A VISUAL BODY CONDITION INDEX FOR BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

By

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To my Father and Mother, Thomas and Marilyn Gryzbek, for supporting me while I follow my dreams

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
CBD	Could not be determined
COD	Cause of death
CIs	Condition Indices
MML	Mote Marine Laboratory
MMPA	Marine Mammal Protection Act of 1972
NMFS	NOAA’s National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
PND	Post-nuchal depression
SDRP	Sarasota Dolphin Research Program
SIP	Mote Marine Laboratory Stranding Investigations Program
UME	Unusual Mortality Event

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Since the early 1990s, concerns about the health of bottlenose dolphin (*Tursiops truncatus*) populations faced with natural and anthropogenic threats have led to the need for a reliable and cost-effective indicator of dolphin body condition. Previous studies on cetaceans have developed methods that monitor the condition of individuals using external features that are visible in photographs. Based on the distribution of fat reserves in bottlenose dolphins, I investigated the post-nuchal region visible in photographs to determine if depressions were indicative of poor body condition. I developed a simple and non-invasive system for determining the presence or absence of post-nuchal depressions (PNDs) from digital photographs of stranded dolphins. Dolphins with PNDs consistently had lower length-weight measurements and body mass index values than those without PNDs. The PND index appears to be a viable tool for assessing bottlenose dolphin body condition.

CHAPTER 1
INTRODUCTION

The Need for Improved Monitoring of Bottlenose Dolphins

From 1991 to the present, the National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) has formally recognized 58 marine mammal unusual mortality events (UMEs) in the United States (NOAA Fisheries Office of Protected Resources, 2013a). The Marine Mammal Protection Act (MMPA) defines a UME as "a stranding that is unexpected; involves a significant die-off of any marine mammal population; and demands immediate response" (MMPA 1972). Causes of marine mammal UMEs have been related to parasites, viruses, bacteria, biotoxins, human interactions, oil spills, and variation in oceanographic conditions (Gulland and Hall 2007). In the Gulf of Mexico alone, eight bottlenose dolphin (*Tursiops truncatus*) UMEs occurred during 1993 through 2008, resulting in at least 930 deaths (Waring et al. 2012). Another UME declared during February 2010 in the northern Gulf of Mexico for cetaceans continues with more than 880 bottlenose dolphins recovered through July 14, 2013 (NOAA Fisheries Office of Protected Resources, 2013b). In light of the frequent occurrence of bottlenose dolphin UMEs, a reliable and cost-effective means of monitoring body condition would be beneficial for the conservation and management of this species. Wells et al. (2004) recognized the need for the improvement of baseline information on dolphin populations prior to die-offs. These authors stressed the importance of a proactive approach to monitoring these populations instead of waiting for large scale strandings to occur.

Tools for Monitoring Wildlife Populations

The determination of a wildlife population's status is important to discerning the level of protection that is necessary for its management (Fowler and Siniff 1992). A population's size depends, in part, upon the health of its individual members, and condition indices (CIs) are a means of quantifying an individual's health (Stevenson and Woods 2006). Therefore, the use of CIs can lead to a better understanding of a population's dynamics, status, and trajectory. CIs are also used to compare the relative health of populations to one another (Stevenson and Woods 2006). Many types of indices have been used to monitor the condition of individuals within wildlife populations for the purpose of management and conservation. A review of CIs by Stevenson and Woods (2006) demonstrates the range of these metrics, including external assessments of size, shape, skin condition, and fat scores; calculations involving body length and weight measurements; examination of the dimensions of internal organs; the analysis of the biochemistry of blood, feces, urine, and tissues; and the direct examination of body composition (percentages of water, fat, and protein in the animal's body). Researchers are typically trying to determine how much energy is available in the form of fat when using these metrics (Stevenson and Woods 2006). CIs have been applied to studies of a variety of species in relation to environmental threats, life history traits, activity cycles, and ecological interactions (Stevenson and Woods 2006). The wide use of CIs in both environmental and conservation biology is a testament to their utility.

Assessment of Body Condition in Cetaceans

Research Involving the Direct Handling of Individuals

Studies on cetacean body (fat) condition include those that involve directly handling live animals or carcasses and those that analyze photographs of individuals.

Morphometric measurements have included girth and blubber thickness at various sites, blubber mass, and total weight (Lockyer et al. 1985, Lockyer 1986, Read 1990, Kuiken et al. 1994, Ichii et al. 1998, Haug et al. 2002, Koopman et al. 2002, Evans et al. 2003, Struntz et al. 2004, Dunkin et al. 2005, Caon et al. 2007, Dunkin et al. 2010, Gómez-Campos et al. 2011, Miller et al. 2011, Christiansen et al. 2013, Hart et al. 2013). Blubber lipid content has also been examined (Lockyer et al. 1985, Lockyer 1986, Kuiken et al. 1994, Evans et al. 2003, Struntz et al. 2004, Dunkin et al. 2005, Dunkin et al. 2010, Montie et al. 2008, Gómez-Campos et al. 2011). The analyses of these measurements have varied greatly. For example, how length is incorporated into the examination of body condition has differed between studies. Researchers have compared raw body condition measurements between reproductive classes that differ in length (Lockyer et al. 1985, Caon et al. 2007). Other studies have adjusted for length when it was correlated with the examined body condition measurement (Ichii et al. 1998, Miller et al. 2011). Length adjustments were made by expressing the body condition measurement as a percentage of length or by taking the ratio of the body condition measurement to length (Ichii et al. 1998, Miller et al. 2011). Furthermore, researchers have examined the residuals resulting from regressions between standard length and a variety of the raw or log transformed body condition measurements (Read 1990, Kuiken et al. 1994, Haug et al. 2002, Evans et al. 2003, Gómez-Campos et al. 2011). Analyses have also included calculating a fat index for blubber (mean body girth * mean blubber thickness * percentage lipid content of blubber) and muscle (mean cross-sectional area of the body inside the blubber layer * percentage lipid content of muscle) of fin whales (*Balaenoptera physalus*) (Lockyer 1986). Another approach taken by Christiansen et al. (2013) involved calculating total blubber volume by dividing the minke whale body into five frustums, calculating the volume of each frustum, and then summing those volumes. In addition, Hart et al. (2013) used length-weight and length-maximum girth models in combination with quantile regression to create baseline 95th percentile reference ranges for bottlenose dolphins. The diversity of methods used to assess cetacean body condition reflects a broad interest within the community to be able to assess this important individual and population parameter. The diversity of methods also reflects the large differences among cetaceans in size, the logistical difficulty inherent in obtaining measurements of these animals due to their size, the limited view of their entire body while in water, their availability for predictable sightings and re-sightings, and their sometimes expansive marine habitats (Pettis et al. 2004). Stevenson and Woods (2006) stated the specific importance of using nondestructive methods to obtain CIs that are easily applied in the field, and photographic analyses are a good example of such methods.

Research Involving Photographic Analyses

Taking photographs of cetaceans is a much easier task than having to handle them to obtain some measurements. In addition to being easier for the researchers, the animals themselves undergo much less stress while being photographed than if they were handled. A few studies on cetaceans have taken advantage of using photographs to evaluate body condition. For instance, in addition to assessing skin condition via photographic analysis, Pettis et al. (2004) examined body condition of the North Atlantic right whale (*Eubalaena glacialis*) by scoring the area caudal to the blowhole in lateral photos using a three point scale based on the concavity or convexity created by the amount of blubber and subcutaneous fat seen there. Similarly, Bradford et al. (2012)

evaluated the concavity or convexity created by blubber and subcutaneous fat in three body regions on the western gray whale (*Eschrichtius robustus*) as seen in lateral photos—the area caudal to the blowhole, the scapular region, and the lateral flanks. A three point scale was used to score the first body region, while a two point scale was used to score the remaining regions. These authors emphasized the area caudal to the blowhole when creating composite scores for overall body condition (Bradford et al. 2012). Both of these studies included a degree of subjectivity in their scoring systems because the score was ultimately dependent upon the scorer's judgment instead of a specific metric. Alternatively, length and width measurements obtained from photos taken during aerial surveys of each of these species, as well as southern right whales (*Eubalaena australis*), have been used to assess body condition (Perryman and Lynn 2002, Miller et al. 2012). These photogrammetric analyses demonstrate how objective measurements can be obtained from photos. The success of all four of these studies in detecting differences in body condition of different reproductive classes lends support to the use of photographic assessments as a viable tool in cetacean body condition analyses.

Post-Nuchal Depression as an Indicator of Bottlenose Dolphin Body Condition

I used a combination of direct morphometric measurements and photographic analyses to determine if post-nuchal depressions are indicative of poor body condition in bottlenose dolphins. A post-nuchal depression (PND) is a concavity on the animal's dorsal surface just caudal to the nuchal crest of the skull that can extend through the cervical region (Figure 1-1). PNDs are a trait seen in dolphins commonly referred to as having "peanut heads" by dolphin trainers because of the peanut-like shape of the anterior portion of their body. In young animals and animals in good body condition, an adipose depot, or fat pad, is located under the blubber layer in this post-nuchal region (Figure 1-2). The depletion of this fat pad likely contributes to the expression of a PND; however, the depletion of blubber and muscle found in and near the post-nuchal area may also contribute to the depression. The physiology behind the depletion of tissues in this region of the dolphin body is poorly understood.

Post-nuchal blubber and post-nuchal fat pad thickness measurements collected via ultrasound during bottlenose dolphin health assessments in Sarasota Bay, Florida (Wells et al. 2004, Wells et al. 2009) during spring and summer 2004-2013 show that both of these tissues range in thickness between individuals (R. Wells, unpublished data). For females (n=62 with some individuals measured multiple times in different years) ranging in total length from 180 to 265 cm, the blubber thickness at the post-nuchal site has ranged from 10 to 20 mm. The post-nuchal fat pad of these females has ranged from 1 to 23 mm. For males (n=67 with some individuals measured multiple times in different years) ranging in total length from 166 to 281 cm, blubber thickness at the post-nuchal site has ranged from 10 to 21 mm. The post-nuchal fat pad of males has ranged from 0 to 25 mm (R. Wells, unpublished data). The range of thickness of these tissues in the post-nuchal region supports the notion that both blubber and the post-nuchal fat pad contribute to the presence of a PND. Furthermore, studies have used atrophied epaxial musculature as a sign of emaciation for bottlenose dolphins (Struntz et al. 2004, Dunkin et al. 2005). Given the proximity of muscle to the fat pad in the post-nuchal region (Figure 1-2) and knowing the depletion of muscle can be viewed externally in the area below the dorsal fin of the dolphin body, depleted muscle tissue also likely contributes to the external expression of a PND.

Although the presence of PNDs has been used to differentiate between bottlenose dolphins in emaciated vs. robust condition by trainers working with bottlenose dolphins in managed populations and in some field studies (Struntz et al. 2004, Dunkin et al. 2005, Fair et al. 2006, Yordy et al. 2010), only two studies are known to have related the PND trait to a quantifiable body condition metric. Both Struntz et al. (2004) and Dunkin et al. (2005), used the atrophy of the post-nuchal fat pad as one of a few characteristics to determine if a bottlenose dolphin was emaciated or not, and the number of dolphins with a PND in their samples of emaciated individuals (n=2 and 5, respectively) was not reported. Struntz et al. (2004) found that the absolute blubber depth of emaciated adult dolphins was significantly less than that of robust adults, and the blubber lipid content of emaciated adult dolphins was significantly less than that of juveniles and robust adults. Dunkin et al. (2005) demonstrated that emaciated adult dolphins had a significantly lower amount of lipid in their blubber than the blubber of robust individuals from all life history categories except fetuses. The blubber of emaciated adults contained less lipid than the blubber of fetuses, but the difference was not significant. Further research using a larger sample size of dolphins with PNDs would be beneficial for understanding how body condition measurements compare between individuals with and without this specific trait.

My two main objectives were to: 1) create a technique to objectively and systematically identify PNDs in photographs of bottlenose dolphins and 2) determine if PNDs were indicative of poor body condition. Stranding case records were used to develop the PND assessment system because they contained both photos and body condition measurements, which allowed for the comparison of these measurements between animals with and without PNDs. Additionally, they included animals dying from a variety of causes with and without a relationship to body condition. The overall goal of this study was to develop a logistically simple and non-invasive method for assessing bottlenose dolphin body condition in the field by using photographs. Although I created a PND index using photos of stranded individuals, I demonstrate how the technique can readily be applied to free-swimming dolphins as well. For the second objective, I hypothesized that animals with PNDs would weigh less for a given length and would have smaller body mass index (BMI) values. To test this hypothesis, I created 95% quantile regression reference ranges based on length-weight models and calculated BMI for stranded animals (Hart et al. 2013).

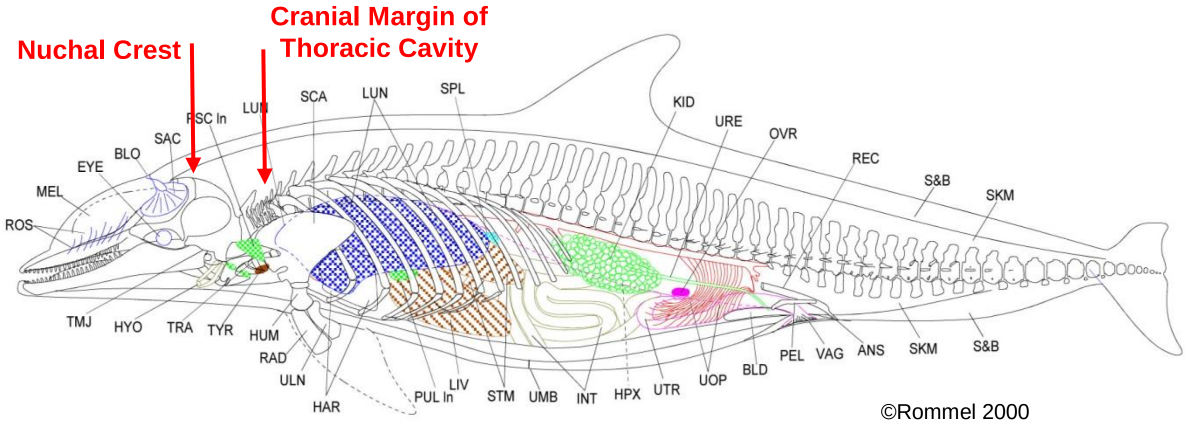

Figure 1-1. The internal anatomy of a bottlenose dolphin depicting the area from the tip of the nuchal crest to the cranial margin of the thoracic cavity, areas that roughly bound the post-nuchal region. Illustration courtesy of Dr. S.A. Rommel.

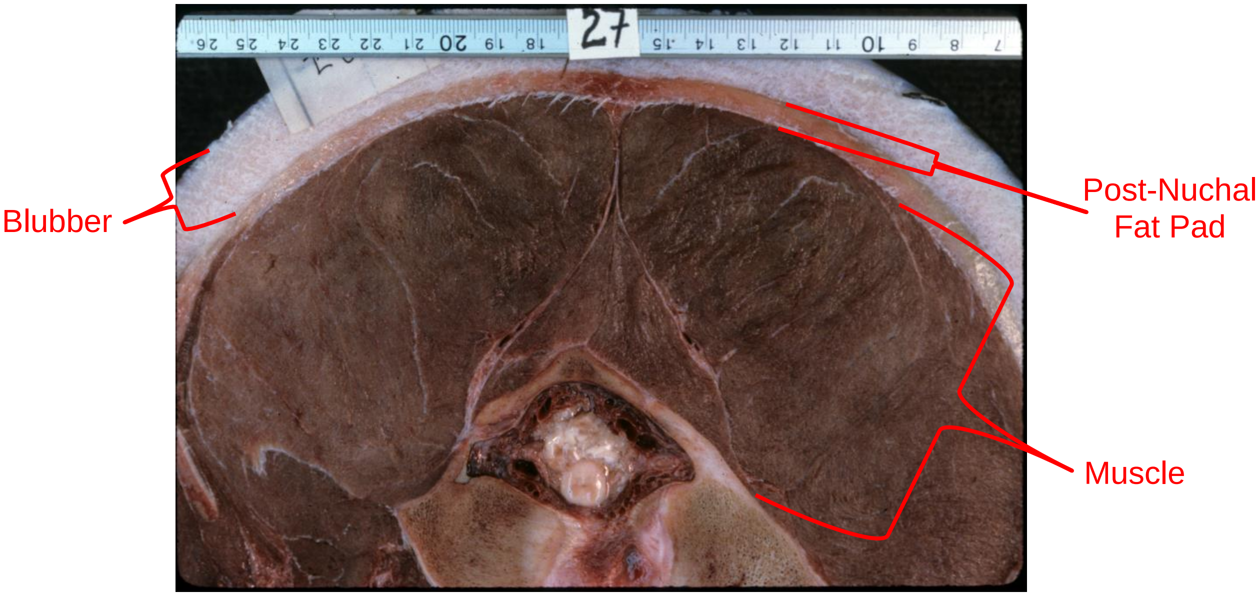

Figure 1-2. A cross section through the cervical region of a 170.9 cm bottlenose dolphin. The ruler is placed at the dorsal surface of the cross section. Brackets denote the location of blubber, the post-nuchal fat pad, and axial muscle. Photo courtesy of Dr. D. Ann Pabst.

CHAPTER 2
MATERIALS AND METHODS

Study Sample Description

Data Sources and Collection

Records from 2000-2012 of 228 stranded bottlenose dolphins with photographic and morphometric data were obtained from the Stranding Investigations Program (SIP) at Mote Marine Laboratory in Sarasota, Florida, USA. Morphometric data included total length (cm), measured from the tip of the upper jaw to the fluke notch (Read et al. 1993), and total weight (kg). Photos were examined to determine if a stranded animal had a PND.

Most of the stranding cases originated from Sarasota and Manatee counties, and some involved long-term resident bottlenose dolphins of Sarasota Bay, monitored by the Sarasota Dolphin Research Program (SDRP) through regular photographic identification surveys and capture-release health assessments (Wells et al. 2004, Wells 2009). For the stranding of MML0904, field survey photographs of the free-ranging dolphin taken eight and ten days prior to its necropsy were used to supplement the SIP record.

Data Organization

Compiled data were examined for potential factors that could confound the assessment of body condition including: carcass condition, missing body parts, pregnancy, life history category, geographical variation, and ecotype. In addition, I eliminated stranding cases with insufficient data for analysis, including cases that did not meet the requirements for photographic quality. Each of these factors is described in detail below.

Carcasses that were too decomposed at the time of examination could yield inaccurate measurements because of bloating or tissue and fluid loss. Therefore, I excluded data for animals that were considered to have a Smithsonian Institution Condition Code of 4 or 5 (Geraci and Lounsbury 2005). A Condition Code 3 carcass was excluded if it: 1) was marked as a late Code 3 in the necropsy report and/or the NMFS stranding network level A form, 2) had a bloated tongue, protruding penis, or distended vulvar region, or 3) was noted as bloated or severely decomposed within the necropsy report and/or the level A form. If any carcass was of marginal condition, I consulted the MML SIP manager. All other Code 3 animals and those with Codes of 1 or 2 were included in the sample, provided that they met the additional requirements described below. Moreover, animals were deleted from the sample if substantial tissue was removed, for example from shark predation or scavenging, since their weight would be inaccurate (Figure 2-1).

Reproductive condition and life history category also influenced inclusion in the sample. All pregnant females were excluded from this study because their weights were not representative of a single individual. Fetuses, stillborn animals, and neonates were excluded because the presence of PNDs in very young animals may be more strongly related to ontogeny than to nutritive condition. Studies that have described neonatal characteristics or analyzed the blubber depth of this age class illustrate how the presence of PNDs in neonates is likely a normal condition of the early developmental stages of a dolphin (Cockcroft and Ross 1990, Dearolf et al. 2000, Struntz et al. 2004). In Sarasota Bay, resident females' calves that were less than a week old at the time of their death, including perinatal mortalities and some stillbirths, were on average about 107 cm (R. Wells, pers. comm., 24 July 2013). To be conservative, I considered animals with a total length ≤ 143 cm to be neonates. This neonatal length is an estimate based on stranded bottlenose dolphins in Virginia with and without selected neonatal characteristics (e.g. rostral hairs, floppy dorsal fins, fetal fold lines, and un-erupted teeth) (Lynott 2012).

The sample used for analyses was restricted to dolphins stranding on Florida's west coast due to geographic variation in size that has been demonstrated in this species (Read et al. 1993, Stolen et al. 2002, McFee et al. 2012). Similarly, I controlled for the two known ecotypes of bottlenose dolphins—inshore and offshore. Offshore animals are larger and more robust than inshore animals (Hersh and Duffield 1990, Mead and Potter 1995). Therefore, I excluded dolphins that were speculated or noted as offshore in their necropsy report.

Stranding cases were excluded when length or weight values were estimated rather than measured or when photos were of insufficient quality. Photo quality was dependent on the angle from which the photo was taken, focus/clarity, contrast, body position, and the amount of the dorsal surface included in the photo. The ideal photo was taken from an angle perpendicular to the animal's sagittal plane and roughly centered on the post-nuchal region, and the focus and contrast of the photo were sufficient so that body landmarks were clearly visible. In addition, the animal was in an upright and flat position (i.e. posture was not affected by the surface that was supporting it). Ideal photos also included the dorsal surface from the blowhole (or the approximate location of the blowhole if it was not directly visible) to at least the point above the anterior insertion of the pectoral fin (see Figure 2-2 for examples). If the best available photo of an individual had one or more features making a PND unrecognizable, that individual was excluded.

Although I examined necropsy reports for potential confounding factors and therefore observed whether or not an animal was considered to be emaciated within some reports during the initial stages of the project, a significant amount of time passed between when I investigated the 228 bottlenose dolphin records and when I applied the PND index (see below) to the finalized sample. Therefore, I believe the PND determinations were not biased.

Development of PND Index

I defined a PND as a concavity on the dorsal surface of the post-nuchal region of a dolphin—an area just caudal to the nuchal crest of the skull that can extend through the cervical region and is associated with the post-nuchal fat pad (Figure 2-2). Preliminary analysis of photos from both stranded animals from SIP records and live animals photographed during Sarasota Bay health assessments showed that identifying PNDs visually without any additional metric applied to the photo could be difficult and subjective. Individuals that seemed border-line to having this trait (for example, when a very slight depression was visible in the post nuchal region) created the most difficulty in terms of confidently identifying PNDs. Additionally, I did not know if the amount of dorsal surface caudal to the nuchal crest visible in the photos would affect the PND outcome.

To help objectively identify PNDs in photographs, I developed a technique that involved drawing a straight, horizontal line across the post-nuchal region. If a space was visible between the line and the animal’s dorsal surface in this region, it was considered to have a PND. For each individual in the study, I tested four different line drawing approaches (see below) to determine whether varying the caudal endpoints (and therefore the amount of the dorsal surface that needed to be included in the photo) would change the PND outcome. I chose the best photo in each individual's record (either the left or right side of the body was acceptable), based on photo quality characteristics, that included the amount of body needed for each of four PND index methods described below. If the PND outcome varied between the different methods, I then compared the results by analyzing length-weight 95% quantile regression ranges and BMI calculations. I made these comparisons to determine whether one method identified dolphins as having PNDs that also had lower weights for a given length and lower BMI values than the PND animals identified by the other methods, examining the results for which method most closely and consistently mirrored body condition results.

Cranial Starting Points Common to All Four Line Techniques

Three cranial starting points for the lines were possible, and these possibilities were the same for each of the four line techniques. For individuals where the nuchal crest was visible, which was generally the case for emaciated animals, I began the line at the tip of the nuchal crest. For more robust individuals where the nuchal crest was not readily visible, I used the external auditory meatus (ear) as a reference point and began the line at the dorsal surface directly above the ear. In most individuals, the nuchal crest is located between the body landmarks of the eye and external ear. The distance between the nuchal crest and each these landmarks is affected by the age of an individual, as the distance between these landmarks increases as an individual grows.   Although the distances between the nuchal crest and each of these landmarks were not recorded in the necropsy reports, the distance between the two landmarks themselves would approximate the maximum distance between the nuchal crest and external ear (i.e. when the nuchal crest is located above the eye). Within my sample, 17 females and 16 males had the measurements necessary to calculate the straight length distance between the center of the eye and external ear available in their necropsy reports. For the females, the straight length distance between the center of the eye and external ear ranged from 5.0 to 8.8 cm, excluding an extreme 10.3 cm observation for a female 171 cm in total length. For males, the straight length distance between the eye and external ear ranged from 5.0 to 8.7 cm. Figure 2-3 shows how the distance between the nuchal crest and the dorsal surface directly above the ear varies between individuals, so the point at the dorsal surface above the ear will better approximate the location of the nuchal crest for some individuals than others. If both the nuchal crest and the ear were not visible, I began the line at the dorsal surface directly above the approximate location of the ear. I used my own judgment to choose a point caudal to the eye that I thought would approximate the location of the ear and then began the line above that point. Examples of the three possible cranial start points are shown in Figure 2-4.

The four line techniques, described in detail below, were differentiated by the amount of body caudal to the blowhole included in the photograph and the caudal endpoints of their lines.

Method #1

The amount of body caudal to the blowhole included in the best photo of an individual for this method varied among individuals, ranging from the dorsal surface directly above the anterior insertion of the pectoral fin to the anterior insertion of the dorsal fin. The caudal endpoint of the line was drawn at the highest point of the dorsal surface at, or caudal to, the anterior insertion of the pectoral fin (Figures 2-5 and 2-6).

Method #2

Photos of each individual included the dorsal surface from the blowhole to the anterior insertion of the dorsal fin. I drew the caudal endpoint of the line at the highest point of the dorsal surface between the anterior insertions of the pectoral and dorsal fins (Figures 2-5 and 2-6). In many cases, the photo considered to be the best for Method #1 included the anterior insertion of the dorsal fin. Therefore, the photo/line combination for these instances was copied and used for Method #2, which required the anterior insertion of the dorsal fin to be included in the photo. However, some animals required different photos for Method #2 to include the anterior insertion of the dorsal fin, and others did not have any photos that included the anterior insertion of the dorsal fin.

Method #3

Photos of each animal included the dorsal surface from the blowhole to the posterior insertion of the pectoral fin, and I drew the caudal endpoint of the line at the dorsal surface above the posterior insertion of the pectoral fin (Figures 2-5 and 2-6).

Method #4

Photos of each animal included the same amount of body as Method #3, and I drew the line caudally to the point directly above the anterior insertion of the pectoral fin (Figures 2-5 and 2-6).

Table 1-1 summarizes the cranial and caudal end points and the amount of visible dorsal surface required for each of the four methods. Figures 2-5 and 2-6 show all four methods applied to dolphins with and without a PND.

Body Condition Analysis

Length-Weight Models

Comparisons of body condition were performed with R version 2.15.2, and Microsoft Excel 2010 was used to give starting estimates for nonlinear model fits in R. I first determined if the length and weight data were related by fitting a simple linear regression model to both the PND and non-PND weight vs. length data from the stranding sample to see if the slope significantly differed from zero. Females and males were analyzed separately because the resident dolphins in Sarasota Bay are known to be sexually dimorphic (Read et al. 1993, Tolley et al. 1995). Residuals of the linear regressions were analyzed relative to assumptions for normality and equal variance. Then I fit a non-linear weight vs. length model to all of the PND and non-PND data, separately for males and females, using ordinary least squares (OLS) regression and the equation:

$$TM = 10^{a} * TL^{b} \tag{2-1}$$

where TM is total mass (kg), TL is total length (cm), and a and b are parameter estimates (Innes et al. 1981, Hart et al. 2013). Starting values required by Program R for parameter estimates when fitting non-linear OLS models were obtained by using values 20% different than Microsoft Excel 2010 solver estimates. Based on visual comparisons, the nonlinear models for both sexes fit better than the linear models. Following the methods of Hart et al. (2013) with the quantreg package in R, I used quantile regression and Equation 2-1 to create 95% quantile ranges for the PND and non-PND length-weight data. I chose quantile regression because it does not assume normality or homogeneity in variance as does simple linear regression (Cade and Noon 2003), and both the female and male weight vs. length regression residuals did not meet the simple linear regression assumptions. The starting values for the a and b parameters for the median fit (tau=0.5) of the quantile range were obtained from the R estimates from the non-linear OLS regression fit. The a and b starting values for the upper (tau=0.975) and lower (tau=0.025) 95% quantile fits were obtained from the median fit's parameter estimates. For each of the four line drawing methods, I plotted the observed weight vs. length points within the 95% quantile ranges and compared the relative locations of the observed points for animals with and without a PND. These plots allowed me to observe general differences in body condition of animals with and without PNDs and to assess which PND index's results most accurately described body condition.

Body Mass Index (BMI) Calculations

Using the b parameter estimate from the nonlinear OLS regression fit of Equation 2-1, I calculated BMI with the equation:

$$BMI = (TM \div TL^{b}) * 10000 \tag{2-2}$$

(Hamill et al. 1995, Harwood et al. 2000, Hart et al. 2013). BMIs of PND and non-PND animals were then compared for each of the four line drawing methods using a Wilcoxon rank sum test in R. I also compared boxplots of the BMIs for PND and non-PND animals for each of the four methods. This BMI model allowed me to further observe both the general differences in body condition of PND vs. non-PND animals and compare the four methods in terms of their PND outcomes.

95% Quantile Ranges for the Recommended PND Index Using Non-PND Data

After determining which PND index consistently reflected the body condition analysis results and was the most flexible in terms of its application, I then created 95% quantile reference ranges using only the non-PND data from this PND index's results. I plotted the observed non-PND and PND data on the same graph as the reference ranges. This plot differs from the other plots because the quantiles were fit to only the non-PND data as opposed to a combination of the PND and non-PND data. I fit the 95% quantile ranges using only non-PND data from the recommended index to see if the PND data would fall outside of the ranges of the non-PND data.

Comparison of BMI Values between Dolphins with a PND and Dolphins Considered to Be Emaciated

I was able to determine whether or not a dolphin was considered emaciated by the professional opinion given in the necropsy reports for a subset of individuals within the sample. I then used boxplots to compare the BMI values for dolphins identified as having PNDs by the recommended index with the BMI values of individuals that were considered to be emaciated. I made this comparison to examine how the body condition of dolphins identified as having PNDs related to the professional opinion of body condition recorded in the necropsy reports.

Table 2-1. Summary of possible cranial start points of the line, amount of dorsal surface required in the photo, and caudal endpoints of the line for all four methods. The three possible cranial start points for each technique were the same, but the amount of the dorsal surface included in the photo and the caudal endpoint of the lines differed for each technique.

Method #	Cranial start point	Dorsal surface included in photo from blowhole to:	Caudal end point at dorsal surface
1	1.Tip of nuchal crest if visible	Point between anterior insertions of the pectoral and dorsal fins (varies)	Highest point of dorsal surface in the photo at or caudal to the anterior insertion of the pectoral fin
2	2. If 1 is not possible, then dorsal surface directly above the ear if the ear is visible	Anterior insertion of the dorsal fin	Highest point of dorsal surface between anterior insertions of the pectoral and dorsal fins
3	3. If 1 and 2 are not possible, then dorsal surface above the approximate location of the ear	Posterior insertion of the pectoral fin	Point directly above posterior insertion of the pectoral fin
4		Anterior insertion of the pectoral fin	Point directly above the anterior insertion of the pectoral fin

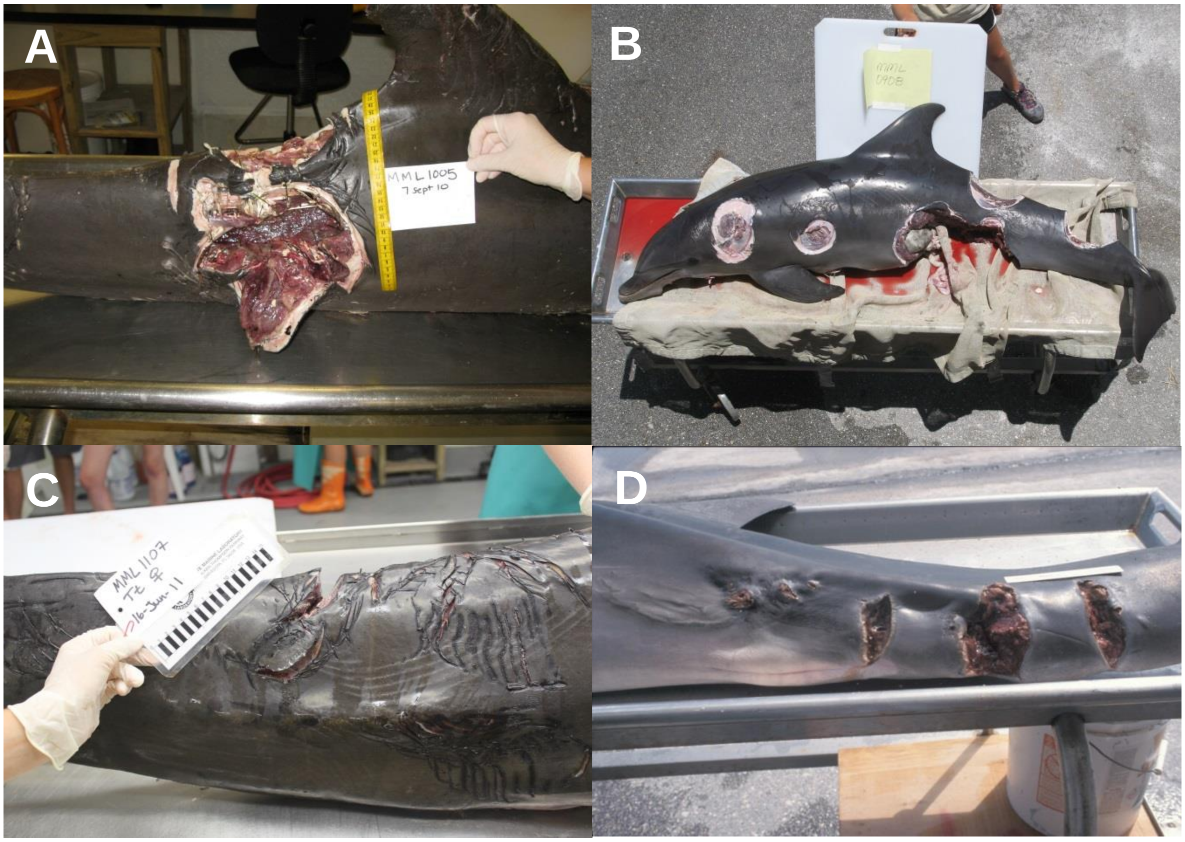

Figure 2-1. Examples of wounds\tissue loss that were thought to have a significant effect on the total weights of the animals (A and B) and wounds that caused minimal tissue loss and were considered to not have a significant impact on the carcasses' total weights (C and D). A) and B) carcasses with deep shark bites C) an animal with a possible cut or bite, D) an animal with propeller wounds. Both animals A and B were excluded from the sample, while C and D were kept in the sample. Photos courtesy of MML SIP.

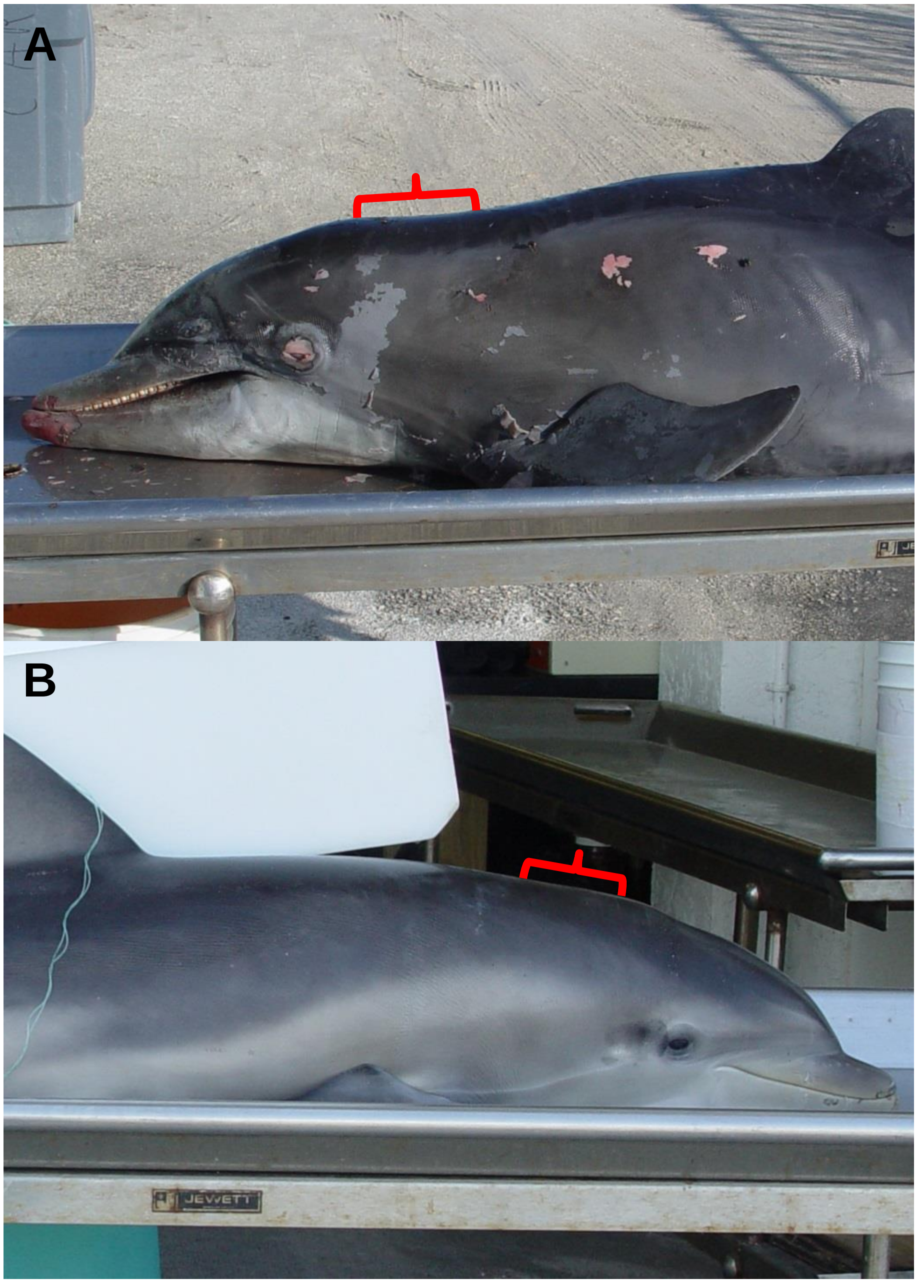

Figure 2-2. Examples of ideal photo quality for PND analysis, including the desired angle from which the photo was taken (perpendicular to the sagittal plane and roughly centered on the post- nuchal region, which is indicated by the red brackets), body position of the animal (upright), amount of the dorsal surface included in the photo (blowhole to at least the point above anterior insertion of the pectoral fin), and focus/clarity, and contrast. A) example 1, B) example 2. Photos courtesy of MML SIP.

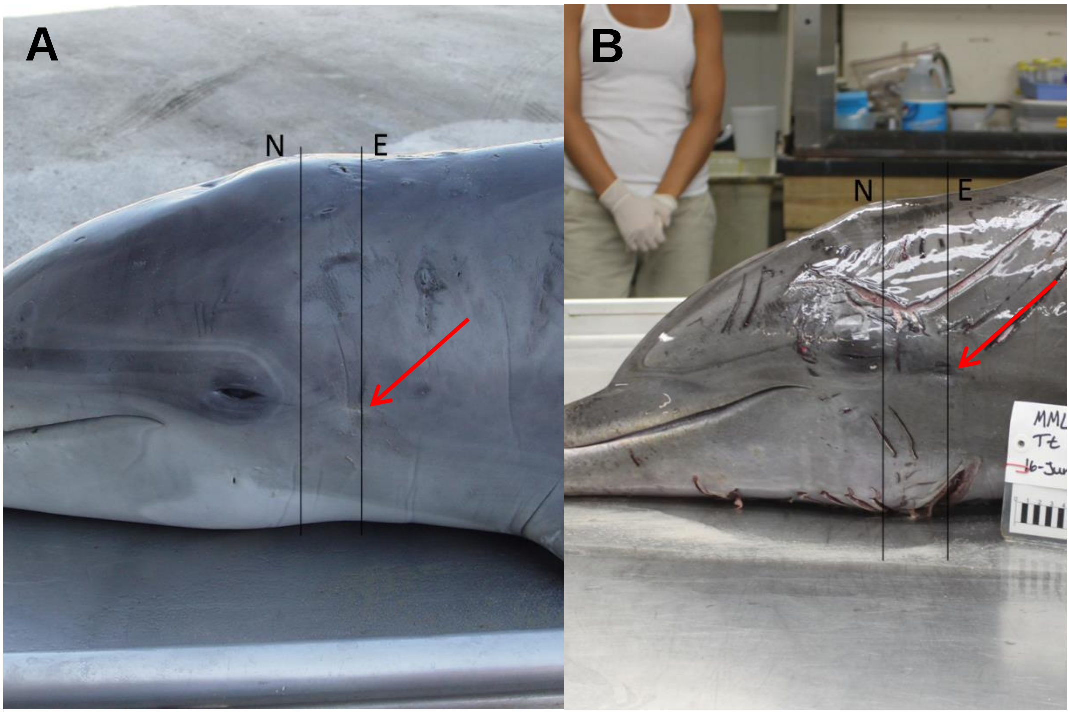

Figure 2-3. Location of the external ear relative to the nuchal crest for two dolphins. The vertical lines marked by the letter “E” go through the ear, and the vertical lines marked by the letter “N” go through the nuchal crest. A red arrow points to the location of each of the ears. For both dolphins, the nuchal crest is located anterior to the ear. A) a 186 cm male, B) a 245 cm female. Photos courtesy of MML SIP.

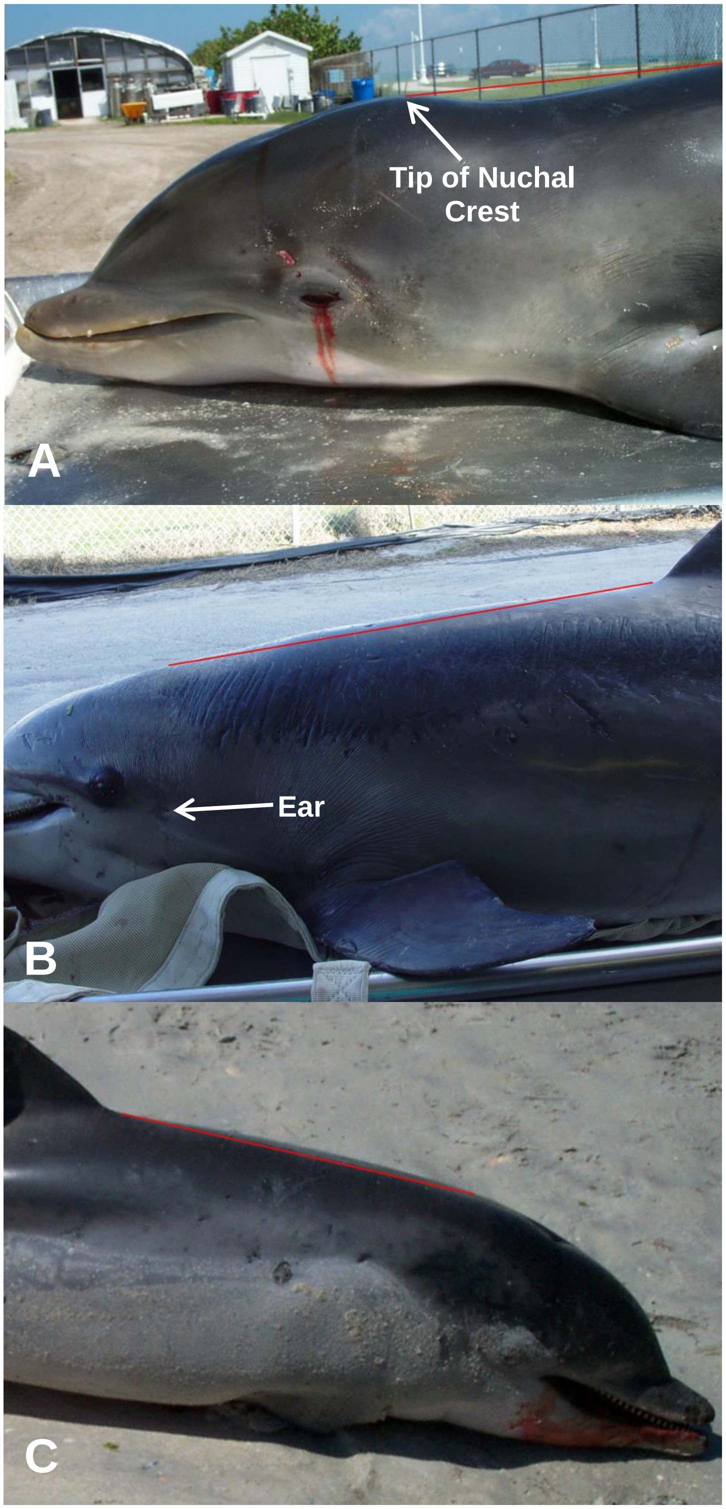

Figure 2-4. Examples of the three possible cranial starting points to the PND lines depending on whether or not the nuchal crest or external ear was visible. A) The tip of the nuchal crest, B) The dorsal surface directly above the ear, C) The dorsal surface above the approximate location of the ear. Photos courtesy of MML SIP.

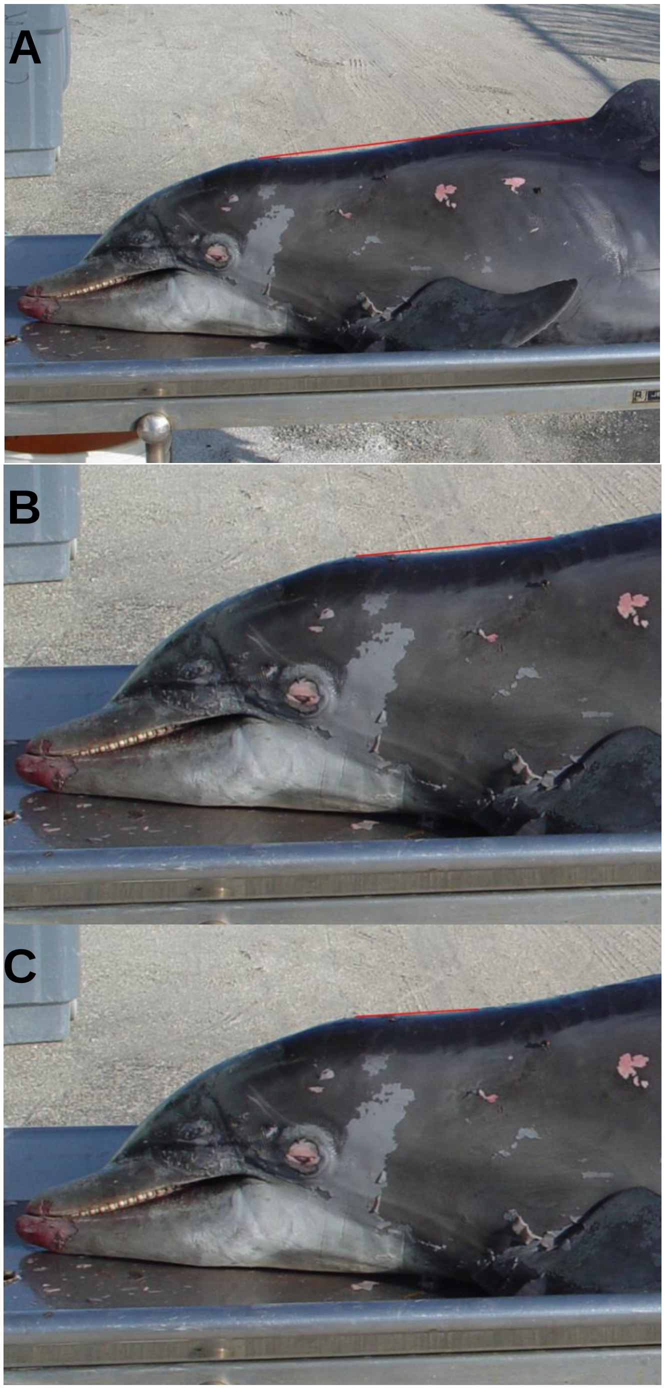

Figure 2-5. Examples of each of the four methods applied to an animal with a PND. A) This photo and line combination was used for both methods # 1 and 2, B) Method #3, C) Method #4. Note the cranial start point for each of the lines is the same, while the amount of dorsal surface included in the photos and the caudal endpoints of the lines differ. Note also that the space between the line and dorsal surface in each of these photos (space is very small in photo C) indicates that all methods identify this animal as having a PND. Photos courtesy of MML SIP.

Figure 2-6. Examples of each of the four methods applied to an animal without a PND. A) Method #1, B) Method #2, C) Method #3, D) Method #4. Note the cranial start point for each of the lines is the same, while the amount of dorsal surface included in the photo and the caudal endpoints of the lines differ. Note also that a space between the line and dorsal surface does not exist in any of these photos, which indicates that all methods identify this animal as not having a PND. Photos courtesy of MML SIP.

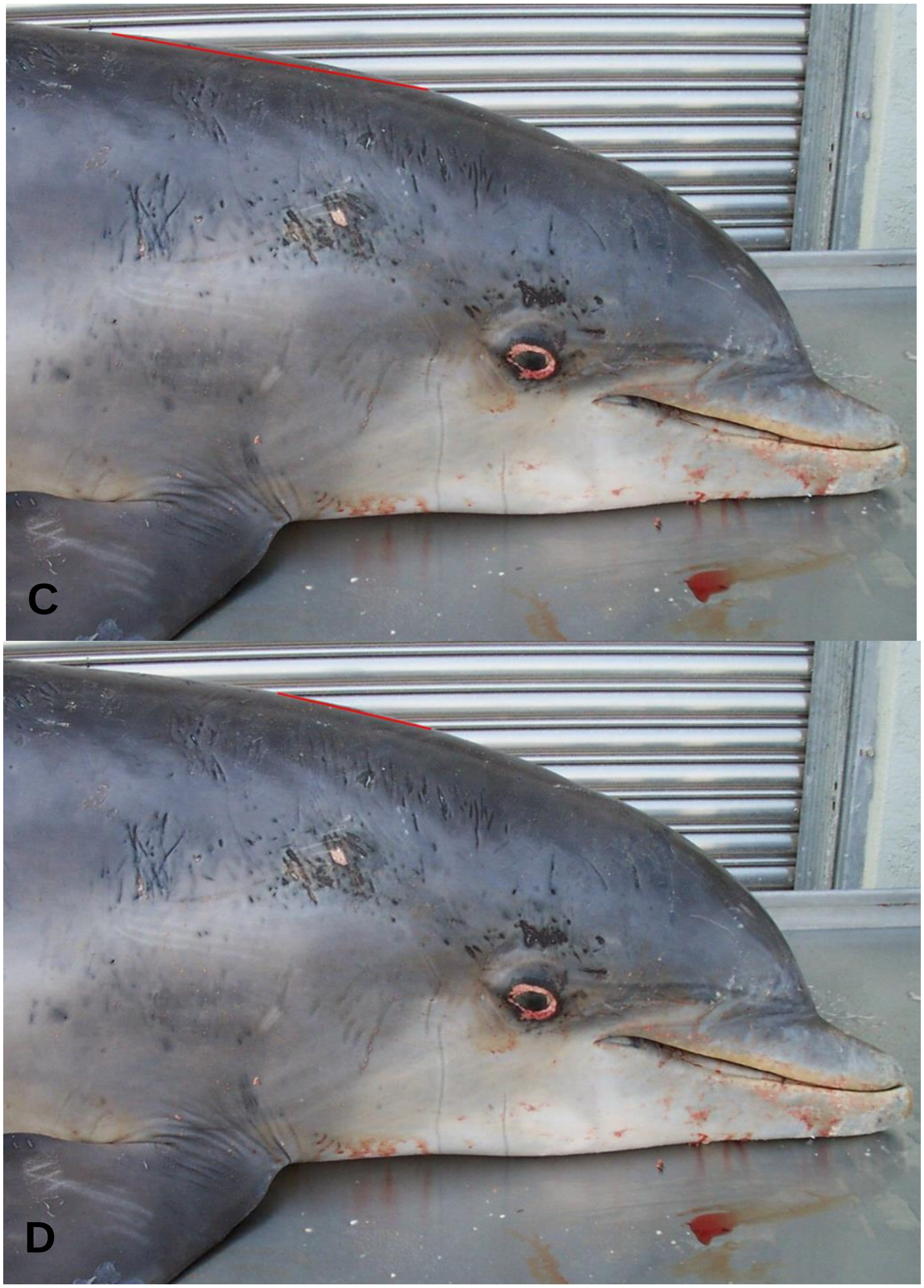

Figure 2-6. Continued

CHAPTER 3
RESULTS

Summary of Length-Weight Data

Of the 228 bottlenose dolphin stranding records from 2000-2012, 20 females and 24 males met the requirements for analysis. Data suitable for the PND index and body condition analyses, including the PND outcomes for each of the four PND indices, are summarized in Tables 3-1 and 3-2.

Simple Linear Regression Model and Assumptions

The slopes of the simple linear regression weight vs. length models for females and males were both significantly different than zero (Females: slope = 1.1837, std. error = 0.1002, $p<0.001$, Figure 3-1; Males: slope = 1.3590, std. error = 0.1281, $p<0.001$, Figure 3-4). Residual analysis showed that neither the female or male models met the assumptions for normality or homogenous variance (Females: Figures 3-2 and 3-3; Males: Figures 3-5 and 3-6).

Nonlinear Model Parameter Estimates and Visual Comparison of the Linear and Nonlinear Model Fits

Table 3-3 lists the parameter estimates that resulted from the nonlinear ordinary least squares (OLS) regression fit of Equation 2-1 for both sexes, and Figures 3-7 and 3-8 show the nonlinear fit of this model for females and males, respectively. Visually comparing the simple linear regression model to the nonlinear OLS regression model indicates that the latter better fit the observed data for both sexes (see Figures 3-1, 3-4, 3-7, and 3-8).

PND Indices Performance Assessment

95% Quantile Regression Reference Ranges

Since the nonlinear models fit the data better, they were used to create 95% quantile regression reference ranges. Tau values and the a and b parameter estimates for each quantile are show in Table 3-4.

Females

Methods #1, 2, and 3 each classified the same six females as possessing a PND (Table 3-1, Figure 3-9). Two females that were identified as negative for possessing a PND by Methods #1,3, and 4 were marked as “Cannot Be Determined” (CBD) for Method #2. Method #4 identified only four of the six PNDs that the other methods identified. Of these two animals that Method #4 did not recognize as possessing a PND, one was on the lower 95% quantile line and the other was on the median line (Figure 3-9). Therefore Methods #1-3 had similar results, while Method #4 was the only index to not identify an animal on the lower 95% quantile range as possessing a PND.

Males

Methods #1 and 2 classified the same seven males as having PNDs (Table 3-2, Figure 3-10). Method #3 identified nine animals as having PNDs, seven of which were shared with Methods #1 and 2. Method #4 recognized seven animals as having PNDs, five of which were the same as the other three methods. Method #3 identified the most animals below the median line as having PNDs, while Method #4 identified the least number of individuals below the median line as having PNDs and was the only method to identify an individual above the median line as having a PND (Figure 3-10).

BMI Calculations

Tables 3-1 and 3-2 list the BMI values calculated using Equation 2-2 and the b parameter estimate from the nonlinear OLS regression fit of Equation 2-1

Females

The Wilcoxon rank sum test comparing the female PND and non-PND BMI values showed that for each method, the BMI values for PND and non-PND animals were significantly different at $p<0.02$ (Table 3-5). Methods # 1 and 3 had the same results with the largest W statistic at the lowest p value (Table 3-5). Regardless of the method, boxplots showed that females with PNDs had lower BMI values than females without PNDs (Figure 3-11). These boxplots comparisons also demonstrated that Methods # 1 and 3 had the same results, and the distance between their PND and non-PND boxes was the greatest (Figure 3-11). Method #4 was the only index to include a BMI value in the non-PND boxplot lower than any BMI value included in the PND boxplot.

Males

The Wilcoxon rank sum test for the males showed that for each method, the BMI values for PND and non-PND animals were significantly different at $p<0.02$ (Table 3-5). Methods #1 and 2 had the same results with the second largest W statistic (Method #3 had the largest) and the lowest p values (Table 3-5). Similar to the females, the male boxplot BMI comparisons indicated that regardless of the method used, BMI values for dolphins that possessed a PND were lower than BMI values of animals that did not possess this trait (Figure 3-12). Methods #1 and 2 had the greatest distance between the PND and non-PND boxes, while Method #4 had the least distance between the PND and non-PND boxes (Figure 3-12).

95% Quantile Ranges for Method #1 Using Non-PND Data

For both sexes, I fit 95% quantile regression ranges using Equation 2-1 and only the non-PND data resulting from Method #1 because this method consistently identified dolphins with relatively low weights for given lengths and dolphins with low BMI values as having PNDs in the body condition analyses (Table 3-6). It is also the most flexible method to apply to photos because it allows for variation in the amount of body required to draw the line. For the females, the 95% quantile regression plots showed four of the six PND observations fell below the lower quantile (Figure3-13). The remaining two PND observations fell below the median. For the males, five of the seven PND points fell below the lower quantile, and the remaining two PND points fell below the median (Figure 3-14).

Comparison of BMI Values between Dolphins with PNDs and Dolphins Considered to be Emaciated

For 15 of the 20 females and for 19 of the 24 males in my sample, body condition (emaciated or not) was consistently described in the necropsy report. Eleven of the 15 females were considered emaciated within the reports. These 11 emaciated females included all six PNDs identified by Method #1, so five emaciated females (45%) were not identified as having PNDs by Method #1. Eleven of the 19 males were considered emaciated within the reports. These 11 emaciated males included all seven PNDs identified by Method #1, so four emaciated males (36%) were not identified as having PNDs by Method #1. For both sexes, boxplot comparisons of BMI values demonstrated that dolphins identified as possessing a PND by Method #1 fell low within the range of dolphins considered to be emaciated within the necropsy reports (Figures 3-15 and 3-16).

Table 3-1. Females that met the requirements for the PND index and body condition analyses, including the results for each of the four PND indices.

Stranding ID females:	Condition code	Date of carcass recovery	Total length (cm)	Total weight (kg)	BMI	#1	#2	#3	#4
MML0115	2	03-Oct-2001	146.7	42.2	1.25	0	0	0	0
MML0202	3	21-Jan-2002	189.0	81.5	1.27	0	0	0	0
MML0305	2	15-Feb-2003	171.0	45.5	0.92	1	1	1	0
MML0309	3	24-Feb-2003	236.0	120.0	1.06	0	0	0	0
MML0403	1	09-Mar-2004*	190.0	90.0	1.38	0	CBD***	0	0
MML0409	2	12-May-2004	246.0	147.0	1.17	0	0	0	0
MML0412	2	02-Jul-2004	238.0	168.0	1.45	0	CBD***	0	0
MML0413	2	04-Aug-2004	250.0	169.0	1.29	0	0	0	0
MML0416	2	12-Sep-2004	267.0	162.0	1.05	0	0	0	0
MML0527	3	13-Sep-2005	236.0	132.5	1.17	0	0	0	0
MML0538	2	15-Dec-2005	252.0	150.5	1.13	1	1	1	0
MML0601	3	9-Jan-2006	206.0	102.5	1.28	0	0	0	0
MML0602	2	12-Jan-2006	151.0	39.0	1.08	0	0	0	0
MML0608	1	01-Apr-2006**	205.0	93.0	1.18	0	0	0	0
MML0611	2	01-May-2006	241.0	114.5	0.96	1	1	1	1
MML0808	2	17-Dec-2008	165.0	65.0	1.43	0	0	0	0
MML0904	2	22-May-2009	235.0	123.0	1.10	1	1	1	1
MML1107	2	16-Jun-2011	245.0	130.0	1.04	1	1	1	1
MML1210	2	08-Dec-2012	266.4	214.5	1.39	0	0	0	0
MML1211	3	28-Dec-2012	169.3	47.0	0.97	1	1	1	1

*Date of rescue from entanglement of live animal. **Date of recovery of live animal that died the same day.
***CBD = Could not be determined

Table 3-2. Males that met the requirements for the PND index and body condition analyses, including the results for each of the four PND indices.

Stranding ID males:	Condition code	Date of carcass recovery	Total length (cm)	Total weight (kg)	BMI	#1	#2	#3	#4
MML0016	2	02-Aug-2000	224.0	69.0	0.20	1	1	1	1
MML0203	2	21-Feb-2002	227.0	146.5	0.41	0	0	0	0
MML0208	2	28-Feb-2002	193.0	94.0	0.41	0	0	0	0
MML0313	3	07-Apr-2003	210.0	85.5	0.29	1	1	1	0
MML0315	3	22-Apr-2003	191.0	85.5	0.38	0	0	0	0
MML0319	2	29-Apr-2003	167.0	42.0	0.27	1	1	1	1
MML0320	3	07-May-2003	177.0	70.0	0.39	0	0	0	0
MML0330	3	26-Aug-2003	172.0	61.5	0.37	1	1	1	1
MML0338	3	30-Nov-2003	162.0	59.5	0.42	0	0	0	0
MML0339	2	14-Dec-2003	190.0	73.0	0.33	0	0	0	0
MML0404	2	03-Mar-2004	200.0	70.0	0.28	1	1	1	0
MML0501	2	24-Jan-2005	200.0	92.5	0.36	0	0	0	0
MML0502	2	27-Jan-2005	255.0	217.5	0.44	0	0	0	0
MML0503	2	05-Feb-2005	186.0	61.5	0.30	1	1	1	1
MML0604	2	09-Feb-2006	156.0	49.5	0.39	0	0	0	0
MML0606	2	03-Mar-2006	263.0	202.5	0.37	0	0	0	1
MML0617	3	03-Jul-2006	151.0	41.0	0.35	0	0	0	0
MML0618	2	06-Jul-2006	257.0	223.0	0.44	0	0	0	0
MML0619	2	13-Jul-2006	255.0	150.5	0.30	1	1	1	1
MML0810	2	30-Dec-2008	161.0	52.5	0.38	0	0	1	0
MML1102	3	02-Feb-2011	276.0	175.5	0.28	0	0	0	0
MML1104	2	15-Mar-2011	214.0	98.5	0.32	0	0	1	1
MML1113	2	27-Dec-2011	252.0	167.0	0.35	0	0	0	0
MML1208	3	13-Nov-2012	157.0	49.0	0.38	0	0	0	0

Table 3-3. a and b parameter estimates from nonlinear OLS regression fit of $TM = 10^a * TL^b$ with corresponding standard error values.

Sex	Parameter estimates*	Standard error
Female	a: -3.93	0.62
	b: 2.55	0.26
Male	a: -4.46	0.64
	b: 2.78	0.27

*All parameter estimates were significant at $p<0.001$.

Table 3-4. Quantiles, tau values, and a and b parameter estimates with their standard errors for the 95% quantile regression fit of $TM = 10^a * TL^b$ including both PND and non-PND data.

Sex	Quantile	Tau	a ± std. error	b ± std. error
Female	Upper	0.975	-3.94 ± 0.71	2.59 ± 0.30
	Median	0.500	-3.49 ± 0.82	2.36 ± 0.35
	Lower	0.025	-4.35 ± 0.60	2.69 ± 0.26
Male	Upper	0.975	-4.55 ± 0.26	2.86 ± 0.11
	Median	0.500	-4.00 ± 0.72	2.59 ± 0.31
	Lower	0.025	-2.13 ± 2.93	1.69 ± 1.27

Table 3-5. Wilcoxon rank sum test W statistic and corresponding p values for each of the four PND indices.

Sex	PND index	W statistic	P value
Female	#1	78	0.0015
	#2	66	0.0032
	#3	78	0.0015
	#4	57	0.016
Male	#1	110	0.00054
	#2	110	0.00054
	#3	120	0.00098
	#4	96	0.019

Table 3-6. Quantiles, tau values, and a and b parameter estimates with their standard errors for the 95% quantile regression fit of $\mathrm{TM} = 10^{\mathrm{a}} * \mathrm{TL}^{\mathrm{b}}$ including only non-PND data.

Sex	Quantile	Tau	a ± std. error	b ± std. error
Female	Upper	0.975	-3.94 ± 0.59	2.59 ± 0.25
	Median	0.500	-3.18 ± 0.79	2.24 ± 0.34
	Lower	0.025	-3.85 ± 0.74	2.50 ± 0.31
Male	Upper	0.975	-4.55 ± 0.41	2.86 ± 0.18
	Median	0.500	-4.22 ± 0.67	2.70 ± 0.30
	Lower	0.025	-3.64 ± 1.07	2.41 ± 0.46

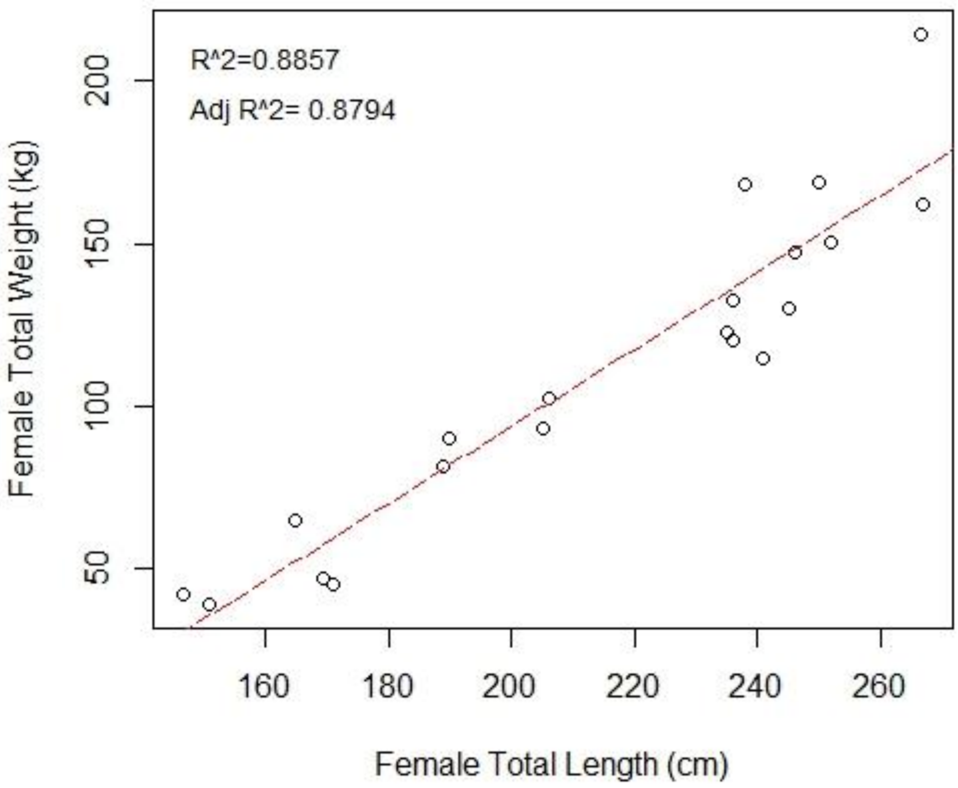

Figure 3-1. Simple linear regression weight vs. length model for females. Red dashed line represents predicted total weight (kg) for a given total length (cm), and circles are observed total weight.

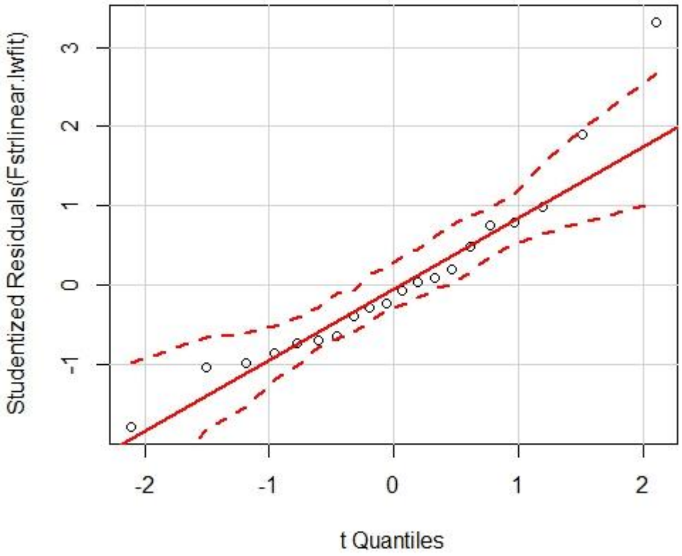

Figure 3-2. Q-q normality plot of studentized residuals of female weight vs. length simple linear regression demonstrating that the studentized residuals do not meet the assumption of normality.

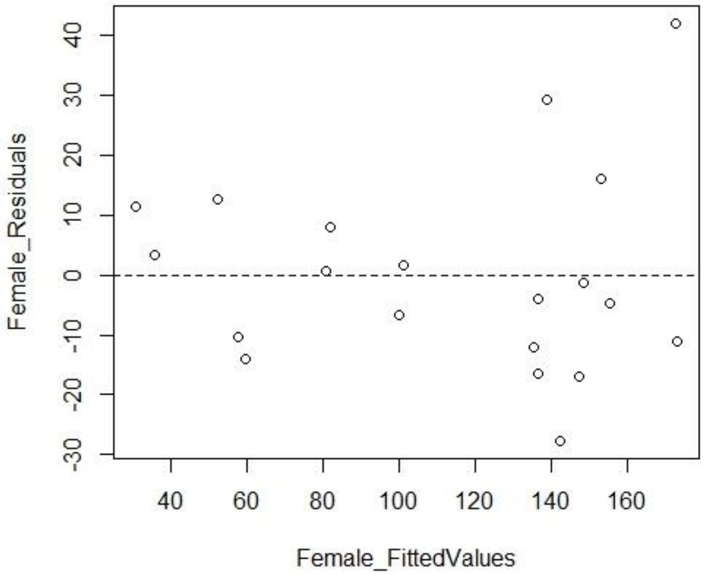

Figure 3-3. Residuals vs. fitted values for female weight vs. length simple linear regression indicating that the residuals do not meet the assumption for homogenous variance.

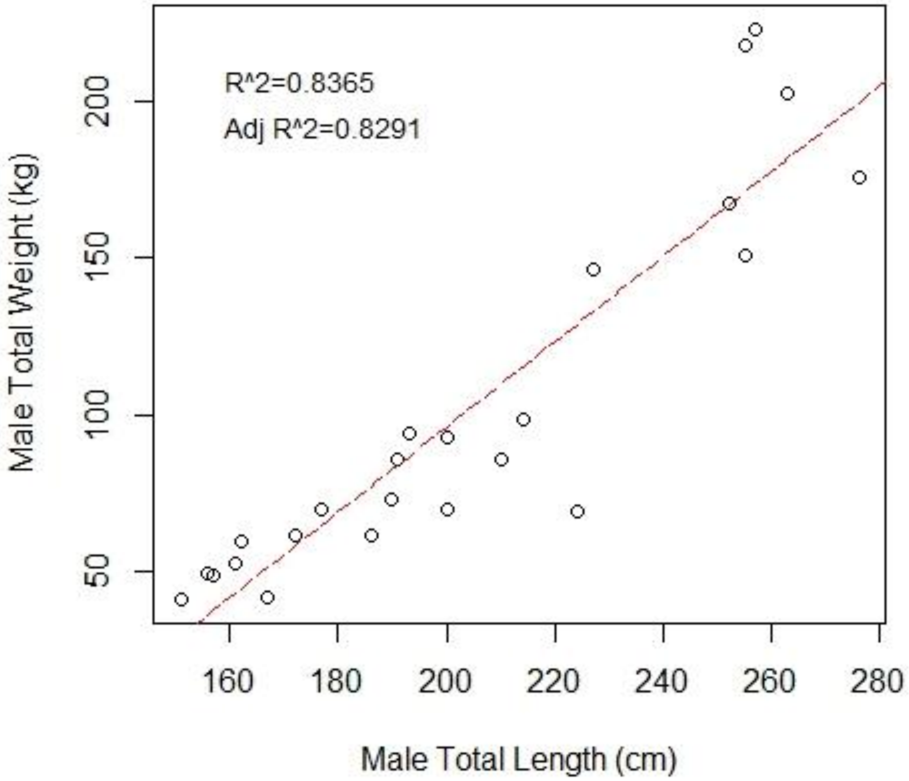

Figure 3-4. Simple linear regression weight vs. length model for males. Red dashed line represents predicted total weight (kg) for a given total length (cm), and circles are observed total weight.

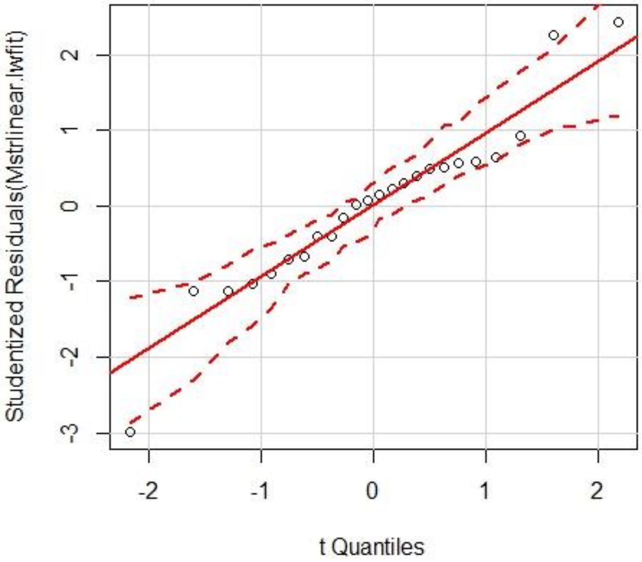

Figure 3-5. Q-q normality plot of studentized residuals of male weight vs. length simple linear regression showing that the studentized residuals do not meet the assumption of normality.

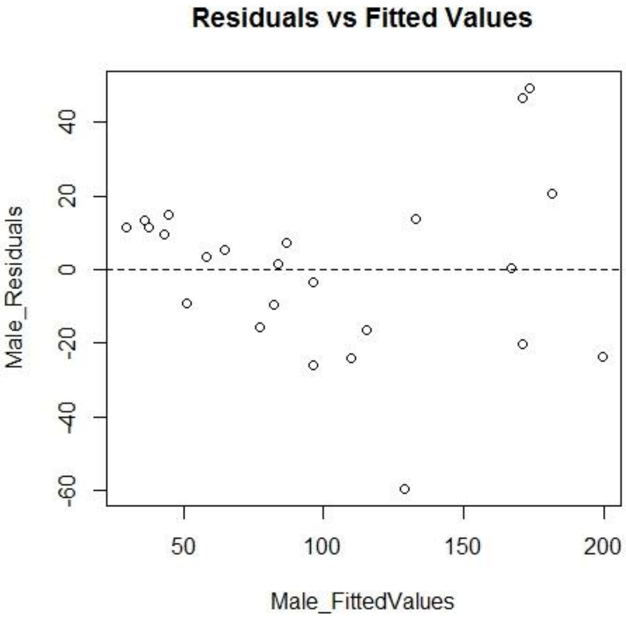

Figure 3-6. Residuals vs. fitted values for male weight vs. length simple linear regression model, showing that the residuals do not meet the assumption for homogenous variance.

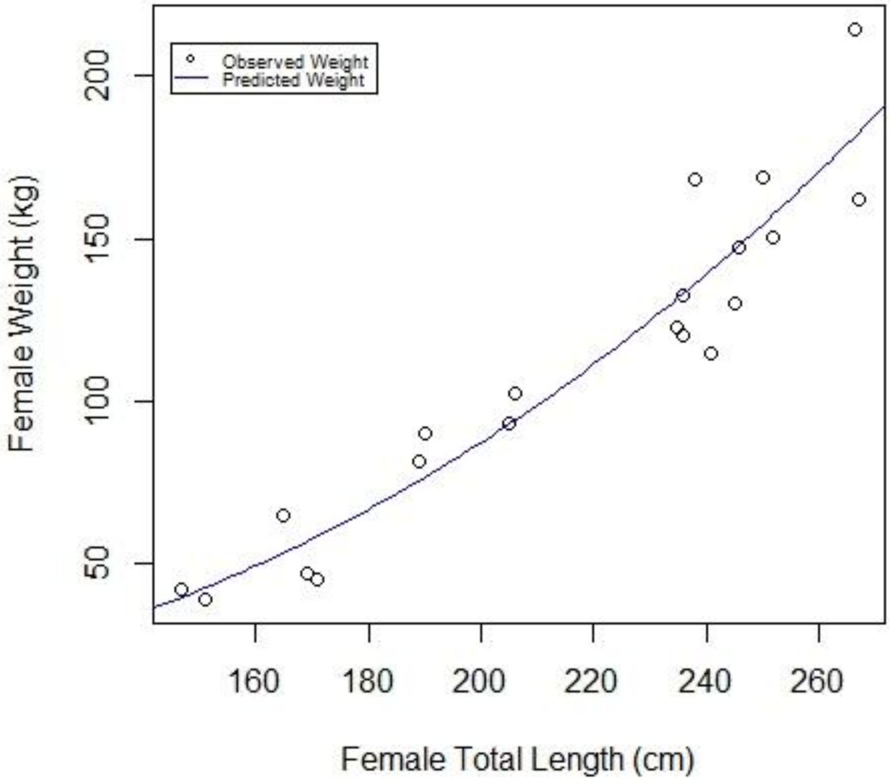

Figure 3-7. Nonlinear OLS regression fit of $TM = 10^a * TL^b$ to female length-weight data.

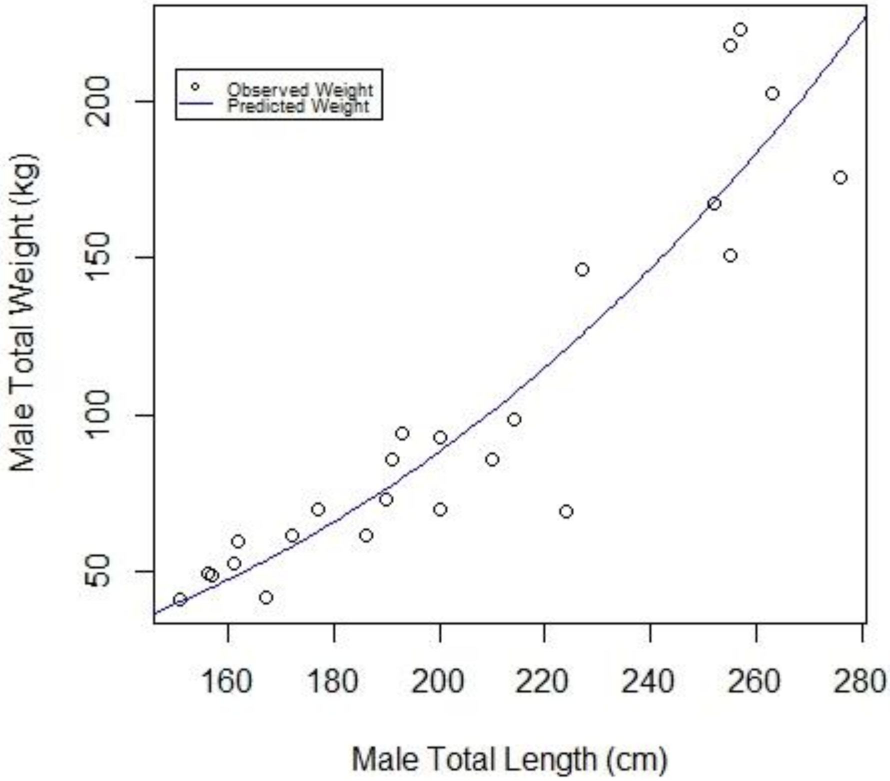

Figure 3-8. Nonlinear OLS regression fit of $TM = 10^{a} * TL^{b}$ to male length-weight data.

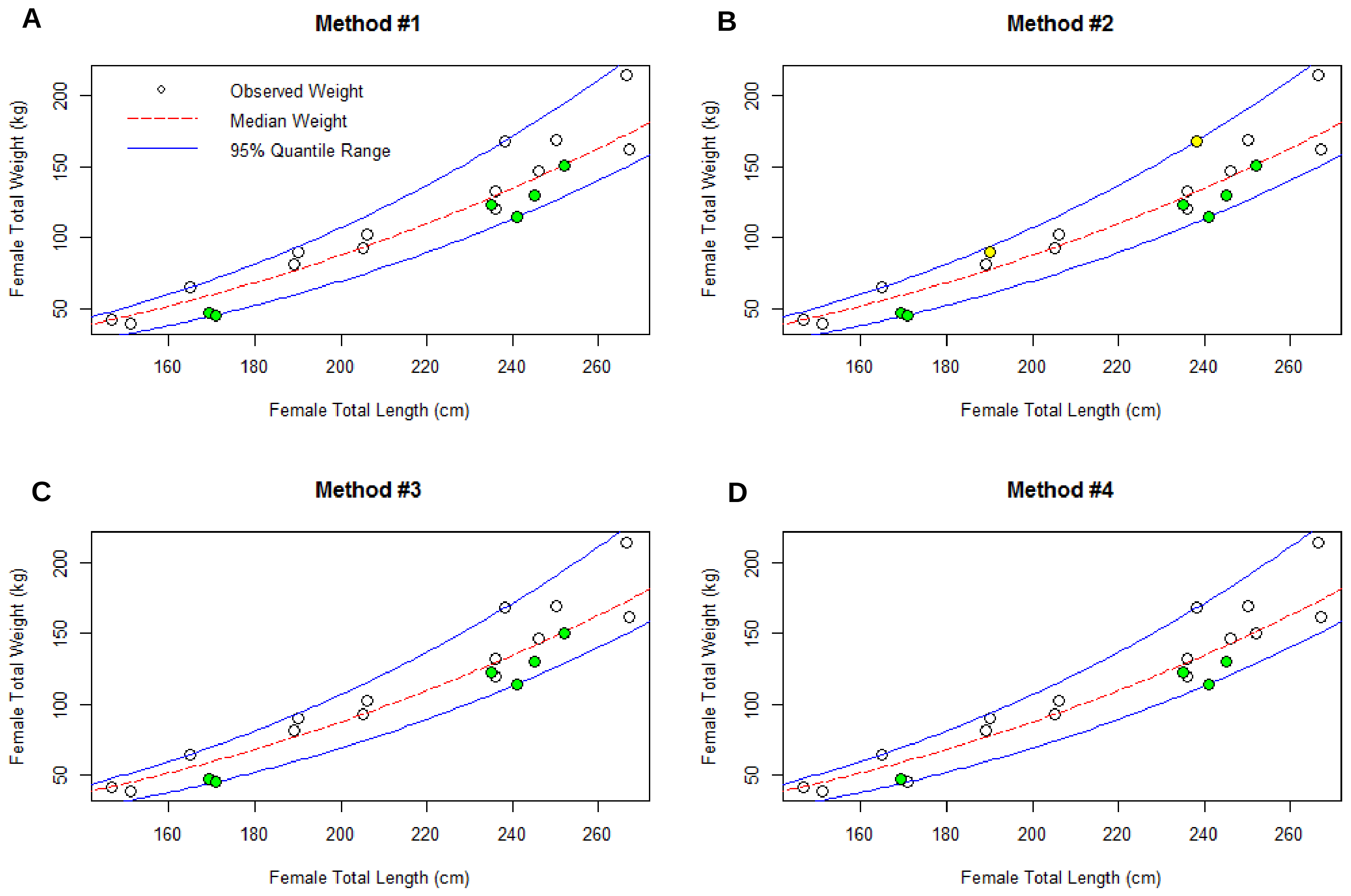

Figure 3-9. 95% quantile regression ranges and observed data for females for each of the four PND indices. Plots differ only in the number of highlighted data points. Green points represent animals with PNDs. The two yellow points represent animals for which Method #2 results could not be determined. A) Method #1, B) Method #2, C) Method #3, D) Method #4.

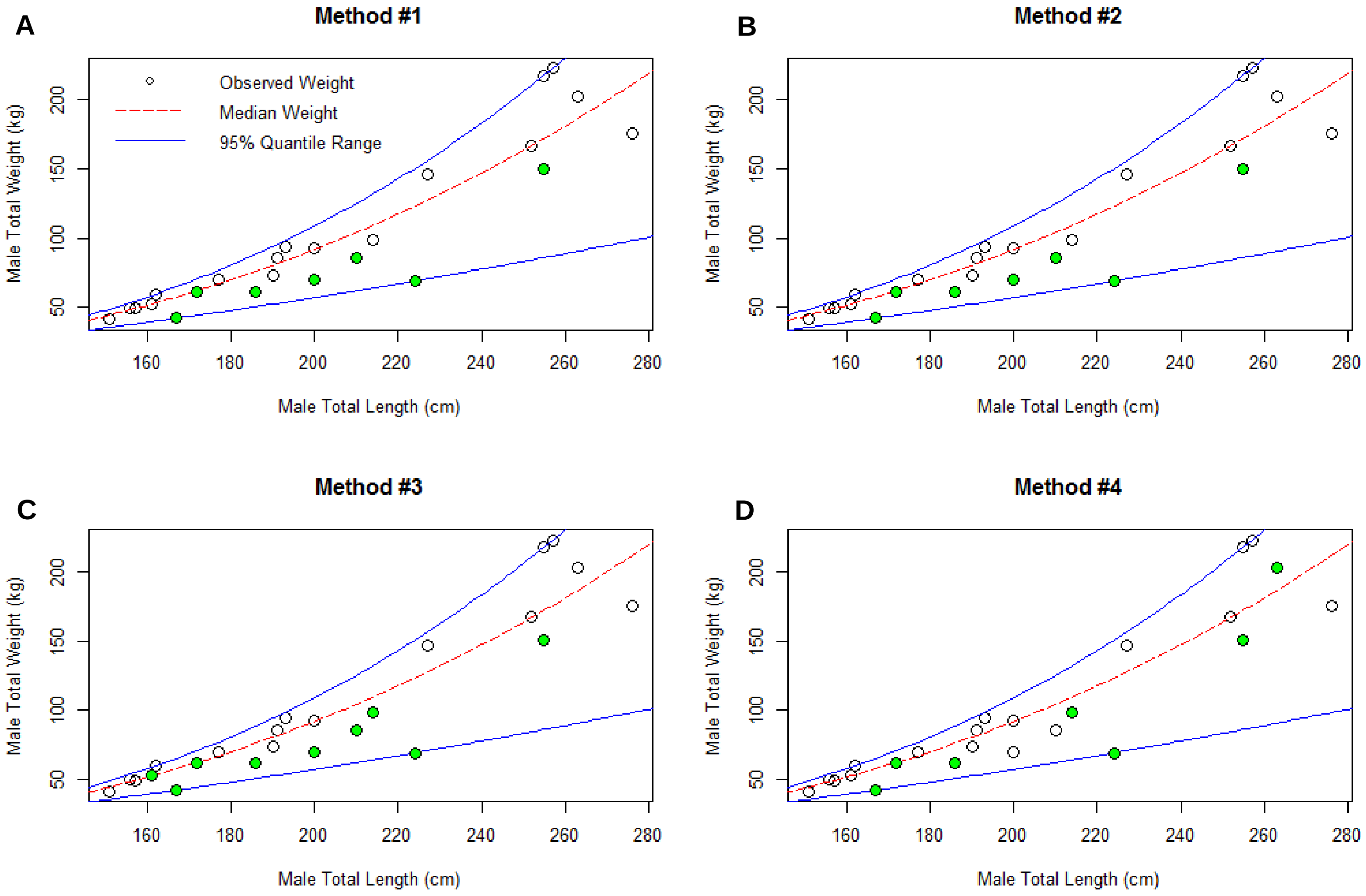

Figure 3-10. 95% quantile regression ranges and observed data for males for each of the four PND indices. Plots differ only in the number of highlighted data points. Green points represent animals with PNDs. A) Method #1, B) Method #2, C) Method #3, D) Method #4

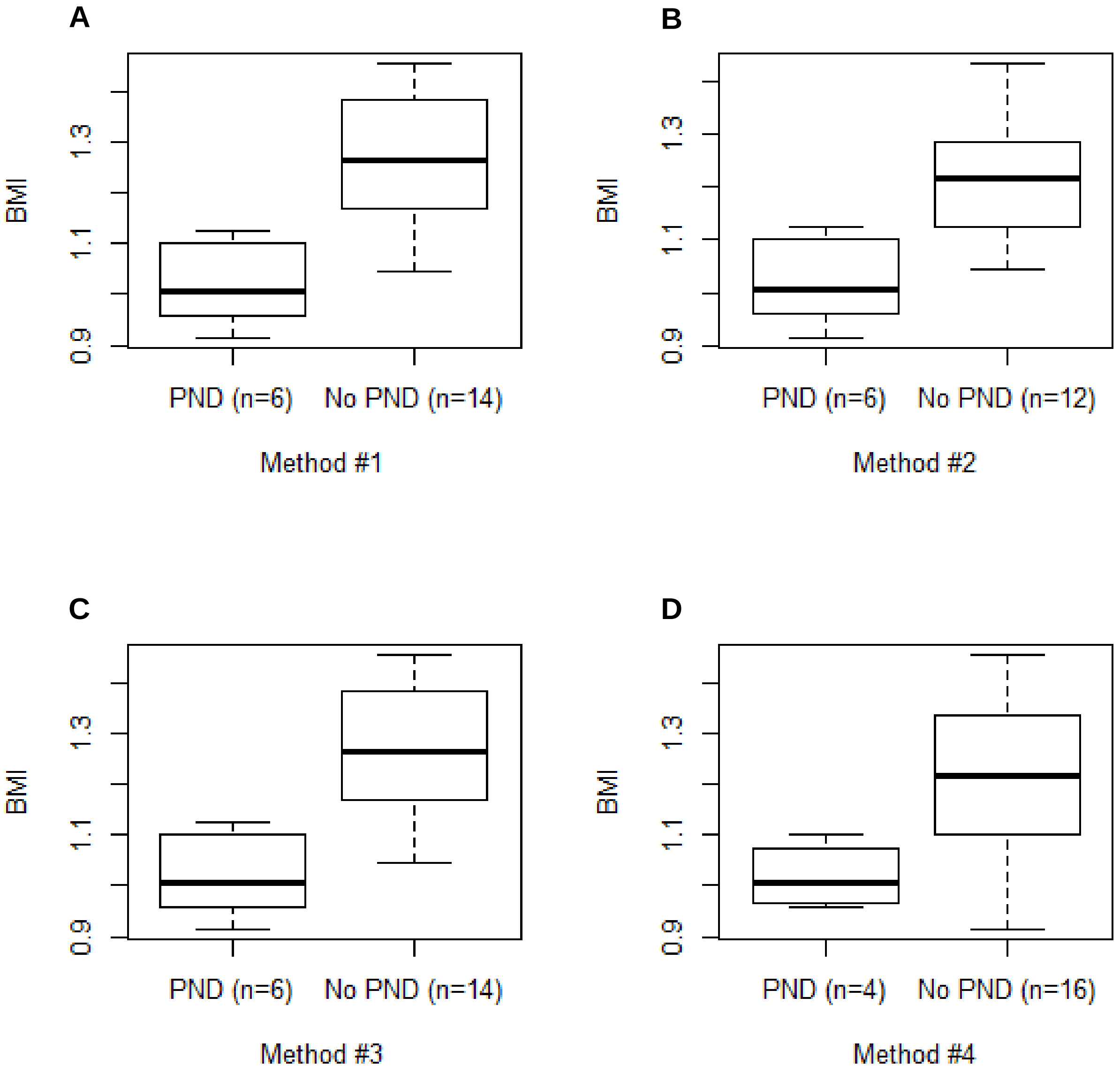

Figure 3-11. Boxplots for female PND and non-PND BMI values for each of the four PND indices. Hinges are versions of the first and third quartiles, and the line within the box is the median. The whiskers show the largest and smallest values that fall within 1.5 times the box size (Dalgaard 2008). A) Method #1, B) Method #2, C) Method #3, D) Method #4.

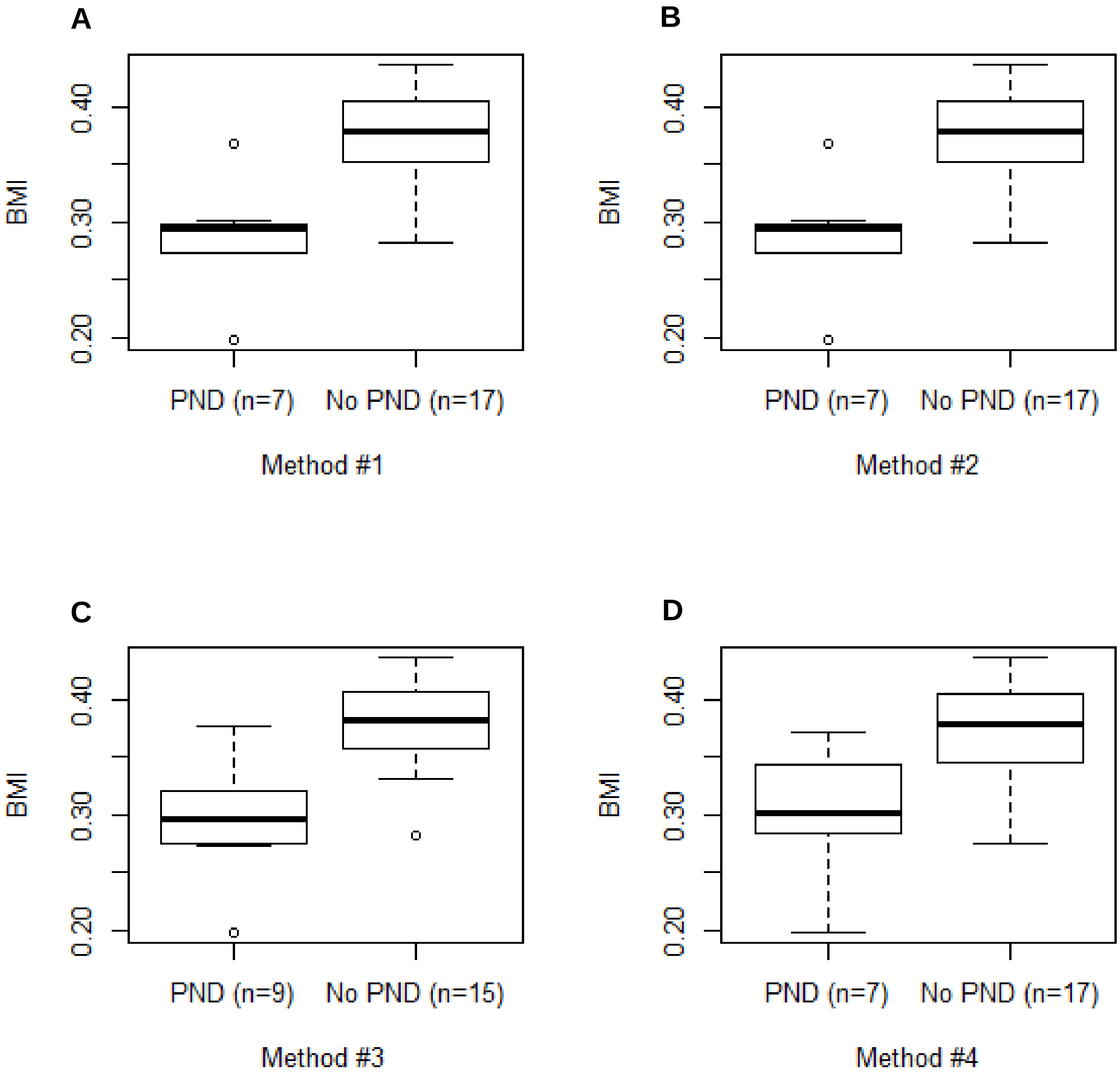

Figure 3-12. Boxplots for male PND and non-PND BMI values for each of the four PND indices. Hinges of the boxplot are versions of the first and third quartiles, and the line within the box is the median. The whiskers show the largest and smallest values that fall within 1.5 times the box size. Values outside of that range are shown separately as circles (Dalgaard 2008). A) Method #1, B) Method #2, C) Method #3, D) Method #4.

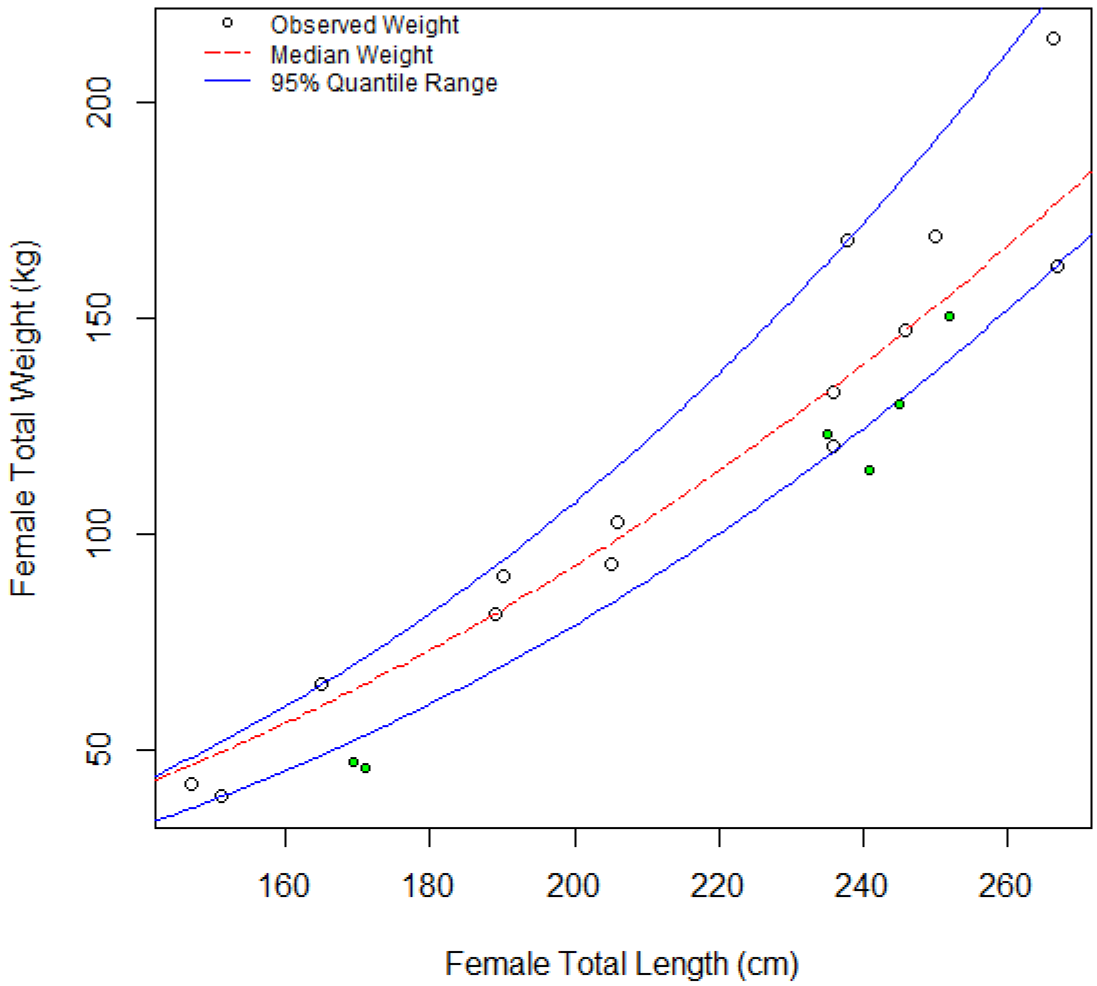

Figure 3-13. Female 95% quantile regression ranges fit to non-PND data of Method #1. Green points represent animals with PNDs.

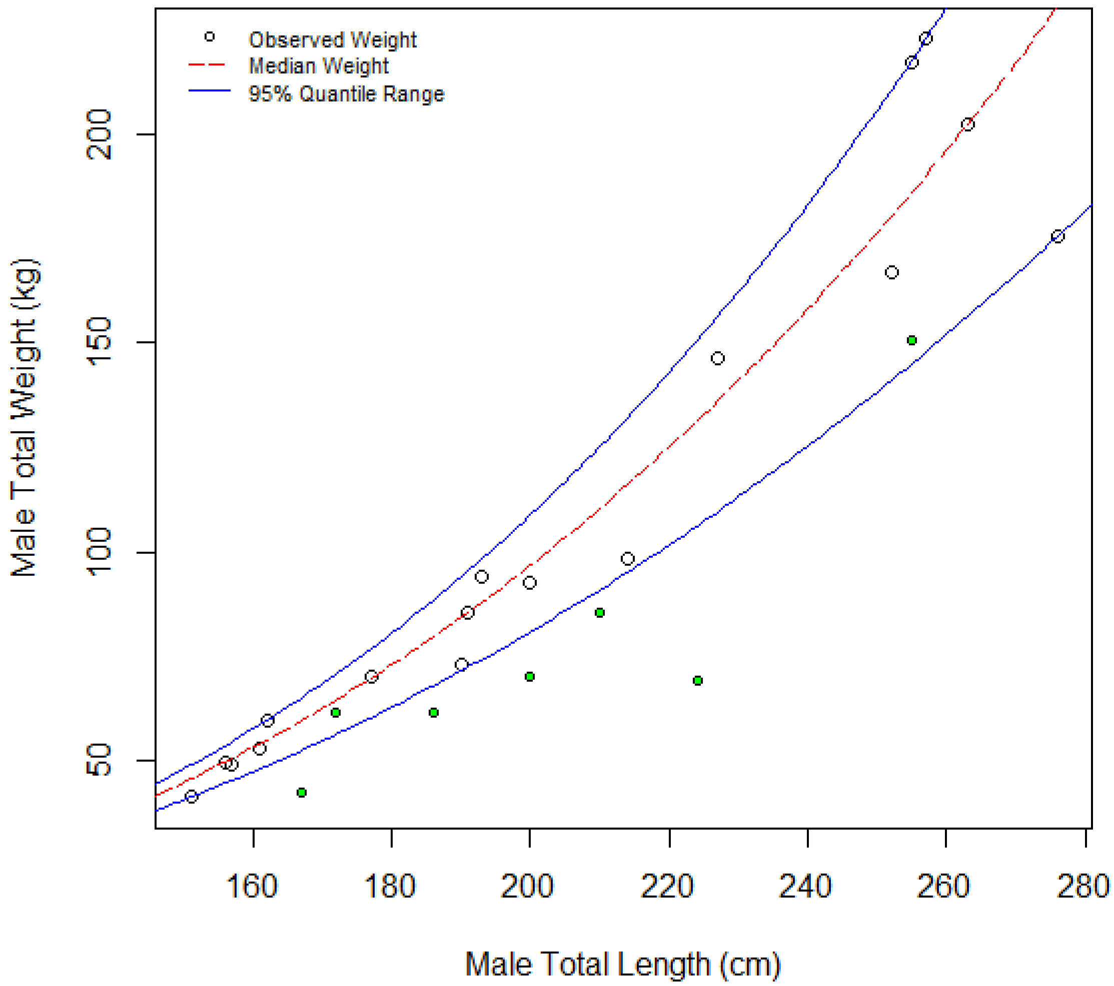

Figure 3-14. Male 95% quantile regression ranges fit to non-PND data of Method #1. Green points represent animals with PNDs.

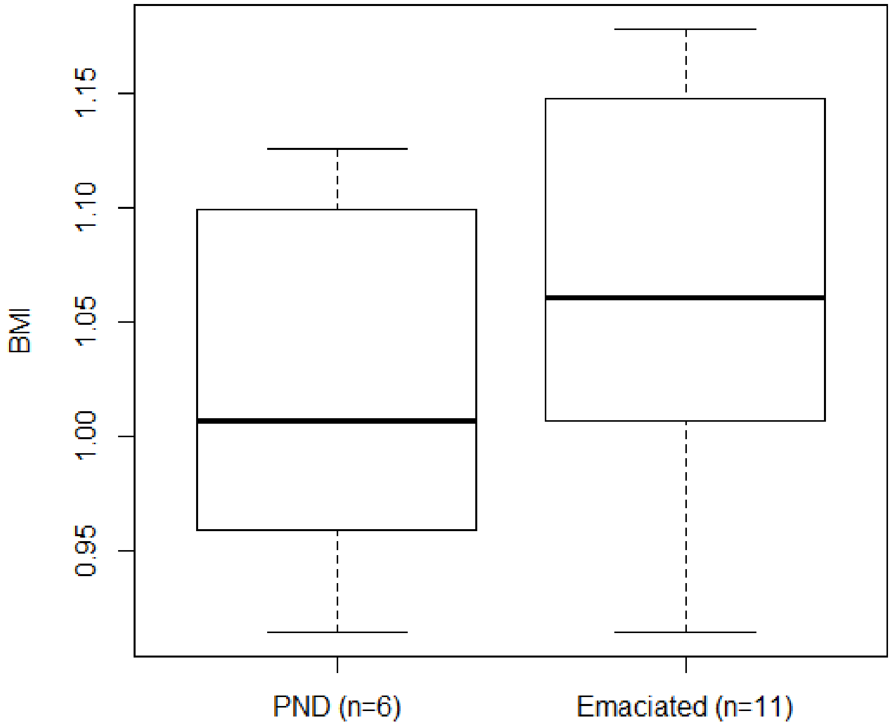

Figure 3-15. Boxplots of female BMI values for individuals with PNDs identified by Method #1 and for individuals noted as emaciated within their necropsy reports. Hinges are versions of the first and third quartiles, and the line within the box is the median. The whiskers show the largest and smallest values that fall within 1.5 times the box size (Dalgaard 2008).

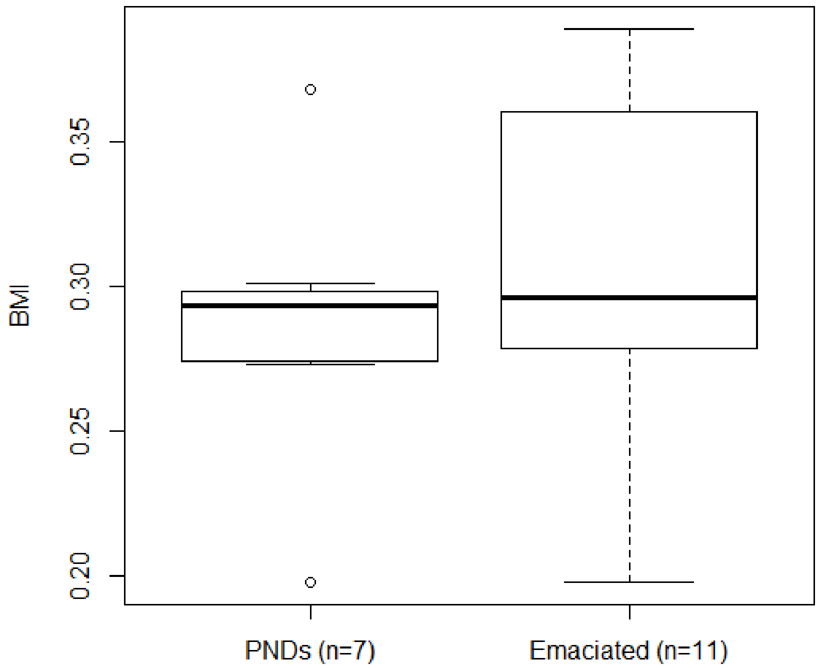

Figure 3-16. Boxplots of male BMI values for individuals with PNDs identified by Method #1 and for individuals noted as emaciated within their necropsy reports. Hinges are versions of the first and third quartiles, and the line within the box is the median. The whiskers show the largest and smallest values that fall within 1.5 times the box size. Values outside of that range are shown separately as circles (Dalgaard 2008).

CHAPTER 4
DISCUSSION

Differences in the Outcomes between the Four PND Indices

Bottlenose dolphin PND indices were developed to decrease the subjectivity inherent in visually assessing this trait based on a researcher's judgment without any systematic metric applied to photos. Preliminary analysis of photos taken both during Sarasota Bay bottlenose dolphin health assessments and stranding events showed that visually classifying dolphins with marginal depressions without applying any metric to the photos was difficult. Furthermore, the amount of the dorsal surface that needed to be visible in the photo in order to determine PND presence was not known. Therefore, I tested four different methods that involved drawing a horizontal line across the post-nuchal region of an individual to determine if a concavity existed in this area of the body. If a space was visible between the line and the dorsal surface in the post-nuchal region, the animal was considered to have a PND. The four methods varied by the amount of dorsal surface they required to be in the photo and the caudal endpoints of their lines (Table 2-1).

The four indices varied in their PND determinations (Tables 3-1 and 3-2). The first three methods identified two females (MML0305 and MML0538) and two males (MML0313 and MML0404) as possessing a PND, while Method #4 did not. MML0305's length-weight point was on the lower 95% quantile range, and MML0538's point was on the median line for the quantile regression ranges fit to both the PND and Non-PND data (Figure 3-9). MML0313 and MML0404's points were both below the median line for the male quantile regression ranges (Figure 3-10). The primary reason for these differences was that the Method #4 line did not span across enough of the dolphin body to incorporate the topography of the dorsal surface caudal to the anterior insertion of the pectoral fin, as can be seen with MML0538 in Figure 4-1. Therefore, the Method #4 line may incorrectly identify some animals as not possessing a PND when they really do (false negative). The differences resulting from using lines that span different lengths across the dolphin dorsal surface can be seen with an upright dolphin (MML0330) in Figure 2-5. Although all four indices indicated MML0330 had a PND, the space between the line and the dorsal surface becomes less evident when Method #4 is applied in comparison to the three other methods.

There were also instances where Methods #3 and/or #4 indicated the dolphin had a PND, while Methods #1 and #2 did not. This occurred for three males (MML0606, MML0810, MML1104) (Table 3-2). MML0606's Method #4 PND length-weight observation fell above the median line, while MML0810's Method #3 PND length-weight observation fell on the median line of the quantile regression ranges fit to both the PND and Non-PND data (Figure 3-10). MML1104's Method #3 and #4 PND length-weight observation fell below the median line (Figure 3-10). For both MML0606 Method #4 and MML0810 Method #3's PND outcomes, the spaces between the lines and their dorsal surfaces were very small (Figure 4-2). These spaces were more likely related to fine-scale changes in the topography of the dorsal surface under the lines in that region of their body than to concavities that are indicative of PND presence (false positive). For MML1104, the slightly skewed angle at which the photo was taken likely contributed to Methods #3 and #4 identifying this dolphin with a relatively low length-weight value as having a PND, while Methods #1 and #2 did not (Figure 4-3).

Effect of Varying the Cranial Start Points for One PND Index

Although the caudal endpoints of the lines differentiated each of the four PND indices, three possible cranial start points were common to each index—the nuchal crest of the skull, the point at the dorsal surface above the ear, and a point at the dorsal surface above the approximate location of the ear (Figure 2-3). Even though I chose these cranial start points because of their relative proximity to one another, the distance between them could potentially affect the PND outcome. In Figures 4-4 and 4-5, I drew the different cranial start points on two animals, MML0503 and MML1107, with Method #1 applied to them and where the nuchal crest and the ear were both visible. Since I could see the ear, I drew two cranial start points for the dorsal surface above the approximation of the ear—one anterior to the ear and one caudal to the ear. For MML0503, changing the cranial start point did not change the PND outcome (Figure 4-4). For MML1107, changing the cranial start point did change the PND outcome to Non-PND when the line was drawn above the point caudal to the ear (Figure 4-5). For the other three cranial start points, the lines identified the dolphin as having a PND, although the spaces under the lines that began at the point at the dorsal surface anterior to and directly above the ear were very small. This example demonstrates that if the cranial start point to the line is drawn too far in the caudal direction, the PND outcome may be a false negative. In general, when the space between the line and dorsal surface is small as in this example, very small changes to the line can affect the PND outcome.

When drawing the cranial start point, I recommend using the tip of the nuchal crest whenever it is visible. If the ear is visible, I suggest consistently drawing the cranial start point at either the dorsal surface above the ear or in between the eye and the ear, as the nuchal crest is most often located at the dorsal surface at some point between the eye and the ear. If both the nuchal crest and the ear are not visible, I recommend drawing the point at the dorsal surface above the approximate location of the ear, using the eye as a reference point (see methods above), but favoring the anterior direction. I would favor the anterior direction because the nuchal crest is rarely seen caudal to the ear in adults.

Body Condition Comparison between Dolphins with and without PNDs

To determine if post- nuchal depressions (PNDs) could be used as an indicator of poor body condition in bottlenose dolphins, I compared length-weight data of stranded individuals with and without PNDs for each of the four PND index methods. I analyzed 95% quantile regression ranges fit to length-weight data of both PND and non-PND animals for all four PND indices by sex. This analysis showed that regardless of the index used to identify PNDs, the length-weight observations of animals with PNDs generally fell lower in the 95% quantile range than those that did not have PNDs (Figures 3-9 and 3-10). Similarly, comparison of BMI values calculated for animals with and without PNDs showed those that display PNDs had lower BMI values for all PND indices used (Figures 3-11 and 3-12). These findings support the common belief among bottlenose dolphin trainers and researchers that animals with PNDs are in relatively poor body condition compared to those that do not have this trait (Struntz et al. 2004, Dunkin et al. 2005, Fair et al. 2006, Yordy et al. 2010).

Studies on harbor porpoises (*Phocoena phocoena*), have also referred to PNDs as indicative of emaciation (Kastelein, van Battum 1990, Cox et al. 1998, Koopman et al. 2002). Kastelein and Van Battum (1990) described PNDs as a sign of emaciation that occurs after depressions lateral to the dorsal fin can be seen (i.e. atrophied epaxial musculature). In other words, they described depressions lateral to the dorsal fin in individuals as an initial indicator of weight loss that is followed by the presence of PNDs in more emaciated individuals. Cox et al. (1998) used PNDs and atrophied epaxial musculature as signs of emaciation to compare the body condition of stranded harbor porpoises with and without signs of entanglement. They found that the percentage of emaciated porpoises in the non-entangled sample was significantly greater than in the entangled sample. Therefore, most of the entangled porpoises were in robust condition. Since a greater proportion of the non-entangled animals were emaciated, they suggested that the non-entangled animals may have died of starvation.

Studies involving the photographic analysis of the post-nuchal region of other cetaceans have also shown that animals with concavities are in relatively poor body condition compared to individuals without concavities. In a study of the North Atlantic right whale, Pettis et al. (2004) showed females had a significantly higher body condition score (higher score = deeper concavity in the post nuchal region) during the years that they were supporting a calf. In addition, animals that were presumed to be dead (were not sighted for five consecutive years) had significantly higher body condition scores in comparison to those that were considered to be living (sighted at least once in five consecutive years). Similarly, Bradford et al. (2012) showed that the body condition of western gray whale females that were lactating was significantly worse than other whales, and the body condition of weaning calves was significantly better than other whales. Although their study used three regions of the body to score body condition, the post- nuchal region had the most influence in their scoring technique.

Recommended PND Index

Of the four indices, I recommend Method #1 for three reasons: 1) it is flexible in terms of the amount of dorsal surface caudal to the blowhole that needs to be included in the photo (can vary from the dorsal surface at the point above the anterior insertion of the pectoral fin to the anterior insertion of the dorsal fin); 2) the animals that it identified as having PNDs had length-weight observations that fell relatively low within the 95% quantile ranges for both sexes (Figures 3-9 and 3-10); and 3) the animals it identified as having PNDs had the greatest difference in BMI values from those that did not have PNDs compared to the other three methods (Figures 3-11 and 3-12). Although other PND indices had similar or better results in one or two of these aspects, Method #1 is the only approach to simultaneously be flexible and have good body condition analyses results.

As the recommended approach, non-PND length-weight data from Method #1 were used to fit 95% quantile regression reference ranges. Both the non-PND and PND observations were then plotted with these reference ranges, and four of six female PND observations and five of seven male PND observations fell below the 95% quantile range of the non-PND data (Figures 3-13 and 3-14). In addition, all of the dolphins identified as possessing PNDs by Method #1 were classified by the necropsy teams as emaciated, and they had BMI values within the lower range of BMIs of emaciated individuals (Figures 3-15 and 3-16). These data further demonstrate that the body condition of animals with PNDs was poor in comparison to the body condition of individuals without PNDs. Furthermore, the causes of death (CODs) given in the necropsy reports listed emaciation or malnutrition as a contributing factor for seven of the 13 dolphins identified as having PNDs by Method #1 (Table 4-1). The remaining six individuals were documented as emaciated elsewhere within the necropsy report, but this was not necessarily considered the cause of death.

Overall, the PND index reduced the subjectivity of assigning PNDs to individual animals by providing a specific metric to evaluate. In addition, the approach is simple and does not require any specialized software or expertise, facilitating its application in funding limited locations. My hope is that if adopted, this simple index will aid in the development of standard approaches to assessing PND presence, allowing for greater comparison of this condition spatially and temporally.

Seasonal Effect on PND Occurrence

Seasonality could potentially play a role in the occurrence of PNDs in wild bottlenose dolphin populations. The waters in Sarasota Bay vary seasonally from less than 13 to 35^{o}C, and Sarasota Bay dolphin residents decrease their blubber thickness by about 38% from the winter to the summer (Wells et al. 2009). Given that blubber thickness in the post-nuchal region of a dolphin most likely contributes to the external expression of a PND (see introduction), PNDs may be more visible in summer than in winter. Three of the six females with Method #1 PNDs stranded in May or June, and the other three stranded in December or February (Figure 4-1A). One male with a Method #1 PND stranded in February, three stranded in March or April, and the remaining three stranded in July or August (Figure 4-2A). These results demonstrate that stranded dolphins with PNDs occur across seasons. However, given that blubber thickness varies by season, I recommend comparing body condition measurements of wild dolphins with and without PNDs within a season, if sample size permits.

Applying PND Index to Field Photos

The major goal of this entire study was to provide a simple and non-invasive technique that can be applied to field photographs of dolphins to monitor the condition of individuals. Although I used stranding photos to create the PND index, the index can readily be applied to field photos as well. Figure 4-7 shows the application of Method #1 to a field photo of female MML0904 (Table 3-1) taken eight days before her carcass was recovered. MML0904 was considered emaciated by the necropsy team. MML0904's field picture is an ideal photo, with good focus and contrast, perpendicular to the sagittal plane, with the animal in a relatively flat position, and including the dorsal surface almost to the anterior insertion of the dorsal fin. The evident space between the line and the dorsal surface in this photo indicates a PND.

Photos taken in the field will not always be as good as in Figure 4-7. For example, a flat, horizontal posture of the dolphin and a perpendicular angle of the photo may be difficult to obtain. Figure 4-8 shows MML0904 photographed at various postures eight and ten days before its carcass was recovered. Although Method #1 indicates that she has a PND regardless of the posture she is in, the space between the line and the dorsal surface is more evident when her posture is flat and horizontal as opposed to at an angle. Furthermore, photos may not always be taken at angles perpendicular to a dolphin's sagittal plane. For instance, in Figure 4-9, MML0907, a 247 cm female not included in the final sample of this study because of a shark bite, is photographed about a month and a half before her necropsy at a less appropriate angle. However, a slight space is still noticeable between the line and the dorsal surface when Method #1 is applied, and therefore MML0907 is considered to possess a PND. MML0907 was considered emaciated at necropsy.

The quality of photos taken in the field varies. For Method #1 to be successfully applied, researchers should try to obtain photos that meet the photo quality guidelines provided. Furthermore, to increase accuracy when assessing PND presence, I suggest including as much dorsal surface in the photo as possible as far caudally as the anterior insertion of the dorsal fin. Both false negatives and positives can result when only the dorsal surface from the blowhole to the anterior insertion of the pectoral fin is included in the photo (see discussion of Method #4 above). Additionally, the cranial start point for Method #1 may not always be visible or obvious in field photos. If an animal's nuchal crest, eye, and ear are all not noticeable in a photograph, I suggest using the blowhole as a reference point and starting the line about 5 cm caudal to the blowhole, which should roughly approximate the site of the nuchal crest.

Future Research

Future research of bottlenose dolphin PNDs should investigate if relationships exist between PND presence and 1) reproductive classes and 2) survival rates for the population, as found by Pettis et al. (2004) and Bradford et al. (2012). If PND presence is found to be related to survival rate, I recommend developing PND proportion baselines for populations where photo identification studies are underway. This data can eventually be used as an indicator of stock trends when considering the overall status of a stock. PND proportion in relation to baseline values can then be included in NFMS Stock Assessment Reports.

Another area of future study that would benefit the understanding of PNDs in bottlenose dolphins is the physiology behind the depletion of the post-nuchal fat pad. Koopman et al. (2002) measured blubber thickness and adipocyte number and size and reported that differences in structure and function of the blubber exist between regions of the porpoise body. They concluded that the inner thorax blubber functions as the primary metabolic energy source for these animals, whereas blubber in the tailstock primarily functions as a structural streamlining component important for locomotion. To the best of my knowledge, no study has measured the presence and\or morphology of the post-nuchal fat pad in porpoises of differing body condition. Struntz et al. (2004) recognized the value of examining blubber across functionally distinct body regions at different stages of bottlenose dolphin development. Studies of bottlenose dolphin blubber and the post-nuchal fat pad that define the relative importance of regions in terms of energy storage would be very helpful.

Conclusions

I have shown that stranded bottlenose dolphins with PNDs consistently had lower length-weight and BMI values than individuals without this trait, supporting the use of PND as an indicator of body condition. The visual assessment of PNDs using photos of animals in the field provides a simple and non-invasive tool for researchers to monitor individual body condition, and could easily be incorporated into ongoing bottlenose dolphin photographic identification studies at many sites within the range of this species.

Table 4-1. Causes of death for dolphins identified as having PNDs by Method #1.

Sex	Stranding ID	Cause of death
MML0305	Female	Emaciation and mild chronic bronchitis with Halocercus
MML0538	Female	Natural, possible intoxication, red tide results pending
MML0611	Female	Human interaction, fisheries, foreign body, ingestion (hook and line); emaciation; foreign body (stingray barb) lung; trauma-fractured processes of spine; skin lesions
MML0904	Female	Natural, malnutrition, lobomycosis
MML1107	Female	Emaciation noted grossly and myocardial fibrosis observed histologically. Combined with the worn and missing teeth also noted grossly, death may have been in part due to age-related generalized decline
MML1211	Female	Presumed to be maternal separation as her mother died due to fishery interaction approximately 20 days before MML1211's carcass was recovered
MML0016	Male	Presumed severe bacterial pneumonia
MML0313	Male	Human related, fisheries (goosebeak) with aspiration pneumonia
MML0319	Male	Natural, foreign body ingestion (rock) esophageal obstruction, malnutrition, infection pneumonia (fungal)
MML0330	Male	Natural, multiple skeletal anomalies, emaciation
MML0404	Male	Natural, foreign body perforation (catfish spine) of lung, diaphragm, stomach, and intestine
MML0503	Male	Natural, foreign body, catfish spines, esophagus, stomach/spleen, lung perforations
MML0619	Male	Human interaction, fisheries, foreign body, ingestion hooks and line; emaciation; pre-mortem shark bites

Figure 4-1. Example of how the PND outcomes differed between Methods #1 and #4 using MML0538. A) Method #1 which indicates MML0538 has a PND, B) Method #4 which indicates MML0538 does not have a PND. Photos courtesy of MML SIP.

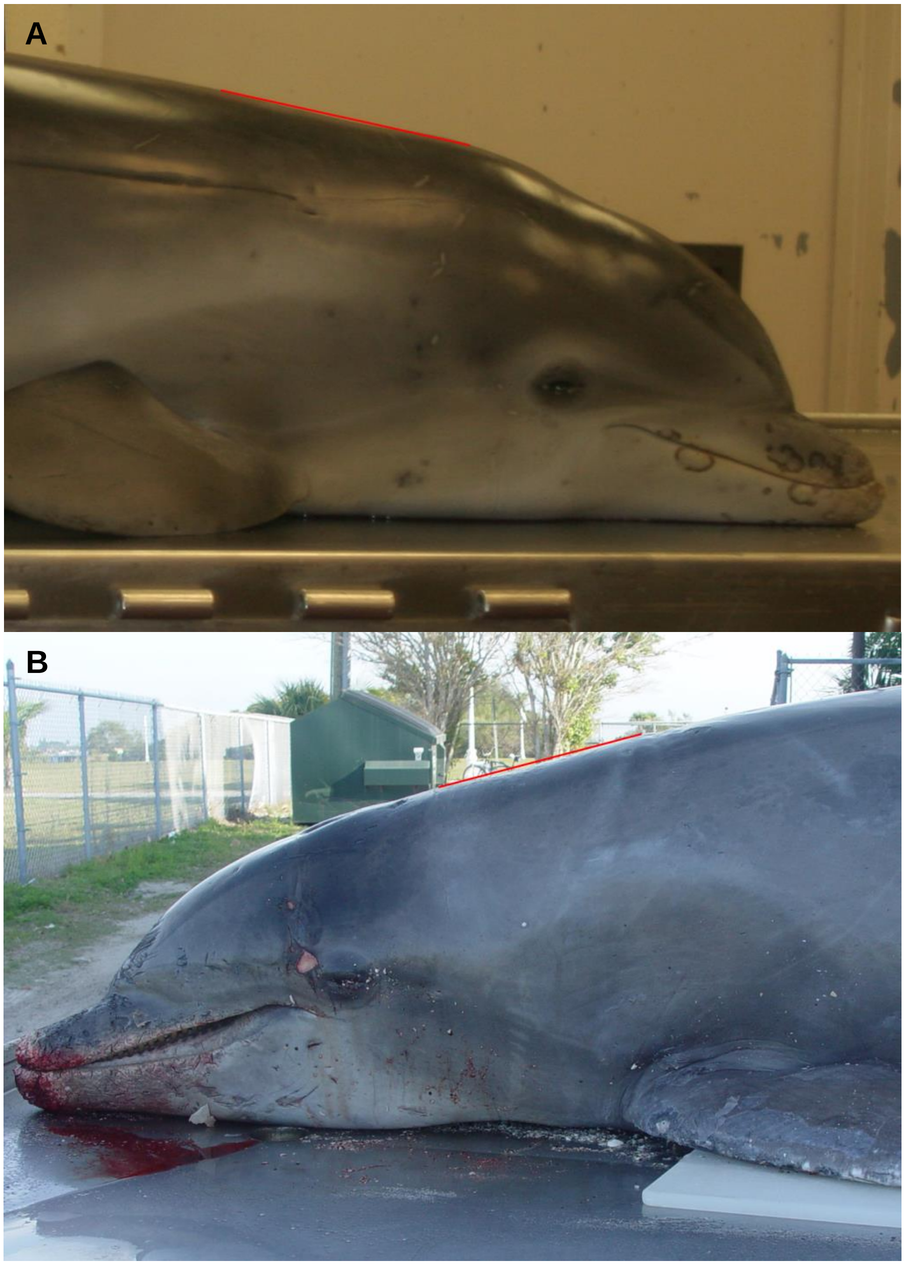

Figure 4-2. Examples of dolphins that were indicated as having PNDs by Methods #3 or 4 but not by Methods #1 and 2. A) Method #3 indicates male MML0810 has a PND, B) Method #4 indicates male MML0606 has a PND. Photos courtesy of Florida Fish and Wildlife Conservation Commission Marine Mammal Pathobiology Lab and MML SIP.

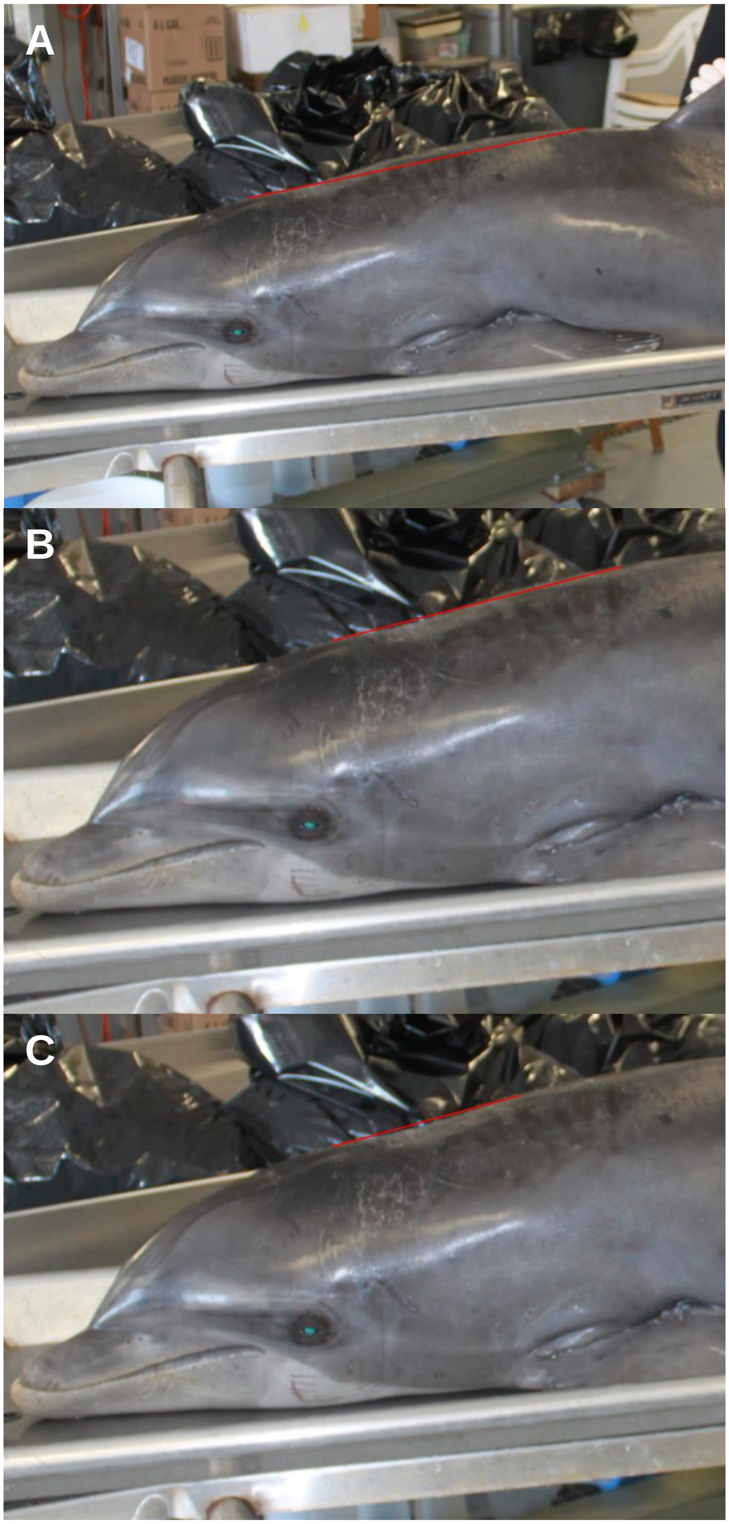

Figure 4-3. Male MML1104, where Methods #3 and 4 indicated it had a PND, while Methods #1 and 2 did not. A) Methods #1 and 2 (same photo and line combination), B) Method #3, C) Method #4. Note, the photo of this dolphin was taken at a slightly skewed angle. Photos courtesy of MML SIP.

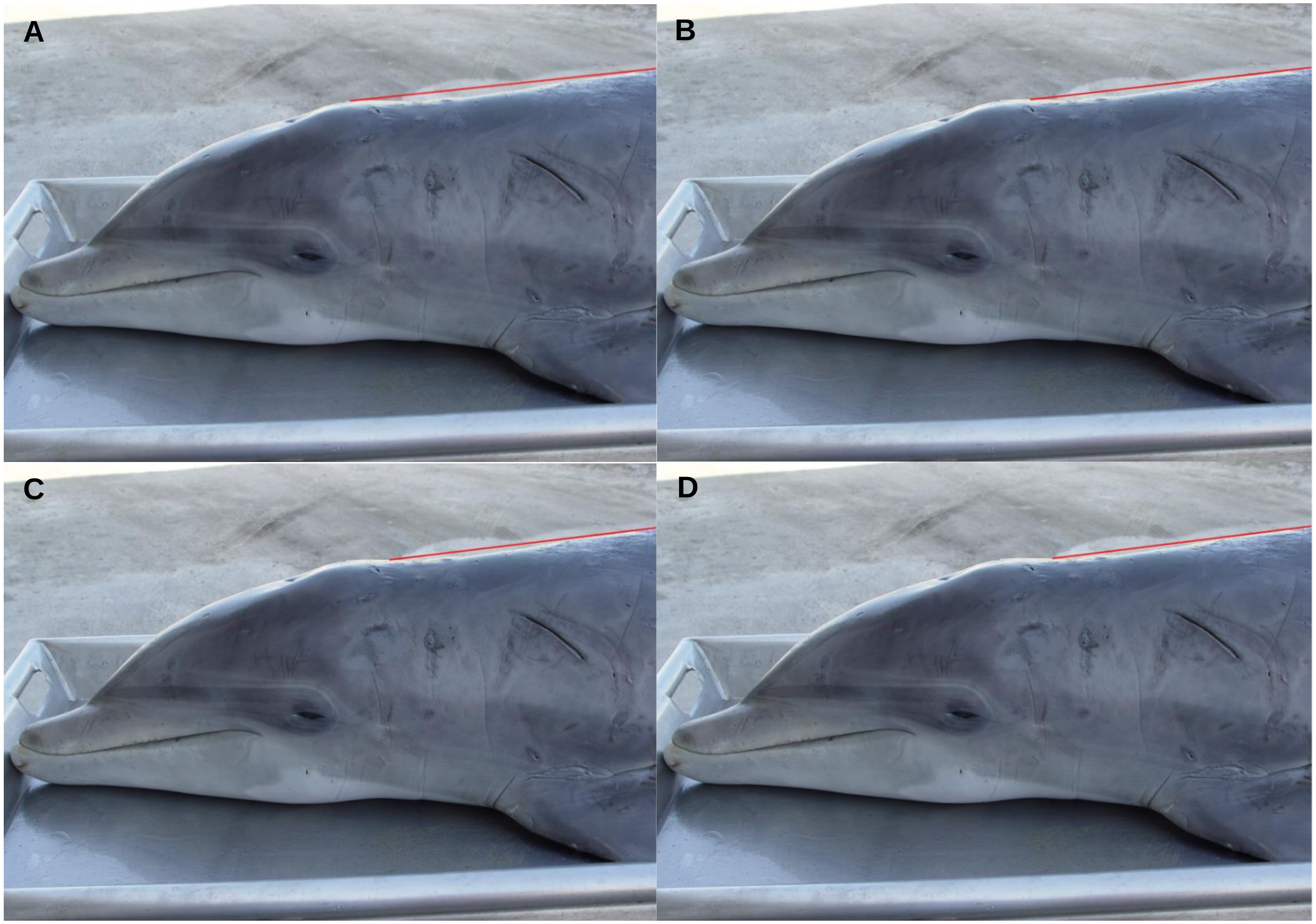

Figure 4-4. Method #1 applied to MML0503 using four different cranial start points: A) at the nuchal crest, B) above a point slightly anterior to the ear, C) above the ear, and D) above a point slightly caudal to the ear. The caudal endpoints in each photo are the same. Cranial start points in B and D were drawn as possible approximations to the location of the point above the ear that could have been made had the ear not been visible. In all four instances, Method #1 shows the animal has a PND, with a space visible between the line and the dorsal surface. Photos courtesy of MML SIP.

Figure 4-5. Method #1 applied to MML1107 using four different cranial start points: A) at the nuchal crest, B) above a point slightly anterior to the ear, C) above the ear, and D) above a point slightly caudal to the ear. The caudal endpoints in each photo are the same. Cranial start points in B and D were drawn as possible approximations to the location of the point above the ear that could have been made had the ear not been visible. For A-C, Method #1 shows the animal has a PND, with a small space visible between the line and the dorsal surface. For D, this method shows the animal does not have a PND. Photos courtesy of MML SIP.

Figure 4-5. Continued

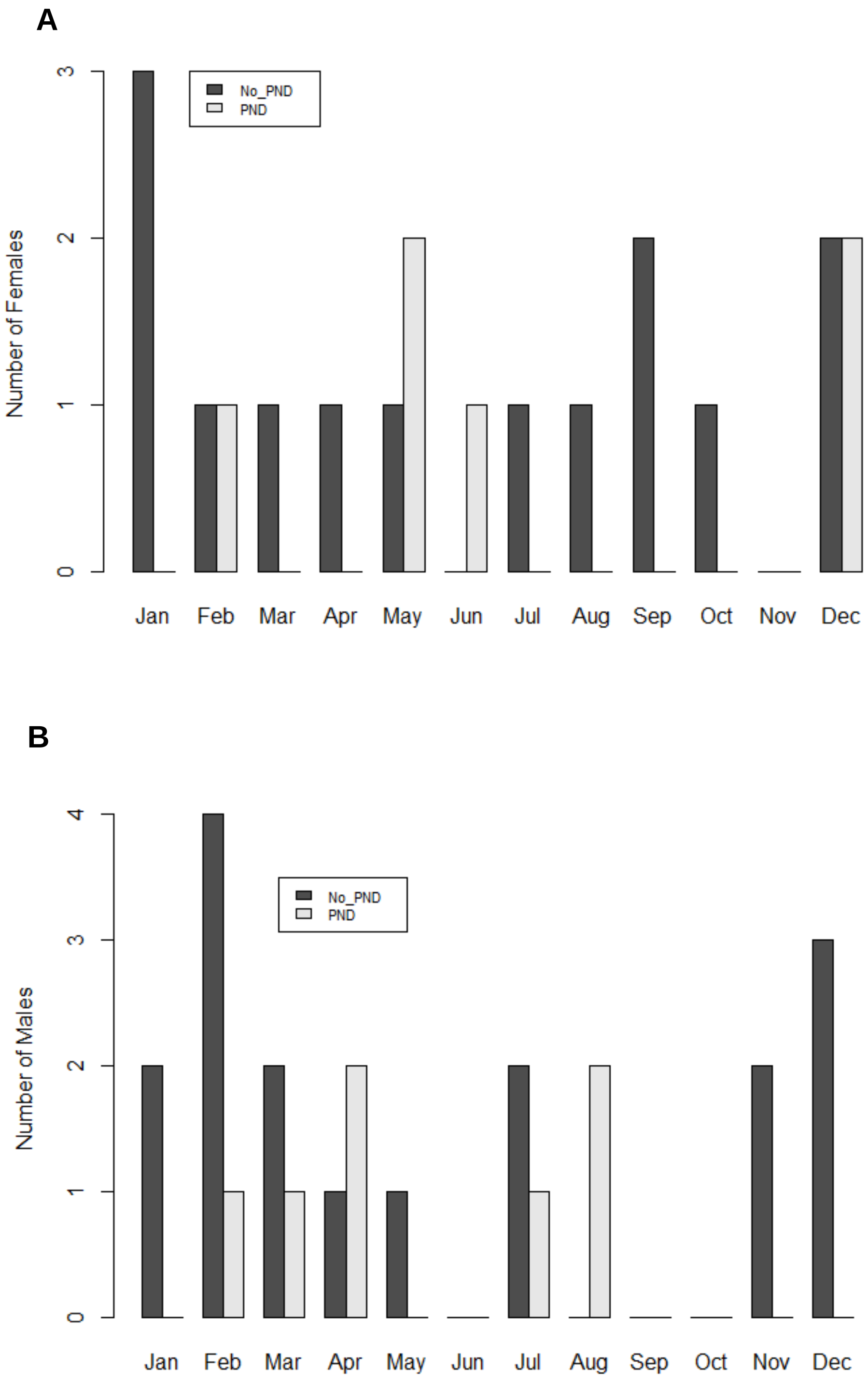

Figure 4-6. Number of individuals with and without PNDs as identified by Method #1 by month for all years pooled together. A) Females, B) Males.

Figure 4-7. MML0904 taken in the field eight days before its necropsy. The space between the dorsal surface and the red line show the animal has a PND. Photo courtesy of the SDRP.

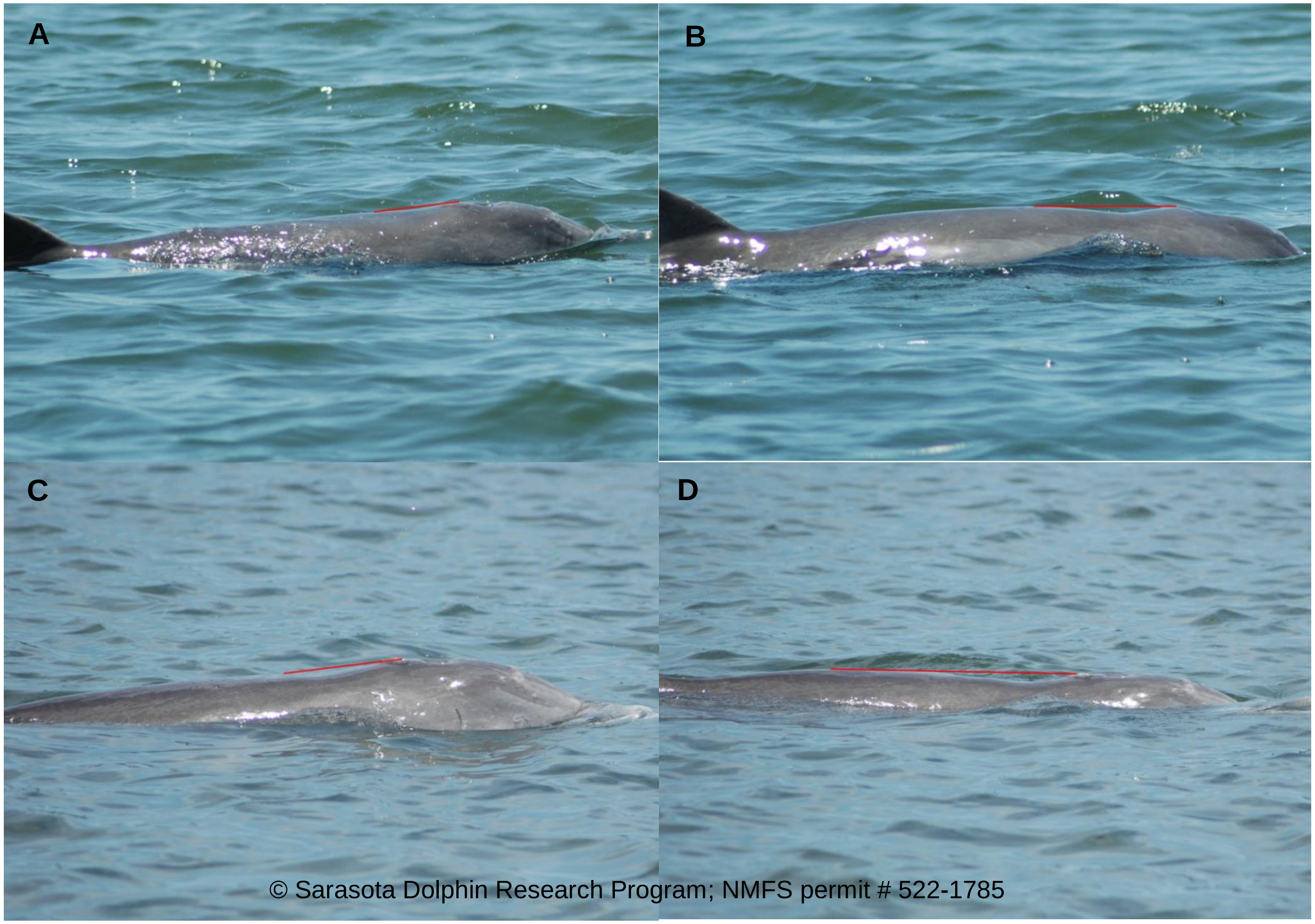

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Figure 4-8. Method #1 applied to photos of the female MML0904 with different postures. A) and B) Photos taken 10 days before MML0904's necropsy. C) and D) Photos taken 8 days before MML0904's necropsy. Regardless of posture, Method #1 identified a PND in each photo; however, the space between the line and dorsal surface is more noticeable when the animal is in a flat position (B and D) compared to an angled position (A and C). Photos courtesy of the SDRP.

Figure 4-9. Female, MML0907, taken at a skewed angle in the field about a month and a half before its necropsy. Although this animal was not included in the body condition analysis because its carcass had a significant shark bite, the photo exemplifies how Method #1 can be applied to a photo taken at a less desirable angle (not perpendicular to the sagittal plane). The space between the line and the dorsal surface indicates that this animal has a PND. Photo courtesy of the SDRP.

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BIOGRAPHICAL SKETCH

Mary Kathleen Gryzbek has had a passion for wildlife and conservation from a very young age. This passion drove her to major in biology and Spanish at Butler University, where she obtained her B.S. in 2010. Shortly afterwards, she interned with the Sarasota Dolphin Research Program for about half of a year. She was also an intern with the Spotted Eagle Ray Project at Mote Marine Laboratory for about three months. After these internship experiences, Mary began a Master's degree program in the Department of Wildlife Ecology and Conservation at the University of Florida in January 2011. She became a staff member of the Sarasota Dolphin Research Program as a graduate student through the support of the Chicago Zoological Society that same year. In August 2013, Mary graduated with a Master of Science degree. She plans to continue her career focusing on wildlife ecology, conservation, public outreach, and education.