent of the population resides in the Upper St. Johns River. Twelve percent is found in the Northwest region. The Southwest region probably contains 37% of the population, and the largest subpopulation is found in the Atlantic, about 47% (U. S. Fish and Wildlife Service 2001). Roughly one third to two thirds of all carcasses collected during the given time periods were less than six months old (including premature births). Such a high percentage reflects the reproductive output of the species combined with low adult mortality rates and probably high mortality rates for the very youngest age class. Animals are not followed from birth, so survival rates of this youngest age class may never be quantified.

Relative detection ratio results for age classes one and two indicate younger (and smaller) carcasses are less likely to be detected than adult carcasses. Although uncertainty is large, carcasses in age classes three and older may have an equal probability of detection. Results are supported by other research. Smaller animals probably have a higher decomposition rate (including scavenging) due to a larger surface-to-volume ratio (Schwarz 2004a). Therefore, smaller carcasses may be more likely to decompose before they are reported. In addition, Gannon *et al.* (2007) showed mothers with calves may be more likely to avoid areas with high boat traffic compared to females without calves. Dependent calf carcasses (age classes one and two) may have a lower detection probability compared to older animals if those carcasses are in a secluded location. Likewise, by age three, animals have probably reached about 84% of their adult asymptotic length and follow the same general movement patterns of older animals without calves (O'Shea & Reep 1990, Deutsch *et al.* 2003). Therefore, equal detection probabilities for age classes three and above may be an appropriate assumption.

The reasons for differences in survival rate estimates are not always obvious. In general, calculated $s_i$s are lower when $C_i^*$ and/or $A_A$ are higher. Age specific survival rates may also be lower when $C_A^*$, $s_A$, $d_i^*$, and/or $A_i$ are lower. The Atlantic and Northwest regions have roughly the same estimated survival rates, while survival rate estimates in the Southwest are considerably lower. Differences in $\underline{C}^*$ and $\underline{A}$ were not remarkable between the three regions. In fact, the Southwest region showed slightly higher proportions alive in non-adult age classes, indicating potentially higher survival rates for young animals compared to other regions. The most apparent reason for differences in young survival rates by region is the difference in regional adult survival rates.

For all regions, $s_4$ appears to be less than $s_3$. The difference is almost eliminated by assuming equal detection probabilities for age classes three and older. Population dynamics modeling has assumed equal survival rates for age classes three and four based on equal survival rates for these age classes in the Upper St. Johns region (Runge *et al.* 2004, 2007a, 2007b). With the assumption of equal detection probability for older age classes, the results presented here show almost equal survival rate distributions for the two age classes.

Estimates of relative detection ratios for age classes 1 and 2 may be biased high. In the Upper St. Johns region, the known-age calves that are most easily tracked belong to experienced mothers that have used the Blue Spring area for a long time and are well known to the biologists. With experienced mothers, mark-recapture survival rate estimates for those calves may be biased high. Overall, such a bias would lead to a positive bias in relative detection ratios which would in turn lead to a positive bias in young survival rate estimates for other regions.

## Future Work

Variability in survival rate estimates is high compared to estimates calculated from mark-recapture studies (Langtimm *et al.* 2004). Given the function used to estimate survival rates (Equation 5.5), uncertainty in more than one parameter multiplies uncertainty in the resulting survival rate estimates. Variability in adult survival rates is the primary source of uncertainty in population growth estimates, and efforts to reduce variability in adult survival rate estimates are ongoing (Goodman 2004, Runge *et al.* 2004, 2007a). Minimizing uncertainty in carcass counts by age class requires more accurate methods to estimate ages of carcasses. The ear-bone method devised by Marmontel *et al.* (1996) may be the most accurate way to estimate ages of Florida manatees, and ear-bone age estimates are already available for roughly one third of all collected manatee carcasses. However, reducing the quantified uncertainty in this method requires more ear-bone age estimates for animals of known age (Chapter 4). Reducing variability in relative detection ratios also requires higher precision in estimates of survival rates for all age classes in the Upper St. Johns region. Of course, direct experiments to measure detection rates by age class and region should test the assumption that relative detection ratios are the same for all regions.

Two methods could provide better estimates of either the proportion alive in each age class or even provide estimates of survival rates. Van Sickle *et al.* (1987) developed an iterative technique to estimate population growth rates using mortality data. The method requires reproductive rate estimates and the assumption of stable age distribution. Survival rates and the stable age distribution are also estimated through the process. Tagging studies focused on young animals could also produce improved estimates of the proportion alive in each age class or survival rates.

This study assumed constant adult survival and reproductive rates over time. However, those rates do display some degree of annual variability. Annual adult survival rates have been calculated for all regions, and annual reproductive rates have also been estimated for at least two regions (Kendall *et al.* 2004, Langtimm *et al.* 2004, Runge *et al.* 2004, Runge *et al.* 2007b). If the techniques presented in this study are used in the future, I recommend that carcass recovery counts be combined with annual estimates of adult survival and reproductive rates for the Atlantic and Southwest regions, where annual carcass counts are high. Mark-recapture studies use an annual cycle from January to December, whereas this study uses March to February. Such a difference does not affect overall survival rate estimates in this study since all years are combined. However, if annual survival rates are estimated, differences in the timing of annual cycles need to be addressed.

# FINAL REMARKS

The necropsy data provided several scientific avenues that were explored in this research. First, given the high quality of the necropsy reports, I was able to redefine and model perinatal mortality (Chapter 3). I was also able to quantify ear-bone age error and estimate age at death given carcass length (Chapter 4). Finally, using the carcass data with age estimates, relative detection ratios by age class and survival rates of younger age classes were estimated (Chapter 5).

The two primary objectives of the Florida Manatee Carcass Recovery and Necropsy Program have been to provide management with information on cause of death and to improve general understanding of manatee physiology and anatomy. The program has yielded large amounts of valuable data about manatee physiology and anatomy. However, the list of questions one could answer with the carcass mortality goes far beyond the subject of physiology. Unfortunately, given current data collection methods, further analysis of carcass recovery data for management purposes, beyond what is reported here, is limited, primarily due to a lack of coordination with other databases and because auxiliary studies to determine detection probabilities have not been performed. However, with some design adjustments and some independent calibration studies, future necropsy data would have the potential to provide an impressive amount of management information, particularly with regards to population dynamics and population censusing. The following suggestions could help shift the intent of the program toward using the data to answer such management questions.

## Shifting the Paradigm

Research should focus on providing as much information as possible to improve or fill in gaps in the population projection model presented in Runge *et al.* (2007b), which currently is at the center of management assessments. So, what improvements and gaps are priorities? First, population size estimates need to be improved. Second, the impacts or effectiveness of management decisions, particularly speed zones, should be quantified. Third, the impacts of current or increased exposure to cold, hurricanes, and red tide on manatee survival and reproduction for all age classes also need to be quantified. Fourth, survival rate estimates of animals younger than 4.5 years old need to be improved. Fifth, uncertainty in adult survival rate estimates needs to be reduced.

If the carcass recovery data are used in such a management context, studies on carcass detection rates and detection probabilities need to take first priority. In general, such studies would need to quantify decomposition rates, carcass reporting rates, and vessel traffic patterns. The studies could be performed using a subset (probably a random sample would be best) of marked, or even satellite-tagged, carcasses that were not necropsied, allowing them to decompose naturally under a variety of environmental conditions while recording the number of times the public reports them. Such a study could also provide quantitative information about carcass drift. A quantification of detection probabilities would lead to a population size estimate independent of aerial and mark-recapture studies.

Consistently connecting the carcass data to other sources of data could be a great benefit. For example, Goodman (2004) provided a Bayesian method to reduce uncertainty in adult survival rate estimates by up to 65% using carcass data cross-referenced with mark-recapture data. PIT tag readings of carcasses have been recorded very consistently in the mortality database, and research to connect the PIT tag and carcass recovery data is currently underway. Such information may be used with traditional mark-recapture analysis methods to estimate population size and survival rates. The effectiveness of rescue and release efforts could also be quantified if carcasses were consistently connected with captivity data.

From a management perspective, specific data need to be recorded consistently during necropsy and entered in the database. The data should include any information about female reproductive status. Indicators of age at death, particularly the indicators mentioned in Chapter 3 for perinatal mortality, should always be recorded. In addition, all indicators that lead to a determined cause of death (some of which have been listed in Chapter 2) should be tallied and entered in the database. Finally, all indicators of human interactions, including scar counts and ingestion or entanglement in debris, should be assayed consistently and have their own fields in the database.

Lastly, as mentioned in Chapter 4, protocols and methods for measuring ear-bone age need to be improved. First and foremost, either all carcasses or a *random* subset of the carcasses need to be ear-bone aged. In addition, estimates of both process and measurement error need to be quantified or improved by cross-referencing ear-bone age estimates with more known-age animals and by measuring uncertainty within and between readers. Any study interested in analyzing mortality data by age class requires improved aging methods.

## Improving Survival Rate Estimates

The research presented here provides one method for estimating survival rates using carcass recovery data. Due to the high level of uncertainty in the parameters, the resulting survival rate estimates have a high level of uncertainty. Reducing uncertainty in survival rate estimates would require better precision in age-at-death estimates, proportions alive in each age class, and detection probabilities.

Given the daunting number of parameters involved in estimating survival rates from carcass recovery data, the most effective way to reduce uncertainty in survival rate estimates for young animals is to use other forms of data in conjunction with carcass data. In the Upper St. Johns region, mark-recapture studies were able to estimate survival rates for young animals with much greater precision than this study using a relatively small sample size (*n* = 115) (Langtimm *et al.* 2004). New studies could estimate survival rates using animals tagged during their first winter, either using PIT tags or perhaps even ear tags attached to their flukes. Even if tag loss is high, if tags were positioned on a different section of the fluke every year, the tag could leave a permanent mark to at least identify the year of birth. Such data could be analyzed using the methods provided in Goodman (2004).

The suggested changes in necropsy data collection protocol and the calibration studies may seem burdensome. However, other sources of manatee population dynamics and population census data present their own challenges, and some management questions can only be answered using the carcass recovery data. Therefore, improving the utility of the carcass data is a subject worth pursuing.

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# APPENDICES

# APPENDIX A

## REARRANGING THE SCHNUTE MODEL IN THE PRESENCE OF PROCESS ERROR

Using the Schnute model to estimate length from age, the equation with normal process error is (see main text for definitions of variables):

$$L_t = \left[ y_1^b + \left( y_2^b - y_1^b \right) \frac{1 - e^{-a(t-\tau_1)}}{1 - e^{-a(\tau_2 - \tau_1)}} \right]^{\frac{1}{b}} + \varepsilon_y \tag{A.1}$$

Rearranging the equation to estimate age from length, the equation becomes:

$$t = -\frac{1}{a} \cdot \ln(x) + \tau_1 \tag{A.2}$$

Where

$$x = 1 - \left[ \frac{(L_t - \varepsilon_y)^b - y_1^b}{y_2^b - y_1^b} \right] \{1 - \exp[-a(\tau_2 - \tau_1)]\} \tag{A.3}$$

The results for Florida manatees show parameter $a > 0$. Therefore, to produce a positive value on age, $\ln(x) \leq 0$. So:

$$0 \leq x \leq 1 \tag{A.4}$$

Substituting and rearranging, we get:

$$0 \leq \left[ \frac{(L_t - \varepsilon_y)^b - y_1^b}{y_2^b - y_1^b} \right] \leq \{1 - \exp[-a(\tau_2 - \tau_1)]\}^{-1} \tag{A.5}$$

By definition, $\tau_1$ and $\tau_2$ are constants, and $\tau_1 < \tau_2$. Since $a > 0$, the right side of the equation ranges between 1 and ∞, depending on the value of $a$. Also by definition, $y_1 < Y_2$. To meet the lower bound requirement:

$$y_1 + \varepsilon_y < L_t \tag{A.6}$$

Such a limitation puts potentially inaccurate restrictions on posterior combinations of $y_I$ and $\varepsilon_Y$ for smaller animals.

# APPENDIX B

## INDEPENDENT CARCASS AGE DISTRIBUTION FOR USE WITH THE SCHNUTE MODEL

To provide an independent estimate of the carcass age distribution, several sources of information from mark-recapture analyses were used, providing separate distributions for each region defined by the Florida manatee recovery plan: Atlantic, Northwest, Southwest, and Upper St. Johns (U. S. Fish and Wildlife Service 2001). First, Rathbun *et al.* (1995) observed noticeably pregnant females 82 times in the Northwest winter range. Subsequently, a calf was observed with those females the following winter 55 times. Likewise, O'Shea and Hartley (1995) observed 35 pregnancies and 21 calves in the Upper St. Johns region. Kendall *et al.* (2004) estimated the probability a first-year calf is missed during an entire observation season given that its mother was seen was 0.22 for the Northwest region and 0.23 for the Atlantic region. Assuming the proportion of first-year calves missed during an observation season is the same for the Atlantic and Upper St Johns region, the observed calf counts above were adjusted to 61 for the Northwest region and 24 for the Upper St. Johns region.

Survival rates from late pregnancy to the following winter ($s_0$) can be estimated from such counts. Using a binomial likelihood with a broad conjugate beta prior distribution of $\beta eta(1,1)$, Bayesian analysis gives a beta posterior distribution of $s_0 \sim \beta eta(62,23)$ for the Northwest region and $s_0 \sim \beta eta(25,12)$ for the Upper St. Johns region (Equations 3.1 and 3.2 in Chapter 3). For the Atlantic and Southwest regions, $s_0$ is assumed to be a uniform distribution between 0.5 and 0.9 ($s_0 \sim U(0.5,0.9)$).

Next, the population dynamics model from Runge *et al.* (2007a) was used to estimate the proportion alive in each age class, not including the youngest age class (in-utero to 0.5 years old). Random samples of all survival and reproductive rates were input in the Leslie matrix and the age distribution of the live population ($E_x$) was calculated as the dominant eigenvector. Known reproductive and survival rates by region were drawn from logit-normal distributions with means and standard deviations shown in bold type in Table B.1. Unknown survival and reproductive rates were estimated using the assumptions and techniques of Runge *et al.* (2004). Those rates were also drawn from logit-normal distributions, but the standard deviations were doubled to represent a higher level of uncertainty in those estimates. As with Runge *et al.* (2007a), $s_3$ was assumed equal to $s_4$, and $r_4$ was less than $r_p$.

Table B.1. Survival and reproductive rate distributions used to estimate the proportion of carcasses in each age class. Unless otherwise noted, samples were drawn from logit-normal distributions with means and standard deviations shown. Bold numbers represent parameter estimates measured directly from mark-recapture data. Non-bold numbers are estimates based on the calculations and assumptions used in Runge *et al.* (2004), doubling the standard deviations.

| Parameter | Atlantic | Northwest | Southwest | Upper St. Johns |
|---|---|---|---|---|
| $s_0$ | $U(0.5, 0.9)$ | ***βeta*(62,23)** | $U(0.5, 0.9)$ | ***βeta*(25,12)** |
| $s_1$ | 1.45 (0.72) | 1.43 (0.72) | 1.18 (0.72) | **1.45 (0.24)** |
| $s_2$ | 2.38 (1.26) | 2.33 (1.22) | 1.85 (1.24) | **2.38 (0.42)** |
| $s_3 = s_4$ | 3.17 (0.58) | 3.07 (0.32) | 2.26 (0.46) | **3.17 (0.29)** |
| $s_P = s_A$ | **3.17 (0.29)** | **3.07 (0.16)** | **2.26 (0.23)** | **3.17 (0.29)** |
| $\gamma_4$ | -6.91 (6.12) | **-6.91 (3.06)** | -6.91 (6.12) | **-1.34 (0.50)** |
| $\gamma_P$ | -0.83 (0.90) | **-0.49 (0.45)** | **-0.83 (0.45)** | **0.45 (0.21)** |
| $\gamma_B$ | **-0.49 (0.19)** | **-0.29 (0.23)** | **0.39 (0.34)** | **0.45 (0.21)** |

To estimate the proportion of carcasses in each age class, conditional reproductive rates, defined as the probability a mature female produces a calf that survives to the first winter, given the female did not have a calf the previous winter ($\gamma_x$), were adjusted to represent the probability a mature female becomes pregnant, given she is not with a calf ($r_x$), where

$$r_x = \frac{\gamma_x}{s_0} \tag{B.1}$$

Survival rates from late pregnancy to about 6 months of age ($s_0$) for a calf given its mother survives were drawn from the distributions described above. The proportion of carcasses seen in the youngest age class ($D_0$) is a function of the proportion of adult females with a calf of that size ($E_x$ and $r_x$), the survival rate of the calf ($s_0$) and the survival rate of the mother ($s_A$). Females in three different stages could have calves in the youngest age class: age class 4 (*4*), pre-breeders (*P*), and resting breeders (*B*).

$$\begin{aligned} D_0 &= E_4 r_4 (1 - s_A) + E_4 r_4 (1 - s_0) s_A \\ &+ E_P r_P (1 - s_A) + E_P r_P (1 - s_0) s_A \\ &+ E_B r_B (1 - s_A) + E_B r_B (1 - s_0) s_A \end{aligned} \tag{B.2}$$

The model assumes the calf dies if the mother dies. All other proportions of dead animals by age class were simply the proportion alive in those age classes multiplied by the mortality rate (1- $s_x$). The proportion alive in age class 1 is equal to the proportion of females seen with a calf ($E_C$). See Runge *et al.* (2007a) or Chapter 5 for more information about the stage-based model.

The proportion of carcasses in age class = 0 estimated above still includes animals that died at less than two weeks old. To correspond with the age class = 0 in the age-length model (two weeks to 0.5 years old), the fraction of carcasses within that age window had to be estimated. Assuming survival rate is constant within that year (in-utero to 0.5 years of age) and all calves are born on July 1st, the proportion of carcasses within age class = 0 is a function of the probability the animal lives to two weeks old and then dies before 0.5 years old.

$$s_0^{\frac{6.5}{12}}\left(1-s_0^{\frac{5.5}{12}}\right) \tag{B.3}$$

The proportion of carcasses in that age class was multiplied by the above equation, and proportions were normalized over all age classes. The resulting marginal proportion of carcasses in each age class is shown in Figure B.1.

Figure B.1. Marginal proportion of carcasses within each age class by region for use with the Schnute model. Points are means, and bars are standard deviations.