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Using Unmanned Aircraft Systems (UAS) to Assess Body Condition of Common Bottlenose Dolphins (Tursiops truncatus truncatus) in the Indian River Lagoon, Florida

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by

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Abstract

Title: Using Unmanned Aircraft Systems (UAS) to Assess Body Condition of Common Bottlenose Dolphins (*Tursiops truncatus truncatus*) in the Indian River Lagoon, Florida

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Common bottlenose dolphins (*Tursiops truncatus truncatus*) in the Indian River Lagoon (IRL) have experienced four Unusual Mortality Events (UMEs). This stock is considered immunocompromised and is routinely subjected to persistent anthropogenic stressors such as fishing gear entanglement, vessel strikes, contaminants, and harmful algal blooms. Previous body condition assessments of this stock have involved invasive capture-release examinations or subjective methods using lateral body images. To improve precision, we investigated the use of photogrammetry data collected from noninvasive unmanned aircraft systems (UAS) combined with models developed from capture-release data to estimate morphometric parameters and subsequently determine the body condition of these free-swimming ecosystem sentinels. Lateral body condition scoring (BCS) and photo-identification methods were simultaneously used to compare body condition measurements and to categorize measurements (ideal, underweight, or emaciated). A total of 29 dolphins were measured using MorphoMetrix. Body Area Index (BAI) measurements were not significantly different across age class ($p = 0.314$) and BCS ($p = 0.232$). Estimated Body Mass Index (BMI) measurements ranged BMI varied significantly by age class ($p < 0.001$) but not by BCS ($p = 0.101$). Lateral body condition evaluation resulted in four ideal dolphins, twenty-four

underweight dolphins, and one emaciated lactating female. Future efforts should be made to increase sample size and efficiency of these methods. This study provides a foundation for future noninvasive UAS studies on bottlenose dolphins.

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List of Abbreviations

AXW	Axillary Width
BAI	Body Area Index
BCS	Body Condition Score
BHW	Blowhole Width
BMI	Body Mass Index
HERA	Health and Environmental Risk Assessment
HSWRI	Hubbs-SeaWorld Research Institute
IRL	Indian River Lagoon
MAXW	Maximum Width
PNW	Post-nuchal Width
SA	Surface Area
TL	Total Length
UAS	Unmanned Aircraft Systems
UME	Unusual Mortality Event

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Chapter 1
Introduction

Monitoring Dolphin Health in the Indian River Lagoon

As apex predators feeding on a diversity of fish and squid, common bottlenose dolphins (*Tursiops truncatus truncatus*) are ideal sentinel species. They can bioaccumulate environmental contaminants and are important in monitoring the health and status of lower trophic levels, as well as ecosystem health (Wells et al., 2004). Common bottlenose dolphins inhabiting the Indian River Lagoon (IRL) along the east coast of Florida are recognized as a strategic stock (NOAA, 2015) and are considered immunocompromised (Bossart et al., 2003) as they are impacted by several natural and anthropogenic pressures (De Freese, 1991). Long-term investigations of bottlenose dolphin stranding events within the Indian River Lagoon allow scientists to gather samples to evaluate health and mortality trends. IRL dolphins are directly impacted by human-induced threats (Noke & Odell, 2002; Durden, 2005; Stolen et al., 2007; Bechdel et al., 2009; Stolen et al., 2013), while also indirectly impacted from exposure to environmental contaminants (Durden et al., 2007; Fair et al., 2010). A recent assessment of IRL dolphin mortality (2002-2020) found that inflammatory disease (41% of strandings; predominantly respiratory illness) and trauma (36% of strandings) were the most common causes of mortality (Durden et al., 2023). The majority of trauma associated mortality (58%) resulted from human activities (propeller strikes, fishing gear entanglements and debris ingestion), while natural trauma (prey-associated esophageal obstruction or asphyxiation, shark bites, and stingray interactions) accounted for 12% of mortality (Durden et al., 2023). As these dolphins are year-round residents exhibiting high site fidelity (Odell & Asper, 1990; Mazzoil et al., 2005), persistent exposure to these threats may amplify susceptibility to disease (Fair & Becker, 2000). For example, IRL dolphins exhibit skin disease, such as Paracoccidioidomycosis, a chronic mycotic disease of the skin and subdermal tissues caused by a yeast-like organism known as *Paracoccidioidomycosis ceti* (Bossart et al., 2017). Moreover, this population has experienced four Unusual Mortality Events (UMEs) that occurred in 2001 (n = 41 mortalities), 2008 (n = 48 mortalities), 2013 (n = 77 mortalities) (Stolen et al., 2007; NOAA Fisheries, 2014) and 2013-2015. The later UME was part of the mid-Atlantic UME and was caused by a morbillivirus epidemic and impacted dolphins in the northern portion of the IRL (NOAA, 2015). In 2013, most mortalities resulted from starvation or inanition (Durden et al., 2023). The recurrence and increasing magnitude of UMEs may indicate a concern for stock decline, therefore, monitoring IRL dolphin health is essential for detecting changes in the population.

IRL dolphins have been monitored using various methods to assess population health, abundance, survival, and dynamics, as well as individual reproductive success (Mazzoil et al., 2008; Bossart et al., 2017; Durden et al., 2017; Durden et al., 2019; Hartel et al., 2020; Durden et al., 2021). Such methods include capture and release health assessments, line-transect distance sampling, mark-recapture, photo-identification, and telemetry studies. The first studies to evaluate IRL dolphin health were initiated in 1979 by Hubbs SeaWorld Research Institute (HSWRI), SeaWorld, and other collaborators (Odell & Asper, 1980). In 2003, the Health and Environmental Risk Assessment (HERA) project began monitoring IRL bottlenose dolphins using capture and release health assessment studies (Bossart et al., 2017). Health assessments established baseline data and enabled comparison of temporal and spatial morbidity patterns between estuaries within the southeastern US (IRL and Charleston SC). Photo-identification, or the identification of individuals based on these unique markings, is widely used to study cetaceans

(Hammond et al., 1990). Likewise, bottlenose dolphins can be individually identified by naturally occurring markings on the trailing edge of the dorsal fin (Würsig & Würsig, 1977; Würsig, 1990). These methods have been utilized to assess the abundance, movement, and survival of IRL dolphins (Durden et al., 2021) and enable long-term population monitoring (Mazzoil et al., 2005; Greenfield et al., 2022).

Methodology for Assessing Body Condition in IRL Dolphins

Assessing marine mammal health is a complex and time-consuming process. Many factors influence the accessibility of accurate data to determine the health of individuals and overall population health including financial and logical constraints. Ideal weather conditions along with the presence of large teams of experienced handlers and veterinary staff are essential to marine mammal capture and release health assessment programs (Fair et al., 2006). Historically, baseline data for bottlenose dolphin health have been collected by encircling the animal (capture and release) to enable a thorough veterinary health assessment to better understand the physiological impacts of chemical, biological, and physical stressors (Fair et al., 2006).

Although dolphin health assessment studies provide a wealth of information regarding individual health, the methods are intrusive, pose an inherent risk to the animal, are cost prohibitive and require post-release monitoring to document survival and behavioral responses (Barratclough et al. 2019). Furthermore, assessments typically only allow for evaluation during one season, making health comparison between seasons difficult (Fair et al., 2006).

Reoccurring bottlenose dolphin UMEs, continuous exposure to anthropogenic stressors, and starvation being the leading cause of mortality for the most recent IRL UME (Durden et al., 2023), illustrate the need for a reliable, cost-effective, and noninvasive method to assess dolphin health.

Body condition scoring is one method that can be used to evaluate animal health. In mammals, individual body condition can be interpreted as an individual's energy reserve (Hanks, 1981). Individuals with better body condition are associated with greater reproductive success, superior thermoregulation capabilities, and an improved survival (Gaillard et al., 2000). In marine mammals, time allocated to feeding and reproduction strongly influences body condition. This has been well studied in large migratory cetaceans such as humpback whales (*Megaptera novaeangliae*; Christiansen et al., 2016) and minke whales (*Balaenoptera acutorostrata*; Ichii et al., 1998). However, cetaceans spend most of their lives submerged making body condition assessment difficult (Bradford et al., 2012). Obtaining free-swimming body condition measurements is difficult, therefore, cetacean body condition is often obtained from stranded carcasses. However, this may induce bias as carcasses may be decomposed at assessment and may only provide data on sick or injured individuals which may not accurately represent the population (Boyd et al., 2010). Therefore, obtaining direct or indirect measurements of free-swimming cetaceans is more commonly used to estimate body condition indices (Amaral et al., 2010; Fearnbach et al., 2018; Burnett et al., 2019). Careful monitoring of cetacean body condition can provide insight into population health which is particularly important for immunocompromised populations such as IRL dolphins (Bossart et al., 2003).

Body girth has been used as an indicator of body condition in cetaceans and has been found to be more accurate than blubber layer thickness (Rice & Wolman, 1971). Variation in body girth and width has been measured for a number of baleen whale species including minke (*Balaenoptera acutorostrata*), fin (*Balaenoptera* *physalus*), gray (*Eschrichtius robustus*), and right whales (*Eubalaena* sp.; Lockyer 1986, 1987a, b; Ichii et al., 1998; Perryman & Lynn 2002; Miller et al., 2012) and has proven reliable for determining body condition (Christiansen et al., 2016). For small cetaceans such as harbor porpoise (*Phocoena phocoena*) and La Plata River dolphin (*Pontoporia blainvillei*), measurements of body mass, girth, and fat layer weight are positively correlated and have been used to monitor body condition and seasonal variation (Caon et al., 2007; Lockyer, 2007).

One noninvasive method to assess bottlenose dolphin body condition involves utilizing lateral photographs taken during photo-identification studies. These images may enable dolphin health assessment based on anatomical indicators including: a post-nuchal depression, epaxial musculature concavity, and exposed ribs and transverse processes (Struntz et al., 2004; Dunkin et al., 2005; Fair et al., 2006). The post-nuchal region is an excellent indicator of marine mammal health as this area tends to lose fat reserves in nutritionally compromised animals (Gryzbek, 2013). In fact, Gryzbek (2013) found that the presence of a post-nuchal depression can reliably predict animals in poor body condition. The evaluation of lateral images can also provide information on the skin disease prevalence, boat strikes, shark bites, and entanglement scars (Durden et al., 2020). Using this method, dolphin images are evaluated using body condition scores (1 - emaciated, 2 - underweight, 3 - ideal, 4 - overweight, and 5 - obese) (Table 5) (Fair et al., 2006). This approach can provide consistent data on nutritional status between seasons and years, where capture-release assessments are lacking, and has been utilized for IRL dolphins during the 2008 and 2013 UMEs (Durden et al., 2020). In 2008, twenty bottlenose dolphins were evaluated in the Indian and Banana Rivers and the results indicated that 35% animals were emaciated, 50% were underweight, and 15% were in ideal body condition. During the 2013 UME, emaciation was the most common finding in stranded IRL dolphins (Durden et al., 2023), thus extensive surveys were conducted to evaluate IRL dolphin body condition utilizing lateral images. The study found that 31% of adults sighted were ideal, 64% were underweight, and 5% were emaciated (Durden et al., 2020). A more recent evaluation of IRL dolphin body condition during a non-UME year (between 19 June and 27 June 2023) found that 93% of dolphins presented in compromised nutritional condition with 68% underweight and 25% emaciated (Durden et al., 2023). Compared to previous studies, this recent evaluation demonstrates that the population appears to be increasingly nutritionally stressed and should be carefully monitored (Durden et al., 2023).

Although helpful as a noninvasive method, the use of lateral images to determine body condition presents several challenges. Lateral image assessment can be relatively subjective and is precluded by poor image quality or the lack of sufficient images (Durden et al., 2020). When body condition is assigned based on only one criterion (excluding the post-nuchal depression), the number of underweight dolphins can be overestimated (Durden et al., 2020). In the 2013 UME study, some animals presented multiple features consistent with ideal body condition, but also presented slightly visible transverse processes or slight depression in epaxial musculature (Durden, 2013). The appearance of transverse processes and epaxial muscle depletion are difficult to evaluate in images if the animal is diving or contorted or if lighting is obscuring the features (Durden et. al., unpub. data). For this reason, the presence of two criteria or the post-nuchal criterion have been utilized to conservatively evaluate individuals (Durden et al., 2020). The lateral image method is a reliable method for visually assessing physical characteristics in free-ranging dolphins, however, a large number of images are needed per individual to accurately assess and categorize nutritional compromise, which may prove impractical.

UAS as a Noninvasive Method for Assessing Marine Mammal Body Condition

Recent advances in unmanned aircraft system technology (UAS; drones) have provided new opportunities for collecting photographic data on free ranging animals for a variety of purposes including abundance estimates, reproductive behavior, and body condition assessments (Christiansen et al., 2016; Elsey et al., 2016; Kiszka et al., 2016; Torney et al., 2018; Allan et al., 2019). UAS are relatively low-cost, are efficient, and provide a noninvasive method to study marine mammal health and behavior (Aniceto et al., 2018; Hodgson et al., 2016). Christiansen et al. (2016) found that noise produced by UAS may be heard underwater by some marine mammals, however, the effect of underwater noise from UAS is minimal. UAS photogrammetry is also beneficial as it allows for large groups of animals to be sampled simultaneously with minimum effort (Booth et al., 2020). Recent studies have successfully used UAS to measure mother-calf energy transfer and body condition in cetaceans, highlighting the potential for long-term monitoring of environmental influences on cetacean populations (Christiansen et al., 2018).

Studies on large cetaceans (Christiansen et al., 2016; Fearnbach et al., 2019; Christiansen et. al., 2020; Glarou et al., 2023), manatees (Ramos et al., 2022), and fur seals (Allan et al., 2019) have used UASs to accurately measure body condition. A study using UASs to estimate body mass of sperm whales found that this method was feasible and noninvasive in estimating mass and body condition of free-swimming sperm whales and can be further used to predict mass-specific metabolic costs such as reproduction, locomotion, and heat loss in animals (Glarou et al., 2023). Obtaining noninvasive morphometrics to estimate body condition is a valuable tool for exploring ecological and physiological questions (Glarou et al., 2023). More recently, UAS have been utilized to aid in identifying pregnancy in bottlenose dolphins (Cheney et al., 2022) as well as the age-structure of small delphinids (Vivier et al., 2023). UAS body condition studies are scarce for smaller cetaceans, likely because of their differing behavior from larger whales (e.g., rapid movements, frequent breaching and arching) (Serres et al., 2024). However, these noninvasive methods can be valuable for assessing trends in health for vulnerable coastal dolphin populations suffering from anthropogenic stress (de Oliveira et al., 2023). Adjusting UAS methods used in large whale studies for small cetaceans are rapidly becoming more common. Christie et al. (2021) tested the precision of total length measurements at different UAS altitudes and position relative to the image frame for two small cetacean species (Australian Snubfin- *Orcaella heinsohni* and Humpback dolphins-*Sousa sahulensis*). The study found that altitude and position of the dolphin in the frame do not significantly affect morphometric measurements derived from images collected between 15 and 50 m, suggesting these methods are feasible and accurate for assessing body condition in small dolphins (Christie et. al., 2021). A recent study assessed the body condition of Tamanend's bottlenose dolphins (*Tursiops erebennus*) in the Charleston Estuarine System (CES) (Perkins-Taylor et al., 2023). The study evaluated spatial, seasonal, and age class differences in measurements by using Body Area Index (BAI), a unitless measurement of the surface area to total length percentage of the animal, which is similar to the Body Mass Index (BMI) used in capture-release studies (Perkins-Taylor et al., 2023). Dolphin BAI values in the CES were significantly greater during winter than spring, indicating that dolphins may shift their energetic priority from survival in winter to reproduction in spring (Perkins-Taylor et al., 2023). A similar study using UAS, compared body condition of resident Indo-Pacific humpback dolphins along the Chinese coastline and found that dolphins experiencing sharp declines in population presented in lower body condition than other populations (Serres et al., 2024). These studies demonstrate the feasibility of assessing body condition on dolphins using UAS. However, methods comparing lateral body condition scores paired with capture-release health assessments have not yet been assessed.

The purpose of this study was to determine if morphometric data obtained from UAS photogrammetry could be used to accurately assess IRL bottlenose dolphin body condition. Establishing these noninvasive baseline techniques to evaluate body condition will aid in IRL dolphin health monitoring as well as conservation efforts for the IRL ecosystem. Given the critical need to monitor IRL dolphin health, established methods will serve as a foundation for future health assessment studies and will enable comparison across seasons, years, and UMEs. Methods will improve our understanding of anthropogenic and environmental stressors on individual dolphin health. Established methods will enable comparison of female body condition during pregnancy and lactation and can be incorporated into long-term estuarine bottlenose dolphin population studies. This is a cost-effective alternative to traditional dolphin health studies that will enhance our knowledge of how morphometric parameters differ across populations and among varying environmental conditions.

This study provides baseline information as well as guidance for body condition assessment in bottlenose dolphins using UAS. While UAS studies have focused extensively on large migratory cetaceans (Christiansen et al., 2016; Fearnbach et al., 2019; Christiansen et. al., 2020; Glarou et al., 2023) few studies have adapted these methods for small cetaceans such as bottlenose dolphins (Perkins-Taylor et al., 2023; de Oliveira et al., 2023; Serres et al., 2024). This study was the first to utilize a UAS equipped with a laser altimeter to obtain precise measurements to assess bottlenose dolphin body condition, while ground truthing methods with measurements of deceased dolphins.

Chapter 2
Materials and Methods

Study Site

The IRL has historically been divided into six segments based on hydrodynamics and geographic features (U.S. EPA, 1996). Efforts for this study focused on the northern portion of the IRL, with only one opportunistic flight in the North Central IRL (Figure 1). Sub-basins included the Halifax River, Banana River, and North Central Indian River which are commonly utilized by IRL dolphin communities. Much of this region of the IRL has low water exchange rates and high residence periods (Smith, 1993) resulting in nutrient accumulation (Lapointe et al., 2020), phytoplankton blooms (Phlips et al., 2021), and seagrass depletion (Morris et al., 2022). Sites were chosen based on locations where photo-identification studies have been conducted (Durden et al., 2021; Greenfield et al., 2022), sites of prior UMEs (Durden et al., 2023), and areas where UAS flights are permitted. Due to the proximity to Patrick Space Force Base, Cape Canaveral Space Force Station, and Kennedy Space Center, there are numerous regions with restricted airspace, which limit the areas where UAS data collection can occur (Figure 1).

UAS Flight Condition Requirements

Conditions for UAS flights were carefully evaluated prior to launch. Flight condition requirements to enable UAS flights included a minimum visibility of three statute miles, a 500 ft clearance below cloud cover, Beaufort Sea State conditions ≤ 2, wind speed less than 12 mph and wind gusts less than 17 mph, cloud cover $< 50\%$, and water turbidity ≥ 0.30 m. Due to these restrictions, only animals observed during ideal weather periods were overflown, providing haphazard sampling.

During data collection, the UAS was flown no less than 5 m above a dolphin to limit potential animal disturbance. The dolphin's behavior and any potential reactions to the UAS during the flight were monitored and recorded.

UAS Measurement Validation

Prior to assessing UAS images, the accuracy of using images extracted from videos paired with the laser altimeter data was validated utilizing opportunistic overflights and measurements of stranded dolphins. An Atlantic spotted dolphin (*Stenella frontalis*) calf that stranded on 16 September 2023 and an entangled IRL bottlenose dolphin calf that was observed free-swimming with its mother on 8 December 2023 were overflown and photogrammetry data were compared with straight line measurements of total length (open reel tape measure). The entangled IRL calf was subsequently recovered deceased on 26 December 2023 and photogrammetry measurement of total length was compared to length at necropsy. The measurement for the entangled dolphin was recorded 18 days after the overflight and was determined to be comparable as animal length should not have changed significantly during that time. Total length, width, and girth measurements were recorded for the spotted dolphin (using slide calipers) and measurements were compared to those collected from the UAS overflight of the carcass. Validation of these methods was important to ensure data collected for the body condition analysis could be reliably measured.

Photographic Collection and Analysis

UAS data collection was conducted in conjunction with lateral body and photo-identification images. Images were collected from a small vessel approximately 5-10 m from the animal using a Canon EOS digital camera with a 100-400 mm telephoto lens (Canon USA, Inc., Melville, New York). Dorsal fin image analysis followed an established protocol (Mazzoil et al., 2004) and were matched to the existing photo-identification catalog. Lateral images of the head, body, and peduncle region were taken of each animal and were evaluated using a standard body condition index (Fair et al., 2006) (Table 1, Figure 2). Body condition evaluation can be subjective, therefore, to avoid bias, straight-line body images were utilized since movement can temporarily alter body concavity or convexity (Durden et al., 2020). Furthermore, individuals were evaluated conservatively, utilizing two or more criteria with an emphasis on the post-nuchal criterion (the only acceptable single criteria) (Durden et al., 2020). Based on these criteria, body condition was assigned into ideal, underweight, or emaciated categories.

Body condition was further assessed by sex and age class (adults and juveniles). Total length varies across age class and sex with adult male TL measuring more than 249 cm, females more than 231, juvenile males generally ranging between 161 cm and 245 cm, juvenile females ranging from 161 cm to 230 cm, and calves under 160 cm (Wells et al., 1987). Dolphins with long-term data were assumed to be males if they were first observed as adults and have a sighting history of > 15 years without calving. Due to the significant energetic investment required during reproduction, lactation, and weaning of calves in marine mammals (Lockyer, 1984), decreased body condition has been observed for females with dependent calves. For example, lactating and post-weaning gray whales had the lowest body condition scores of any demographic unit (sex, age, and reproductive status) (Lemos et al., 2020). Similarly, Christiansen et al. (2016) found that during breeding months, the rate of body condition decline was greater for lactating humpback whales than other mature individuals. Likewise, lactating southern right whales lose approximately 25% of their body volume during the first three months of the breeding season (Christiansen et al., 2018). Although bottlenose dolphins do not exhibit specific migratory, feeding, and breeding seasons as observed in mysticetes, bottlenose dolphin mothers produce milk for their calves for one and a half to three years, and calves often stay with their mothers for three to six years or until another calf is born (Shane et al., 1986). For this reason, females observed with dependent calves were considered lactating (not always confirmed) and evaluated separately from adult females without dependent calves. Dependent calves (calves seen in swimming in the infant or echelon position, or <2 years old, or <75 % of the proximate adult’s size) were not used in the analysis to avoid biasing data toward underweight body condition.

The UAS was launched to retrieve corresponding video for the same individuals that lateral images were taken. A DJI Mavic 3 Cine equipped with a hasselblad camera (20 - megapixel, 17.3 sensor width, and 12 mm focal length), laser altimeter logger (O3ST), and catch handles (Upshot Photos) was used for image collection. The UAS is relatively small (347.5 × 283 × 107.7 mm; length × width × height), weighs 899 g at takeoff (without the laser altimeter), and the laser altimeter and catch handles can be quickly attached, making it ideal for efficient field data collection. Without the additional weight from the laser altimeter and catch handles, the UAS has a maximum flight time of 46 minutes and a maximum hovering time of 40 minutes (with no wind) (DJI Mavic). At least three UAS batteries were available during each survey. Flights were conducted from a vessel, therefore, to ensure adequate time for a safe landing, the UAS returned to the vessel at ~ 50% battery level and was hand caught with catch handles to avoid dislodging the laser altimeter attachment. Therefore, the average flight was ~20 minutes (laser altimeter and catch handle weight may have decreased drone efficiency). The camera was orientated at 90 degrees to enable accurate measurements. Obtaining straight-line photographs of free-swimming bottlenose dolphins can be difficult as they only surface briefly and are often curved when diving. Furthermore, water quality during UAS flights was usually poor, prohibiting visualization of dolphins. For this reason, we utilized the video feature on the UAS and subsequently extracted still images of the dolphin in a straight-line while surfacing in Adobe Photoshop. All UAS flights were flown by a U.S. Federal Aviation Administration (FAA) licensed remote pilot (FAA Part 107 operator) with extensive experience operating a drone. Dolphin overflights were conducted under a National Marine Fisheries Service permit (# 25574). Dolphins were overflown for < 20 minutes to retrieve adequate video footage while limiting potential disturbance. Videos were reviewed and dolphin images where the animal was aligned in a straight line were extracted. Images where the animal was in center frame were favored over images where the animal was not directly centered. Since Christie et al. (2021) found that the position of the dolphin in the frame does not significantly affect morphometric measurements, dolphins that only had one image to measure but were not directly in center frame were still measured. The exact time of the image was recorded and used to obtain the corresponding data from the laser altimeter log file. The laser altimeter records data every second during flight and includes date, time, latitude, longitude, laser distance (cm), and tilt angle. Images were sorted by quality prior to measurement. Images with minimal dolphin body curvature or angled contortion and where the body outline was clearly visible were considered ideal for measurement. Images where overexposure or effects from water ripples limited the ability to clearly visualize body edges were excluded from analysis. For final data analysis, measurements from a single image with the best quality were used. Only images where the tip of the rostrum, fluke notch, and lateral edges of the body were clearly present were used for analysis.

MorphoMetrix is a graphical user interface used for photogrammetry and morphometry used to calculate piecewise/arc lengths and widths along segments/curves and areas for polygons (Torres, 2020). MorphoMetrix has been successfully used to calculate body length and widths for both large and small cetaceans (Bierlich et al., 2022; Bierlich et al., 2023; Christiansen et al., 2018; Cheney et al., 2022; Torres et al., 2022; Fernandez et al., 2023; Perkins-Taylor et al., 2023). The input frame for MorphoMetrix includes focal length of camera, pixel dimensions (pixel dimensions = senor width ÷ image width), altitude, and number of width segments desired. The true altitude, or the altitude of the UAS given the tilt angle of the camera, is calculated based on the laser distance recorded by the altimeter using the following equation:

$$\text{True Altitude} = (\text{Laser Distance}) \times \text{Cosine}\ (\text{Tilt Angle})$$

Once the true altitude is calculated, the image and corresponding data inputs can be imported to MorphoMetrix. The total length and width segments can then be measured using the Bezier fit application which applies a smooth fitting curve to the points selected (Torres, 2020). Total length was measured from the tip of the rostrum to the fluke notch (Norris, 1961) (Figure 3). Width segments were divided into 25-30 segments to enable measurement of the width at the blowhole, width at the post-nuchal, width at axilla, and width anterior to the dorsal fin (maximum width) (Figure 3). Locations for width measurements were chosen based on measurements taken during bottlenose dolphin necropsies since they are areas that are most likely to change in animals with poor body condition (Geraci & Lounsbury, 2005; Fair et al., 2006; Gryzbek, 2013) and the anterior part of the body is the most informative for the assessment of body condition (Cheal & Gales, 1991; Hart et al., 2013; Joblon et al., 2014). Similar locations for width measurements have also been used in other UAS studies on small cetaceans (de

Oliveira et al., 2023; Serres et al., 2024). Width at the post-nuchal region was estimated to be the area behind the blowhole, typically around 15%-17% of total length (segment 5 of 30 segments). The axillary girth measurement is easily identified during necropsy by utilizing the pectoral flippers as a landmark. To mimic necropsy methods, the location of pectoral flippers was used as a landmark for width measurements. If pectoral flippers were clearly visible, axillary width was measured just caudal to the scapular joint, to remain consistent with necropsy protocol. If the pectoral flippers weren't seen, axillary width was estimated roughly at 35%-45% of the TL. Opportunistically, height measurements were obtained from animals that were observed swimming laterally underwater and paired with maximum width to estimate girth measurements using the elliptical circumference formula (Raman, n.d.):

$$\text{Circumference} \approx \pi(a+b)(1+\frac{3h}{10+\sqrt{4-3h}})$$

Where a is the radius of height and b is the radius of the maximum width. Height measurement was recorded just anterior to the dorsal fin at the same landmark where the maximum width measurement was recorded (Figure 3). Body condition has been inferred through width to length ratios in previous UAS studies (Miller et al., 2012; Fearnbach et al., 2018; Noren et al., 2019; Cheney et al., 2022; de Oliveira et al., 2023; Serres et al., 2024) where ratios are used as indices for body condition assessments that can be compared across seasons, age class, and sex. Width to length ratios were calculated by dividing the each of the four width segments (blowhole width, width at post-nuchal, axillary width, and maximum width) by the total length for each dolphin to obtain four indices for body condition analysis and compared across age class and lateral body condition score.

Body Area Index (BAI) was also calculated for each dolphin. BAI is a two-dimensional body condition measurement metric that was developed based on the body mass index (BMI) formula that is frequently applied to humans (BMI = mass[kg] ÷ height [m^2]; Gallagher et al., 1996). In contrast to the BMI formula, BAI uses surface area (SA) of the animal (Burnett et al., 2018) across a defined Head-Tail Range (Bierlich et al., 2021a) and was calculated using the following equation:

$$\mathrm{BAI} = \frac{\mathrm{SA}}{(\mathrm{Head} - \mathrm{Tail}_{\mathrm{Range}}\ \mathrm{x}\ \mathrm{TL})^2}\ \mathrm{x}\ 100$$

Cetacean energy reserves are not stored in their head, tail flukes, or pectoral fins (Brodie, 1975; Christiansen et al., 2016), so the optimal Head-Tail Range should only include areas of the body that vary in size in response to the individual's energy storage. The Head-Tail Range used in this study compromised 50% of the total length measured between 20-60% of each dolphin's TL (Figure 3-D). Although previous studies have not included the blowhole and post-nuchal region in SA measurements, previous body condition analysis emphasized the importance of these regions (Gryzbek, 2013) and therefore the head was included in SA measurements. BAI has been used to study the body condition of larger cetacean species such as gray whales (Burnett et al., 2018; Lemos et al., 2020; Torres et al., 2022), humpback whales (Bierlich et al., 2021a; Bierlich et al., 2022), blue whales (Burnett et al., 2018; Bierlich et al., 2021a), Antarctic minke whales (Bierlich et al., 2021a), and was recently applied to smaller cetaceans to study body condition of Tamanend's bottlenose dolphins (Perkins-Taylor et al., 2023).

Estimating Unknown Girth of UAS Dolphins from Width and Height Measurements

Body Mass Index has been used to determine the body condition of bottlenose dolphins during capture-release health assessments (Hart et al., 2013). Actual BMI values can only be obtained when dolphin mass is available. In this study, estimated BMI was calculated by using estimated mass obtained from nonlinear OLS regression models for total length (Model 1) and estimated girth (Model 2) (see below) that were created from historical capture-release health assessments. Assuming the maximum girth of bottlenose dolphins can be represented as an ellipse, girth could be estimated by calculating the circumference of an ellipse using the width anterior to the dorsal fin measurement and the height in the same area of the dolphin as parameters (Hiromitsu et al., 2021). When data were deficient for determining dolphin height (needed for the ellipse equation), girth estimation was precluded, and thus the sample size for girth measurements is considerably low. The estimated girth assumption based on width and height measurements was validated on one adult stranded bottlenose dolphin. During necropsy, straight height and width anterior to the dorsal fin were measured, (using slide calipers), along with the maximum girth (open reel tape measure). The elliptical circumference was then calculated by using the circumference equation.

Estimating Unknown Mass of Dolphins

To calculate a Body Mass Index (BMI), mass was estimated for all dolphins. Using capture-release data from IRL dolphins collected during the dolphin HERA project (2003-2015), nonlinear OLS regression models with 95% reference ranges for body condition (upper, median, lower), following previously established methods (Hart et al., 2013; Gryzbek, 2013), were used to determine the relationship between mass, girth, and total length. These models were then used to estimate dolphin mass based on photogrammetry (total length and estimated girth measurements). Since IRL dolphins exhibit sexual dimorphism (Stolen et al., 2002), models were created for each sex and dolphins measured with the UAS that had undetermined sex were not able to obtain estimated weight measurements. Data from 119 males and 36 females that were physically evaluated and measured during HERA evaluations (2003-2015) were utilized for model development. The dataset initially contained 68 females but was refined before modeling to avoid bias (pregnant or suspected pregnant females excluded), resulting in a smaller female sample size.

From the model, Equation 1 was used to determine the unknown mass where Mass is in kilograms, total length in centimeters, and a and b are estimated parameters from the OLS regression (Hart et al., 2013). Dolphin body mass index was calculated using Equation 2, as it is the most appropriate morphometric index for making inferences on small cetacean body condition (Kershaw et al., 2017) and has been successfully used to compare morphology and BMI in two rough-toothed dolphin mass stranding events in Florida (Karns et al., 2019).

$$\text{Equation 1: Mass} = a * \text{Total Length}^{b}$$

$$\text{Equation 2: BMI} = \text{Mass} \div \text{Total Length}^{2}$$

HERA data used to develop OLS regression models were collected from animals healthy enough to undergo capture-release assessments, therefore, these data do not include emaciated individuals, which could potentially cause bias in the model.

Statistical Analysis

All statistical analyses were conducted in R version 4.2.2 (R Core Team 2022). For nonlinear OLS regression models, the R package *Quantreg* was used to model the relationships between weight and length, and weight and girth of capture-release data. In cases where multiple images were extracted of the same individual during image, the two straightest images with the best focus were used to determine total length, widths, estimated mass, and girth. Measurements were compared to determine accuracy by calculating the coefficient of variance (CV). Similar to other cetacean studies, CVs below 5% indicated consistent measurements (Perryman & Lynn 1993, Miller et al., 2012, Durban et al., 2016). To assess how IRL dolphin body condition measurements changed across sex, age class, and lateral body condition score, two-way ANOVA tests were performed for each measurement (BAI, BMI, and width to length ratios). Residuals of the ANOVAs were analyzed relative to assumptions for normality and equal variance. The R package *dplyr* was used for data manipulation and the package *ggplot2* was used for creating detailed boxplots for all assessments. Principal Component Analysis (PCA) was used following previous studies (Lockyer, 2007; Miller et al., 2012; Noren et al., 2019; de Oliveira et al., 2023) to categorize photogrammetry measurement correlations and determine which indices best explains the distribution of lateral body condition scores. The R package *Vegan* was used for PCA-related functions and ordination plotting.

Chapter 3
Results

Measurement Validation

The total length measurement obtained from a UAS overflight of the entangled IRL dolphin calf was the same from the still image (TL = 181 cm) as the length measured (open reel tape measure) during necropsy 18 days later (TL = 181 cm). The spotted dolphin TL measured 109 cm from the UAS still image and 112 cm using calipers. Width measurements at two locations were compared for the spotted dolphin. BHW was 14 cm from the UAS image and 14.7 cm from the calipers. MAXW was 19 cm from the UAS flight and 18.7 cm from the calipers.

The height of the stranded bottlenose dolphin that was used to test the elliptical circumference was 42.8 cm (radius = 21.4 cm) and MAXW was 34.2 cm (radius = 17.2). The elliptical circumference using these measurements was calculated as 121 cm while the true girth was measured as 115 (mean: 118 ± 4.48, CV: 3.79%).

Photographic Collection and Analysis

UAS video collection took place simultaneously with lateral body condition methods during survey efforts. A total of 26 flights, predominately from the northern IRL (Halifax River = 19, Banana River = 9) were conducted from August 2023 to March 2024 during nine dolphin surveys and 1 opportunistic flight from land (north-central Indian River Lagoon) (Figure 1). A total of 21 videos were recorded and 150 still images were extracted that contained 45 dolphins. UAS and lateral body image processing later excluded 16 dolphins due to poor image quality, body curvature, or inability to determine lateral body condition score. To avoid body condition bias, a suspected pregnant dolphin was excluded from the study and the pregnancy was subsequently confirmed when the female was observed with a neonate three months after the UAS flight. This resulted in a total of 29 dolphins being measured for complete analysis. After the exclusion of inadequate images, 99 images were measured in MorphoMetrix. The number of images measured per dolphin ranged 1-5 (2.5 ± 1.33).

Sex was determined for 25 dolphins (86%) based on previous observations from long-term photo-identification studies where the genital region was directly observed or by the animal calving (≥ 3 observations with a dependent calf). Of the 25 dolphins with known sex, 12 were female and 13 were male.

A total of 20 adults (9 males, 10 females, 1 unknown sex) and 9 juveniles (4 males, 2 females, 3 unknown sex) were included in the study. A total of eight lactating females (observed nursing or accompanied by a dependent calf) and two adult females were included.

Individuals with two sufficient images for accurate measurement were used to assess measurement precision for each dolphin. Measurement precision was evaluated for 22 dolphins (75%; seven adult males, two adult females, six lactating females, and seven juveniles) (Table 5). CVs ranged 0.9% (TL) to 9.4% (MAXW) for adult males, 1.4% (TL) to 7.2% (MAXW) for adult females, 1.8% (TL) to 6.5% (BHW) for lactating females, and 0.9% (TL) to 4.9% (PNW) juveniles. TL had the lowest CV among measurements for each age class. TL had the lowest CV compared to other measurements across all age classes (Table 5).

Body Measurements and Ratios

The total length, width at blowhole, post-nuchal, axilla, maximum width, and surface area at 50% of the body were measured for all 29 dolphins. The height anterior to the dorsal fin was opportunistically measured for seven dolphins and paired with MAXW to obtain an estimated girth (circumference of an ellipse).

TL measurements were greatest for adult males (range: 244-274 cm; 257 ± 11.05 cm), followed by adult females (range: 224-257 cm; 243 ± 10.13 cm), and juveniles (range: 199-234 cm; 215 ± 10.63 cm). Lactating females and adult females were evaluated as different age class groups for width segments. Width at blowhole was greatest for adult males (range: 24-28 cm; 26 ± 1.66 cm), followed by adult females (range: 22-27 cm; 25 ± 3.54 cm), lactating females (range: 20-26 cm; 25 ± 1.63 cm), and juveniles (range: 21-25 cm; 23.44 ± 1.74 cm). Width at post-nuchal was greatest for adult males (range: 28-33 cm; 31 ± 1.64 cm), followed by adult females (range: 29-30 cm; 30 ± 0.71 cm), lactating females (range: 24-29 cm; 27 ± 1.73 cm), and juveniles (range:24-29 cm; 25.89 ± 1.83 cm). Width at axilla was greatest for adult males (range: 32-43 cm; 36 ± 3.80 cm), followed by adult females (36 cm), lactating females (range: 31-39 cm; 34 ± 2.43 cm), and juveniles (range: 31-35 cm; 31.89 ± 2.15 cm). Maximum width was greatest for adult females (range: 36-42 cm; 39 ± 4.24 cm), followed by adult males (range: 31-47 cm; 36 ± 4.82 cm), lactating females (range: 31-43 cm; 35 ± 3.85 cm), and juveniles (range:28-36 cm; 33 ± 2.51 cm) (Table 2).

Juveniles had the greatest mean BHW/TL (0.109 ± 0.008; range: 0.098-0.123) followed by adult males (0.101 ± 0.008; range: 0.088-0.115), lactating females (0.101 ± 0.008; range: 0.083-0.112), and adult females (0.099 ± 0.017; range: 0.087-0.111). Juveniles had the greatest mean PNW/TL (0.120 ± 0.008; range: 0.111-0.133) followed by adult males (0.12 ± 0.008; range: 0.109-0.131), adult females (0.118 ± 0.001; range: 0.118-0.119), and lactating females (0.111 ± 0.009; range: 0.99-0.125). Juveniles had the greatest mean AXW/TL (0.149 ± 0.008; range: 0.137-0.161) followed by adult females (0.145 ± 0.004; range: 0.142-

0.148), adult males (0.141 ± 0.014; range: 0.119-0.160), and lactating females (0.140 ± 0.012; range: 0.121-0.164). Adult females had the greatest mean MAXW/TL (0.156 ± 0.012; range: 0.148-0.165) followed by juveniles (0.151 ± 0.006; range: 0.138-0.160), lactating females (0.146 ± 0.015; range: 0.132-0.181), and adult males 0.142 ± 0.021; range: 0.115-0.181) (Table 3).

BAI ranged 22.17-32.12 (25.47 ± 3.33) for adult males, 27.1-27.22 (27.16 ± 0.09) for adult females, 22.1-30.51 (25.81 ± 2.69) for lactating females, and 26.01-28.97 (27.34 ± 1.08) for juveniles (Table 2).

Mass Model Results

The relationship between total length (cm) and mass (kg) of HERA dolphins was assessed in Model 1 (Female: R-squared = 0.75, Male: R-squared = 0.79) (Figure 4). The equation generated by the model can be used to estimate mass from total length measurements from all 29 dolphins measured in MorphoMetrix (Female: $Mass = (2.04 \times 10^{-4}) \times TL^{2.47}$; Male: $Mass = (7.67\times10^{-5}) \times TL^{2.65}$). In addition, the relationship between maximum girth (cm) and mass (kg) of HERA dolphins was assessed in Model 2 (Female: R-squared = 0.60; Male: R-squared = 0.76) (Figure 5) and the model equation can be used to estimate mass based on the calculated estimated girth from the seven dolphins where opportunistic height measurements were available (Female: $Mass = (2.67\times10^{-3}) \times Girth^{2.26}$; Male: $Mass = (1.45\times10^{-3}) \times Girth^{2.40}$).

Using the equation given in Model 1, mass estimates ranged 163.7-222.7 kg (188.1 ± 21.5 kg) for adult males, 157.5-175.6 kg (166.5 ± 12.9 kg) for adult females, 128.8-180.8 kg (156.5 ± 17.0 kg) for lactating females, and 119.1-143.5 kg (131.3 ± 17.2 kg) for juveniles. BMI was estimated using Equation 2. BMI ranged 0.0027-0.0030 (0.0028 ± 0.0001) for adult males, 0.0027 (0.0027 ± 0.0000) for adult females, 0.0026-0.0027 (0.0027 ± 0.0001) for lactating females, 0.0024-0.0026 (0.0025 ± 0.0001) for male juveniles, and 0.0025-0.026 (0.0026 ± 0.0001) for female juveniles.

Model 2 was used to estimate mass from estimated girth for two females and five males. Estimated girth ranged 118.2-126.0 cm (122.1 ± 5.5 cm) for females, and 104.3-149.7 cm (128.1 ± 18.7 cm) for males. Estimated mass ranged 126.9-146.4 kg (136.7 ± 13.8 kg) for females, and 100.6-239.3 kg (180.7 ± 65.1 kg) for males. BMI for the two females was 0.0027 and 0.0024-0.0036 (0.0031 ± 0.0005) for males. One male and one female had two different height measurements and therefore two estimated girth and mass values and were used to evaluate precision. The female girth was 126.0 cm (first height image) and 124.2 cm (second image). The first measurement gave a mass of 146.4 kg (BMI 0.0027), while the second resulted in a mass of 141.9 kg (BMI 0.0028). The male's girth (first height image) was 139.1 cm and 142 cm (second image). The first measurement resulted in a mass of 200.8 kg (BMI of 0.0034), while the second gave a mass of 210.9 kg (BMI 0.0036).

Dolphins with estimated mass from both models (i.e., the seven dolphins that had girth measurements) were used to compare model accuracy in estimating mass. For most dolphins, an estimated BMI range was determined using both models, except for one animal where both models estimated the same BMI. Overall, Model 1 had lower mass estimates than Model 2. Coefficient of variance ranged from 0 to 15% for measurements between the two models (Table 4).

Lateral Body Condition Scoring

Using lateral images to evaluate body condition, four dolphins were ideal (three adult males, one unknown sex adult), twenty-four were underweight (six adult males, two adult females, seven lactating females, nine juveniles), and one was emaciated (lactating female).

Statistical Analysis

Residuals of the ANOVAs were analyzed relative to assumptions for normality and equal variance, and all met the assumptions. Since all juveniles were considered "Underweight", analysis between BCS was not performed for the juvenile age class.

BAI measurements did not significantly differ across age class and BCS (age class: $p = 0.330$; BCS: $p = 0.221$). BAI also did not differ significantly between the ideal, underweight, and emaciated BCS levels within the adult age class ($p = 0.753$) or within lactating females ($p = 0.151$) (Figure 6).

Using a two-way ANOVA, BMI differed significantly between BCS ($F = 6.103$ $p = 0.00853$) and age class ($F = 16.335$ $p < 0.001$). Post-hoc testing indicated ideal dolphins had a significantly higher BMI than underweight dolphins (p-adjusted = 0.006). However, there was no significant BMI difference between underweight and emaciated ($p = 0.935$) for lactating females, or between ideal and emaciated (p-adjusted = 0.287) for adults. When comparing BMI across age class, juveniles (p-adjusted = 0.0001) and lactating females (p - adjusted = 0.033) had significantly lower BMI compared to adults. Lactating females also had significantly higher BMI compared to juveniles (adjusted p = 0.0072). A one-way ANOVA was used to assess the effect of BMI on BCS and age class without including interactions between the two variables. Results from the one-way ANOVA found no significant difference in BMI across BCS ($F = 2.549$ $p = 0.101$) but yielded a significant difference by age class ($F = 22.26$ $p < 0.001$). When assessing BCS within each age class, BMI did not differ significantly between ideal and underweight within adult age class (p = 0.428) or between emaciated and underweight within lactating females (p = 0.867) (Figure 7).

There was not a significant difference in BHW/TL across age class (p = 0.131) or BCS p= 0.910). BHW/TL was not significantly different between ideal, underweight, and emaciated BCS within the adult age class (p = 0.843) or within lactating females (p = 1) (Figure 8).

Using a two-way ANOVA, PNW/TL measurements approached significance across age class (F = 3.042 p = 0.07) but not BCS (F = 0.460 p = 0.637). Results from the post-hoc Tukey multiple comparisons of means test also indicated a difference that approached significance in PNW/TL ratios between lactating females and juveniles (adjusted p = 0.09) and no significant difference between adult and juvenile PNW/TL (adjusted p = 0.90) or lactating female and adult (adjusted p = 0.163). Although age class differences are not significant, lactating females tend to have a lower PNW/TL compared to adults and juveniles, with the differences approaching significance. A one-way ANOVA was then used to test the effect of age class on PNW/TL independently of the BCS variable and found PNW/TL to be significant across age class (F = 3.793 p = 0.036). Thus, PNW/TL varied significantly across age class when not accounting for the interaction between BCS and age class. There was not a significant difference between ideal, underweight, and emaciated BCS within the adult age class (p = 0.977) or within lactating females (p = 0.978) (Figure 8).

There was not a significant difference in AXW/TL across age class (p = 0.165) or BCS (p = 0.845) or between ideal, underweight, or emaciated AXW/TL measurements for the adult age class (p = 0.405) or within lactating females (p = 0.968). Likewise, there was not a significant difference in MAXW/TL measurements across age class ($p = 0.801$) or BCS ($p = 0.598$) or between ideal, underweight, or emaciated MAXW/TL measurements within adult age class ($p = 0.769$) or lactating females ($p = 0.507$) (Figure 8).

Results from the PCA found that PC1 explained only 34% of variance with BAI, AXW, and AXW/TL as the dominant variables (Figure 9). PC2 explained 31% of variance with TL, PNW, and BAI as the dominant variables (Figure 10). PC3 explained 19% of variance with BHW/TL, PNW/TL, and BHW as dominant variables (Figure 11). Combined, PC1, PC2 and PC3 explain 82% of variance. These components together explain a significant portion of the variance and provide meaningful insights into overall body size and body proportions. While there was not a clear pattern in underweight and emaciated individuals for any combination of the three components, ideal individuals all had PC2 scores of 0 or greater, indicating that PC2 can help explain how individuals with ideal BCS are correlated by TL, PNW, and BAI.

Chapter 4
Discussion

UAS Measurement Validation

Total length measurements from still images exacted from UAS video proved to be accurate for the IRL dolphin calf, and within 3 cm of the true TL for the spotted dolphin (UAS TL = 109 cm; tap measure TL = 112). It is important to note that the carcass was measured while frozen which may have impacted the accuracy of measurements. Since MorphoMetrix does not measure to the nearest mm, we can only confirm that UAS measurements were accurate within 1 cm for BHW (UAS = 14 cm; caliper = 14.7 cm) and MAXW (UAS = 19 cm; caliper = 18.7 cm). Although this method was only confirmed with two calves, it provides evidence that it is feasible to obtain accurate measurements from UAS videos and that this methodology can be a reliable method for assessing the body condition of small cetaceans.

Previous morphometric studies on large and small cetaceans tend to favor the photo burst feature as a method for capturing still images for measurements (Christiansen et al., 2016; Fearnbach et al., 2018; Cheney et al., 2022). This method can provide better image quality than extracting still images from videos in Adobe Photoshop. The image pixel width and Dots Per Inch (DPI)/Pixels Per Inch (PPI) values are greater when capturing images on the UAS during the flight (pixel width = 5280, DPI = 96) than subsequently extracting still images from videos (pixel width = 3840, DPI = 72). Higher DPI/PPI values typically provide higher resolution images with a less granulated appearance than images with lower DPI values. Because of the lower image resolution and the added image processing time, extracted images from videos are rarely used for body condition studies.

Overall, utilizing burst images is more efficient for image analysis and processing. However, capturing burst images of IRL dolphins at the surface in a straight-line position proved to be extremely difficult. Dolphins are fast moving and the frequent lack of IRL water clarity made predicting dolphin surfacing difficult. Still images extracted from UAS videos were successfully utilized to evaluate body condition in sperm whales (Glarou et al., 2022), and other small cetaceans (Perkins-Taylor et al., 2023; de Oliveira et al., 2023; Serres et al., 2024). Likewise, following these methods, still images extracted from videos were found to be more efficient for IRL dolphins. Overall, the utilization of UAS videos rather than burst images to enable photogrammetry proved reliable and did not hinder accurate dolphin measurements.

Photographic Collection and Analysis

Although 35% of overflown dolphins were excluded due to factors prohibiting accurate measurement (body curvature, etc.), photogrammetry and body condition assessment was successful in most cases. Thus, confirming these methods can be used as a more detailed assessment of IRL dolphin health than the subjective lateral body condition scoring method alone while providing a noninvasive alternative to capture-release health assessments. Furthermore, field methods can be conducted in tandem with photo-identification studies, consuming minimal additional field time, while providing useful supplementary morphometric details on individuals throughout seasons, UMEs, reproductive cycles, and life history stages.

Video footage provided multiple opportunities to measure each dolphin with the goal of comparing measurements from two or more images. Only seven dolphins were measured in one image which prohibited measurement error estimation in those individuals. For the dolphins with replicated measurements, the mean coefficient of variation for TL measurements were lowest compared to other body measurements (for all age classes), with lowest CV values averaging 0.9% for juveniles and adult males, suggesting measurement was consistent and reliable. A clear trend was not evident in the error of measurements, except for in lactating females where there was a decrease in CV as area of the body increased in size from BHW to MAXW. This is comparable to large cetacean studies that found measurement error increased as the body area decreased (Miller et al., 2012). For example, width measurements from 60-80% (caudal region of the whale) had higher error than measurements taken at the 20-50% areas where the animal is the widest (Miller et al., 2012). Since the current study only compared width segments at four locations as opposed to the eight locations used in previous studies (Miller et al., 2012), a trend in measurement accuracy across different width segments may not be as obvious.

Sex could not be determined for four of 29 dolphins in the study. Three of the four unknown sex dolphins were juveniles that have not yet reached sexual maturity. Data from long-term photo-identification and capture-release research on IRL dolphin demographics enabled the classification of most individuals by sex and age class. The UAS videos were also helpful in determining the sex of unknown individuals by offering a top-down view while dolphins were socializing allowing enhanced opportunities to visualize the genital region and confirm lactation than photo-identification observations alone. The continuation of these methods will provide additional demographic information for IRL dolphins. Exposure to long periods of poor foraging or environmental stress can lead to adverse effects on individual body condition, fecundity, reproductive success, and survival in marine mammals (Lockyer, 1986; Perryman et al., 2002). Since body composition can vary by age, sex, and reproductive status (Koopman et al., 1996; McLellan et al., 2002; Dunkin et al., 2005; Beck et al., 2007; Koopman, 2007) evaluating these variables can help determine how body measurement trends may vary during periods of high metabolic investment such as mating, pregnancy, lactation, and weaning. As IRL dolphins are considered an immunocompromised stock (Bossart et al., 2003), it is important to monitor body condition changes by age and sex demographics.

Body Measurements and Ratios

Girth is a variable commonly used to monitor and assess marine mammal health (Caon et al., 2007; Lockyer, 2007; Gómez-Campos et al., 2011; Hart et al., 2013; Joblon et al., 2014). Girth measurements are commonly collected during dolphin capture-release health assessments and during necropsy, enabling assessment across varied health status and age class. However, noninvasive measurement of free-swimming dolphin girth is still relatively unexplored. A method to estimate the girth of free-swimming right whales (*Eubalaena sp*.) was developed by using width and height measurements collected from UAS overflights (Christiansen et al., 2019). Width and height measurements were then fitted to infinite ellipses across the total length of the whale, and body volume was predicted from models based on historical catch records (Christiansen et al., 2019). Estimating girth for fast-swimming, small cetaceans that spend significantly less time at the surface compared to large whales, can be more difficult. Hiromitsu et. al. (2021) tested a method for estimating ellipticity of the cross-sectional view around the girth of wild dolphins using underwater video footage to obtain width measurements. This methodology was found to be effective (Hiromitsu et al., 2021), suggesting that the evaluation of the ellipticity of dolphin widths can be an accurate way to estimate girth. Similar assumptions were successfully utilized to estimate fur seal girth as the diameter of a circle to enable an estimate of axillary girth from axillary width obtained from UAS measurements (Allan et al. 2019).

New noninvasive methods to estimate girth and mass of dolphins are needed, and to our knowledge, this is the first study to accurately estimate bottlenose dolphin girth and mass using UAS photogrammetry paired with capture-release models. Capturing images of dolphins positioned laterally in a straight orientation is difficult. The lack of water clarity in the northern IRL can be attributed to nutrient enrichment, eutrophication, and seagrass die-off (Briel et al., 1973, Bricker et al., 2007) and often prohibits dolphin visibility from the UAS except for at or very close to the surface. In this study, images that enabled opportunistic height measurement typically occurred on clear days with increased water clarity, while the dolphin was in shallow water and typically engaging in probable feed or social behavior. However, even if the dolphin was observed laterally positioned, if the body was curved or angled and precluded an accurate height measurement, the image was excluded. A fresh dead bottlenose dolphin, with little decomposition, stranded during the study and provided an opportunity to test the assumption that elliptical circumference can be used as a proxy for girth. While only a single sample results were encouraging with only a 6 cm measurement difference between the elliptical circumference and true girth at necropsy. The difference could potentially be explained by the slightly flattened abdomen succumbing to gravity while being measured on a necropsy table, and slightly skewing the width and height measurements (Hiromitsu et al., 2021). Future studies should collect additional measurements to compare elliptical circumference to girth in stranded IRL dolphins and to help confirm assumption reliability as well as its utilization to assess IRL dolphin body condition.

As IRL dolphins are considered sexually dimorphic (Stolen et al., 2002), TL measurements were as expected for sex and age class. Adult males had the greatest mean TL followed by adult females, and juveniles. One lactating female was measured at 224 cm in both images which would be considered juvenile by total length (Wells et al., 1987). Since this female has been previously observed with dependent calves (Durden unpub. data), was 13 years old at overflight (approximate date of birth 16 November 2009) and females can be considered reproductively mature between 6 and 12 years of age (Lacy et al., 2021), we are confident that she is a mature adult female. The low sample size of this study prohibited evaluation of older calves, however, future efforts should consider evaluating calves to examine differences in body measurements since dependent calves are vulnerable to environmental threats (Lacy et al., 2021).

Girth at blowhole, axilla, and maximum girth are routine measurements taken during dolphin necropsies (Geraci & Lounsbury, 2005). Widths at these locations were used to determine if potential differences across body condition scores or age class could be observed. During necropsy, width measurements in these areas are not typically collected in leu of girth, however, these areas are the most significant for body condition measurements (Geraci & Lounsbury, 2005; Fair et al., 2006; Gryzbek, 2013) and were thought to be more useful in assessing changes in health than at 10% increments across the TL as in previous studies (Perkins-Taylor et al., 2023; Miller et al., 2012; de Oliveira et al., 2023). Future efforts to measure the width at the corresponding girth locations during necropsy can enable comparison between the two methods.

Width to length measurements were assessed for all 29 dolphins. Although data are scarce to enable comparisons across populations, data may enable future comparisons for the IRL dolphins as efforts continue. These ratios have been assessed for Guiana dolphins, franciscanas dolphins, and Indo-Pacific humpback dolphins (de Oliveira et al., 2023, Serres et al., 2024) and could potentially be compared to IRL dolphin ratios with further population-wide sampling. Previous ratios for bottlenose dolphins are not available and therefore, we cannot compare our findings to known appropriate ratios for ideal or compromised body condition. Lactating females had the lowest recorded BHW/TL ratio and juveniles had the highest. Although adult females without dependent calves had the lowest mean value for BHW/TL, there were only two adult females in that age class. Therefore, increasing this sample size may potentially show a trend with lactating females possessing smaller ratios than those without dependent calves. The larger BHW/TL ratios observed in juveniles compared to adults can likely be explained by juveniles having shorter TL measurements, as BHW did not drastically change across age classes and does not necessarily equate to better juvenile body condition. Serres et al. (2024) found that BHW/TL ratios for Indo-Pacific humpback dolphins did not vary across age class or status but were significantly lower in areas where populations of dolphins were rapidly declining compared to BHW/TL from dolphins in other locations. This trend indicates the BHW/TL index can provide important insights on individual and population body condition and can be used to help explain rapid declines in populations exposed to continuous anthropogenic stress (Serres et al., 2024). Although our results do not show a specific trend, incorporating a larger sample size, similar to the size in previous studies ($n > 40$ for each location, Serres et al., 2024), may aid in the analysis and significance of the BHW/TL index.

The post-nuchal region is not commonly measured in dolphin necropsies or in previous UAS studies, however, Gryzbek (2013) assessed this specific area on deceased dolphins and found that it tends to lose adipose in nutritionally compromised animals and therefore, this landmark was deemed an important measurement in this study to identify differences between age class or BCS. To our knowledge, this is the first study to include PNW/TL ratios as an index for body condition scoring, prohibiting the ability for comparison of measurements to other species. PNW/TL ratios were lower for lactating females when compared to adult males, adult females without dependent calves, and juveniles. We found the PNW/TL measurement to be an important variable that can potentially be used as indicator to assess changes in body condition across age classes. Future studies should evaluate how the post-nuchal region changes across age class and reproductive status with a larger sample size to aid in understanding of adipose deposit fluctuation during periods of energetically expensive metabolic investment. A larger dataset will be needed to confirm findings as the current study only measured one emaciated animal and therefore a clear distinction between PNW/TL measurements was not indicated between BCS. A previous study evaluated the lateral body condition of grey whales (*Eschrichtius robustus*) and found that lactating females presented in significantly worse body condition than other age classes with emphasis given to the visual adipose depletion in the postcranial region (Bradford et al., 2012). Although Bradford et al. (2012) was assessing lateral images rather than UAS measurements, it provides similar findings to IRL dolphins, further emphasizing the significance of post-nuchal depletion in lactating female cetaceans. Since depletion of the post-nuchal region is emphasized in the lateral body condition methods for IRL dolphins, smaller PNW/TL measurements from the UAS may correlate with emaciated lateral BCS.

The axillary region is near the widest portion of the dorsum and girth at axilla can be monitored for changes in body condition (Hart et al., 2013; Joblon et al., 2014). The axillary girth measurement is easily identified during necropsy by utilizing the pectoral flippers as a landmark. However, UAS images were not always clear enough to visualize pectoral flippers. We would expect that this uncertainty may have caused errors in AXW measurements. However, when looking at measurement precision, AXW did not have the highest measurement error for any age class, indicating it can still be a reliable measurement. Juveniles had the lowest mean AXW, but the highest AXW/TL ratio compared to other age classes. This is likely because juveniles tend to have thicker blubber layers compared to older animals (McLellan et al., 2002; Struntz et al., 2004; Noren & Wells, 2009; Mallette et al., 2016) and overall shorter total length measurements, resulting in higher AXW/TL values. Aside from the one adult (unknown sex) that had the lowest individual AXW/TL ratio, lactating females had the lowest mean AXW/TL ratio compared to other age classes. However, there was no significant difference between age class or BCS for AXW/TL ratios. Further evaluation is needed to confirm the significance of utilizing the AXW measurement for body condition analysis in UAS studies. Lactating females had lower AXW/TL ratios following the same trend we observed in PNW/TL values. This further contributes to the hypothesis that lactating females may trend toward lower body condition compared to other age classes, however, further comparisons are needed. Similar to ratios at BHW, Serres et al. (2024) found that ratios of measurements taken near the pectoral flippers of Indo-Pacific humpback whales were also lower in declining populations. De Oliveira (2023) also assessed width at axilla to length ratios for two species of small cetaceans and found that AXW/TL differed significantly across season, with higher values occurring in the winter months (de Oliveira et al., 2023) reconfirming that this proportion can be an important index for assessing changes in body condition for small cetaceans. Axillary girth and total length ratios are known to be good indicators of body condition in bottlenose dolphins, (Cheal & Gales, 1991; Hart et al., 2013; Joblon et al., 2014). Modifying the axillary girth to length ratios from necropsy and capture-release health assessments to width to length for UAS measurements can provide an effective means to noninvasively assess body condition of IRL dolphins.

From a top-down perspective, the MAXW area may not be considered the widest portion of the dolphin as dorsally the area looks narrower than areas closer to the axilla, however laterally it is the widest, hence the measurement location. To enable future comparison to necropsy data and for sample consistency, MAXW was measured just anterior to the dorsal fin. MAXW values were lowest for juveniles and greatest in adult males, although adult females had the highest mean MAXW and the highest mean MAXW/TL ratio. It is important to note that there were only two adult females without dependent calves, therefore these samples may not be representative for adult females. Likewise, pregnancy was not determined for adult females, and it is uncertain if MAXW and MAX/TL values could be affected by early pregnancies. MAXW is a useful measurement for girth estimates when paired with height, however, CV between images was highest for MAXW in the adult male (9.4%) and female (7.2%) age classes (excluding lactating females) when compared to other width measurements. Therefore, MAXW may not be as reliable as the PNW for adult males and females. Similar to our study, Serres et al. (2024) found no significant difference in width at dorsal fin to length ratios (MAXW/TL) between animals at different geographical location or age class confirming that this index alone may not be as powerful for determining body condition (Serres et al., 2024), but may be helpful for pregnancy determination (Cheney et al., 2022).

BAI was not significant across age class and BCS. Similar studies have found BAI to be a reliable measurement for assessing body condition using UAS, however, these studies have typically compared changes in BAI across spatial and seasonal patterns (Burnett et al., 2019; Lemos et al., 2020; Bierlich et al., 2022; Perkins-Taylor et al., 2023). For example, dolphin BAI was compared by region, season, and age class and age class was found to be the most significant BAI predictor, followed by seasonality (Perkins-Taylor et al., 2023). Specifically, dolphins had significantly higher BAI values in the winter than in the spring (no significant change in summer or fall) and calves had higher BAI values than adults (Perkins-Taylor et al., 2023). Compared to other age classes, Pacific Coast Feeding

Group (PCFG) gray whale calves had significantly higher BAI values (Lemos et al., 2020). The current study did not consider how season or location may affect body condition, but future efforts should attempt to address these changes. Future efforts should also aim to evaluate changes between different sub-basins of the IRL. Data were collected opportunistically from August to April, encompassing both the wet and dry seasons of Florida (Adams, 1996). Although IRL dolphins do not undergo energetically stressful seasonal migrations and taxing reproductive periods that larger marine mammal species do, there has been evidence that blubber thickness increases during the winter months in small cetaceans (Perkins-Taylor, 2023; de Oliveira et al., 2023), implying body measurements could potentially be greater in winter. While calves were also not included this study, future efforts should incorporate calf BAI to compare to results found in similar studies.

Mass Models

Estimating mass from TL proved to be an efficient way to assess BMI. BMI values are not typically utilized in UAS studies since there is not a reliable method to estimate BMI without accurate mass measurements. Instead, BAI measurements are used for body condition evaluation. Hart et al., 2013 developed models with 95 percentile reference ranges for relationships between TL, girth, and mass from capture-release studies that can be used to aid in assessing body condition. In those models, individuals with mass and TL, or girth, measurements that fall below the lower 95-quantile range would be in a compromised condition, while dolphin measurements above the upper 95-quantile range would be in healthy condition (Hart et al., 2013). Instead of using the models to compare individual measurements to the population as in Hart et al. (2013), we used these models to estimate mass from the model equations for the relationship between TL and mass (Model 1) and girth and mass (Model 2).

Obtaining mass from dolphins during necropsies is not always possible, therefore, inability to test the accuracy of these models was a limitation to the study. When given the opportunity, future IRL dolphin strandings should be measured for mass, girth, TL, MAXW, and height, for model validation. Comparing the true mass to the estimated mass could provide important feedback on the validity and can help improve the statistical power of the models.

Both models had high R-squared values for males and females. In model 1, 75% of the variability in the female weight data could be explained by the model using female length as a predictor and 60% by the second model; suggesting model 1 provides a significantly better fit for predicting female weight. For males, 79% of the variability in the male weight data can be explained by model 1 using male length as a predictor, and 76% by model 2, suggesting that both models provide a reasonably good fit for predicting male weight (model 1 being slightly better). Performance of the models show promise for utility in future studies for estimating mass and BMI for IRL dolphins.

For the seven dolphins that had girth estimates, predicted mass for each model was compared. Overall, Model 2 predicted a greater mass for each dolphin. The greatest difference in mass between the two models occurred for an adult male that was considered ideal with a difference of 47 kgs (CV = 15%). For this male, Model 1 predicted a mass of 192 kg while Model 2 predicted a mass of 239 kg. This provided a large range for estimated BMI varying from 0.0029 to 0.0036. Similarly, another adult male with an underweight BCS, had a difference of 37 kgs (CV = 14%) between the two models providing a BMI range of 0.0027-0.0034. The larger variation in estimated mass and BMI ranges implies more uncertainty for these individuals and estimates may not accurately represent true mass measurements. In contrast, both models predicted the same mass for a juvenile male (101 kg). Since mass was equivalent for both models, this dolphin did not have a range for BMI estimation therefore we can assume this BMI score to be accurate. Only two of the seven dolphins were in ideal body condition, but both had higher BMI ranges than the underweight dolphins. BMI scores for ideal animals ranged 0.0029 to 0.0036 while underweight BMI scores ranged 0.0024 to 0.0034. Although the sample size was low and there was not a distinct pattern of BMI ranges, it appears that greater BMI scores (> 0.0029) may correlate with ideal body condition. BMI scores were not significantly different across BCS, however, this study established baseline ranges for estimated BMI by BCS. The potential to accurately categorize dolphins by the corresponding lateral BCS using estimated BMI measurements collected from the UAS could be an extremely powerful tool for assessing the body condition of IRL dolphins. Continued efforts are needed to increase the sample size of BMI scores for each BCS will improve confidence in BMI intervals.

While results from the two-way ANOVA found BMI to be significantly greater for ideal dolphins than underweight dolphins, a one-way ANOVA found no significant difference in BMI across BCS when not including the interaction between BCS and age class. This suggests that the interaction between BCS and age class is significant when assessing difference in BMI. The F statistic was slightly higher for the one-way ANOVA indicating that evaluating BCS independently explains more of the variation than the interaction between BCS and age class and therefore, there is likely not a significant difference in BMI across BCS. When comparing BMI scores across age class, males had the highest BMI scores, followed by lactating females and juveniles. Since mass for each age class was dependent on TL from Model 1 and TL varies across age class, the results are not surprising. It is interesting that juveniles were observed with a lower BMI compared to adults and lactating females. Newly dispersed from their mothers, juveniles have less experience with dependence and are at a higher risk from conspecific aggression, predation and starvation than adults (Krzyszczyk et al. 2017) since may influence BMI. Equations for calculating BMI vary across studies. For example, Hart et al. (2013) and Gryzbek (2013) used estimated parameters from the OLS quantile regression models to calculate BMI (BMI = $Mass/Length^b$ x 10000; Hart et al., 2013, Gryzbeck, 2013). In the current study, estimated parameters from the OLS quantile regression did not provide BMI values that could easily be interpreted and compared across age class and BCS. Instead, we used the equation that was found to be the most informative out of 10 indices tested (BMI = $Mass/Length^2$; Kershaw et al., 2017) which was also used by McFee et al., 2013. However, other studies have successfully used simplified equations (BMI = Mass/Length; Karns et al., 2019) to calculate BMI. The variation in BMI calculation for small cetaceans hinders efforts to accurately assess and compare BMI across populations and methods. Future studies on BMI should evaluate differences in BMI calculations to enable comparisons across populations.

Lateral Body Condition Scoring

Lateral body condition scoring is a subjective method that requires a large quantity of detailed images of the head, thorax, and caudal regions for accurate assessment. In this study, assessing lateral images was challenging for several reasons. Tracking which individuals, from a larger group, were photographed in both the UAS and lateral images proved to be challenging. This was especially complicated for animals that did not have specific markings on their dorsum or dorsal fin and made the identification individuals from both platforms extremely difficult. At the beginning of the study, lateral images were taken before flying the UAS but later were taken simultaneously throughout the UAS flight. This enabled timestamp comparisons from lateral images and UAS video to confirm overflown animals and aided in excluding lateral shots of animals that were not successfully measured from the UAS. Using this adjusted method, the remote pilot would signal to the photographer when a dolphin was about to surface (only in clear water), and the photographer could more accurately get images of the head and thorax region while recording the animal's ID or distinguishing dorsal fin features. Simultaneously, the photographer would announce the known ID of the individual each time the animal surfaced while notes were recorded with the time that each individual was overflown. This adjusted method was extremely helpful for processing lateral images and identifying individuals in the UAS videos.

The post-nuchal region is known to be the most accurate criteria visualized for animals in compromised body condition (Gryzbeck, 2013). For this study, most animals were categorized as underweight (n = 24) which corresponded with a slight post-nuchal depression or other criteria in lateral images. Emaciated animals presented a depressed post-nuchal region and often other corresponding criteria. It is possible that some animals categorized as underweight could be considered emaciated if additional images could have been assessed. The animal that was categorized as emaciated had an apparent post-nuchal depression, exposed scapular ridge, and exposed ribs, making the body condition score evident. Ideal dolphins did not have a post-nuchal depression, however, due to the subjectivity of lateral body condition methods, it is possible that these dolphins could have also been considered underweight if additional images were available. However, the flat appearance of the post-nuchal region was the most reliable factor for determining BCS and scores can be considered as accurate as possible. While there were not distinct UAS measurement variations across lateral BCS, continued efforts and increased sample sizes for each BCS may provide enough variation to establish measurements and indices for each BCS.

Statistical Analysis

Previous UAS studies have used Principal Component Analysis to identify which body measurements or indices are most helpful for detecting correlations between seasons, populations, or species (Miller et al., 2012; de Oliveira et al., 2023). PCA is commonly used with multivariate data sets to reduce the number of variables to be interpreted by only interpreting the variables with the highest eigenvalues that can best explain the variance of that data. In this study, one objective was to use PCA to identify which measurements were most helpful for determining BCS. The cumulative proportion of the first three components was 82%, making this PCA robust for analysis. However, since there was only one emaciated dolphin, correlations for the emaciated BCS category could not be interpreted. Dolphins with underweight BCS did not follow any specific pattern for PC1, PC2 or PC3. This is likely because the majority of dolphins in this study were categorized as underweight and therefore, had the most measurement variation and could not be correlated. Dolphins categorized as ideal all have PC2 scores of -1 or more. PC2 was mostly explained by positive factor loadings of total length, PNW, BAI and seems to be a composite measure of overall body robustness and represents a general size factor where larger values indicate larger overall body sizes. This was as expected with dolphins in ideal body condition documented with larger body measurements. The inclusion of additional emaciated and ideal samples will aid in confirming these findings and allow for a more powerful evaluation for which body measurements and indices group BCS together. A prior study (de Oliveira et al., 2023) utilized PCA for proposed body condition indices and determined that anterior widths (widths taken anterior to the dorsal fin) had the highest correlation suggesting that these indices were good proxy to assess seasonal patterns in body condition. Serres et al. (2024) also found width to length ratios to represent clusters of similar body condition. Although our sample size was considerable smaller than the previous studies mentioned, there are some similarities in that width to length ratios are important components for grouping small cetaceans of similar body conditions. Understanding which measurements are most important for grouping individuals with the same BCS will assist in quantifying body measurements to eventually eliminate the need for subject lateral BCS methods by instead relying on more quantifiable morphometrics.

Chapter 5
Conclusions

The objective of this study was to determine if the body condition of free-swimming bottlenose dolphins could be accurately evaluated using new noninvasive methods. Using unmanned aircraft systems and MorphoMetrix, we were able to accurately measure the total length, width and height of free-swimming dolphins. Morphometric measurements from stranded dolphins were used to ground-truth measurement accuracy and were found to be reliable. This was the first study to utilize historical capture-release data for a specific population to estimate the mass and BMI of dolphins from measurements collected with a UAS. These methods proved to be innovative and powerful in assessing IRL dolphin health. By increasing the sample size and efficiency of these methods, we may be able to eliminate the need for subjective lateral body condition methods by establishing baseline measurements for each body condition score. Likewise, measuring a larger proportion of IRL dolphins will allow for a more robust PCA analysis for which UAS measurements could be used to accurately assign BCS. Once measurements can be categorized to the correct BCS, lateral BC methods will not be necessary. However, pairing lateral BC methods with UAS measurements can provide a more detailed examination of individual dolphin health, as lateral images can be used to assess skin lesions and injuries (Durden et al., 2020)

Utilizing a UAS in tandem with photo-identification studies allowed for a new perspective on identifying individuals. A top-down view of dolphins allows for a better view of dorsum scratches and scars that may not be observed in photo-identification images. Using both lateral images of the dorsal fin paired with UAS images, we can more accurately determine individual ID while also adding morphometric measurements for each individual that can be used to track body condition over time. UAS measurements will be helpful for assessing IRL dolphins entangled in fishing gear as well as body measurements comparison following disentanglement efforts. Future studies should aim to collect UAS footage from different areas of the lagoon to assess if there are differences in body condition based on location. Since capture-release methods are typically limited in comparing dolphin health seasonally, UAS measurements will also enable seasonal comparison for IRL dolphins.

Overall, the success of this study provides a solid foundation for future studies to build upon. Using UAS to assess bottlenose dolphin body condition has proven to be a reliable method with a wide variety of implications. UAS can be utilized not only to assess body morphometrics, but provide a vantage point to evaluate foraging, mating, maternal care behavior, as well as the social structure of IRL dolphins.

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Tables and Figures

Table 1: Standardized method and criterion for determining body condition index from lateral images (Fair et al., 2006).

	Body Condition Score	**1**	**2**	**3**	**4**	**5**
Body Area	**Description**	**Emaciated**	**Underweight**	**Ideal**	**Overweight**	**Obese**
Head	Nuchal crest	depressed	slightly depressed	flat	slight mid-dorsal indentation	mid-dorsal indentation
Head	Cervical region (lateral)	concave	mild concavity	flat	broad	broad
Head	Prominent facial bones	exposed	slightly exposed	not observed	not observed	not observed
Head	Ear os	exposed	no dimpling	slight dimpling	dimpled	significantly dimpled
Head	Chin skin folds	not present	not present	not present	present	present
Body	Epaxial muscle definition	concave	slightly concave	flat	convex	convex
Body	Dorsal ridge of scapula	exposed	slightly exposed	not observed	not observed	not observed
Body	Ribs	exposed	slightly exposed	not observed	not observed	not observed
Tail	Transverse processes	exposed	slightly exposed	not observed	not observed	not observed

Table 2: UAS measurements of Indian River Lagoon bottlenose dolphins including total length (TL) (cm), body width measurements taken at the blowhole, post-nuchal region, axillary, and maximum (anterior to the dorsal fin) (cm), the calculated Body Area Index (BAI), estimated mass (kg) from Model 1, and estimated Body Mass Index (BMI) by age class, sex, and sample size. Each measurement has the mean ± standard deviation reported.

UAS Measurements									
Class	Sample Size	Total Length (cm)	Blowhole Width (cm)	Postnuchal Width (cm)	Axillary Width (cm)	Maximum Width (cm)	BAI	Mass (kg)	BMI
Lactating Females	8	242 ± 10.68	25 ± 1.63	27 ± 1.73	34 ± 2.43	35 ± 3.85	25.81 ± 2.69	156.53 ± 17	0.0027 ± 0.0001
Females	2	248 ± 7.78	25 ± 3.54	30 ± 0.71	36	39 ± 4.24	27.16 ± 0.09	166.54 ± 12.85	0.0027 ± 0.0000
Males	9	256 ± 11.05	26 ± 1.66	31 ± 1.64	36 ± 38	36 ± 4.82	25.47 ± 3.33	188.12 ± 21.5	0.0028 ± 0.0001
Unknown Sex Adults	1	254	28	30	34	39	26.97		
Female Juveniles	2	225 ± 12.02	24 ± 1.41	27 ± 1.41	34 ± 2.12	35 ± 2.12	27.49 ± 2.09	131.26 ± 17.23	0.0026 ± 0.0001
Male Juveniles	4	214 ± 9.46	24 ± 1.89	27 ± 2.08	33 ± 1.73	32 ± 2.99	27 ± 0.61	115.64 ± 13.66	0.0025 ± 0.0001
Unknown Sex Juveniles	3	210 ± 9.45	23 ± 2.08	24 ± 0.58	30 ± 1.73	32 ± 2.08	28 ± 1.2		

Table 3: Ratios of body width (cm) measurements to total length (TL) (cm) ratios calculated at blowhole width (BHW/TL), post-nuchal width (PNW/TL), axillary width (AXW/TL), and maximum width (MAXW/TL) for age class and sex and sample size for Indian River Lagoon bottlenose dolphins. Each measurement has the mean ± standard deviation and range reported.

		Body Width/Total Length Ratios			
Class	Sample Size	BHW/TL (cm)	PNW/TL (cm)	AXW/TL (cm)	MAXW/TL (cm)
Lactating Females	8	0.101 ± 0.008 (0.083-0.112)	0.111 ± 0.009 (0.99-0.125)	0.140 ± 0.012 (0.121-0.164)	0.146 ± 0.015 (0.132-0.181)
Females	2	0.099 ± 0.017 (0.087-0.111)	0.118 ± 0.001 (0.118-0.119)	0.145 ± 0.004 (0.142-0.148)	0.156 ± 0.012 (0.148-0.165)
Males	9	0.101 ± 0.008 (0.088-0.115)	0.12 ± 0.008 (0.109-0.131)	0.141 ± 0.014 (0.119-0.160)	0.142 ± 0.021 (0.115-0.181)
Unknown Sex Adults	1	0.11	0.118	0.134	0.154
Juveniles	9	0.109 ± 0.008 (0.098-0.123)	0.120 ± 0.008 (0.111-0.133)	0.149 ± 0.008 (0.137-0.161)	0.151 ± 0.006 (0.138-0.160)

Table 4: Comparison of nonlinear OLS regression models used to estimate the mass (kg) based on total length measurements (Model 1) and estimate girth measurements (Model 2) for the seven Indian River Lagoon bottlenose dolphins that had estimated girth measurements. Coefficient of variation (%) are given for each pair of estimated mass along with the range of estimated BMI calculated based on estimated mass given by the two models, and Lateral Body Condition Score (BCS) for each animal.

Mass Models Comparison					
Sample ID	Mass (kg) Length Model (Model 1)	Mass (kg) Girth Model (Model 2)	CV (%)	BMI Range	Lateral BCS
2	223	239	5	0.0030-0.0032	Ideal
6	119	127	5	0.0025-0.0027	Underweight
11	116	124	5	0.0025-0.0027	Underweight
13	101	101	0	0.0024	Underweight
15	164	201	14	0.0027-0.0034	Underweight
27	143	146	2	0.0026-0.0027	Underweight
28	192	239	15	0.0029-0.0036	Ideal

Table 5: Precision of measurements for Indian River Lagoon bottlenose dolphins that were measured in more than one image (*n* =22). Coefficient of variation were calculated (%) for each individual and averaged across each category of measurement.

Precision of Measurements							
Age Class	no. of dolphins with 2 images	TL	BHW	PNW	AXW	MAXW	SA
Adult Male	7	0.9	8.5	3.0	8.0	9.4	7.2
Adult Female	2	1.4	5.9	3.6	3.8	7.2	3.0
Lactating Female	6	1.8	6.5	5.3	3.1	2.5	3.6
Juvenile	7	0.9	4.3	4.9	3.5	3.7	4.4

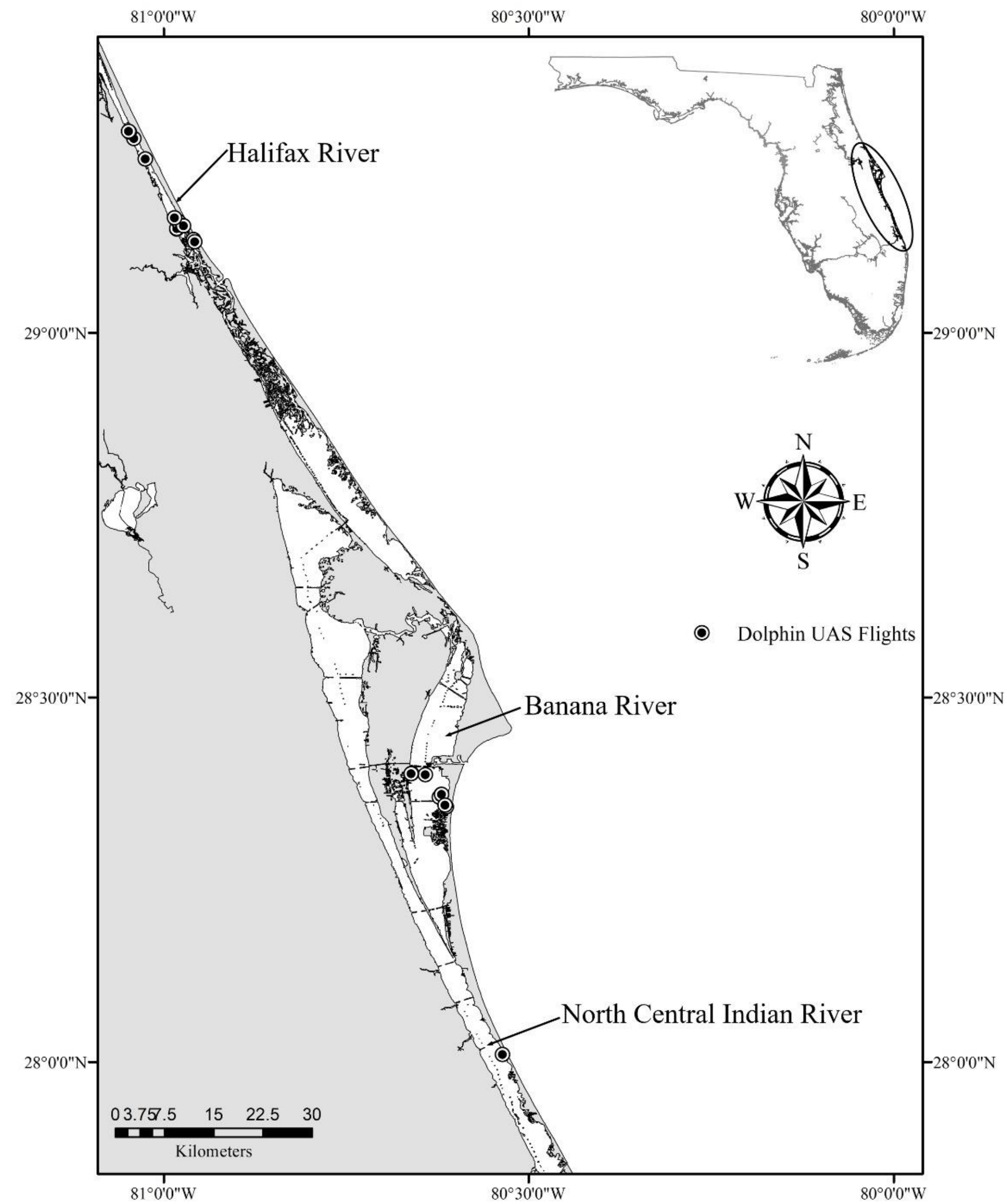

Figure 1: Inset map of Florida illustrating the Indian River Lagoon encircled along the east coast of Florida. The main map depicts the Indian River Lagoon with sub-basins labeled and points indicating where UAS flights occurred.

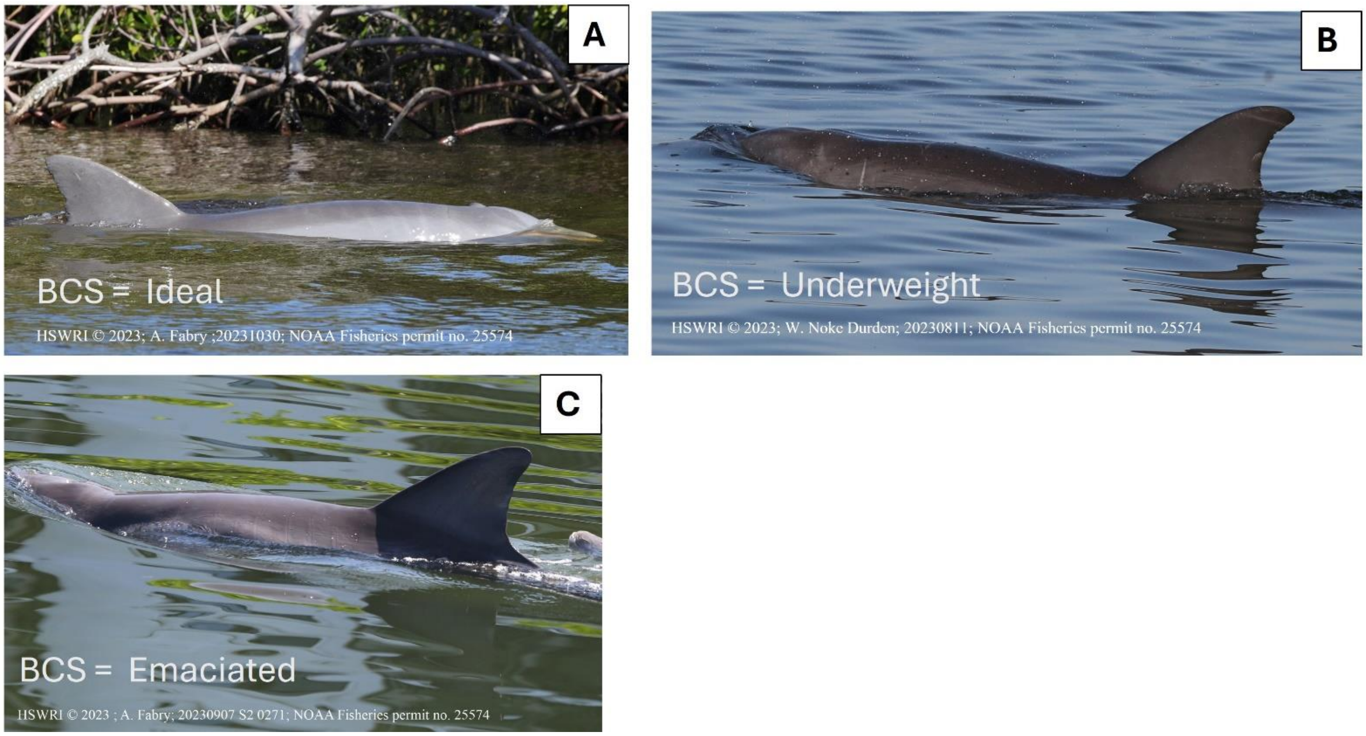

Figure 2: Examples for each body condition category based on lateral images of Indian River Lagoon bottlenose dolphins: A. Ideal, B. Underweight, C. Emaciated.

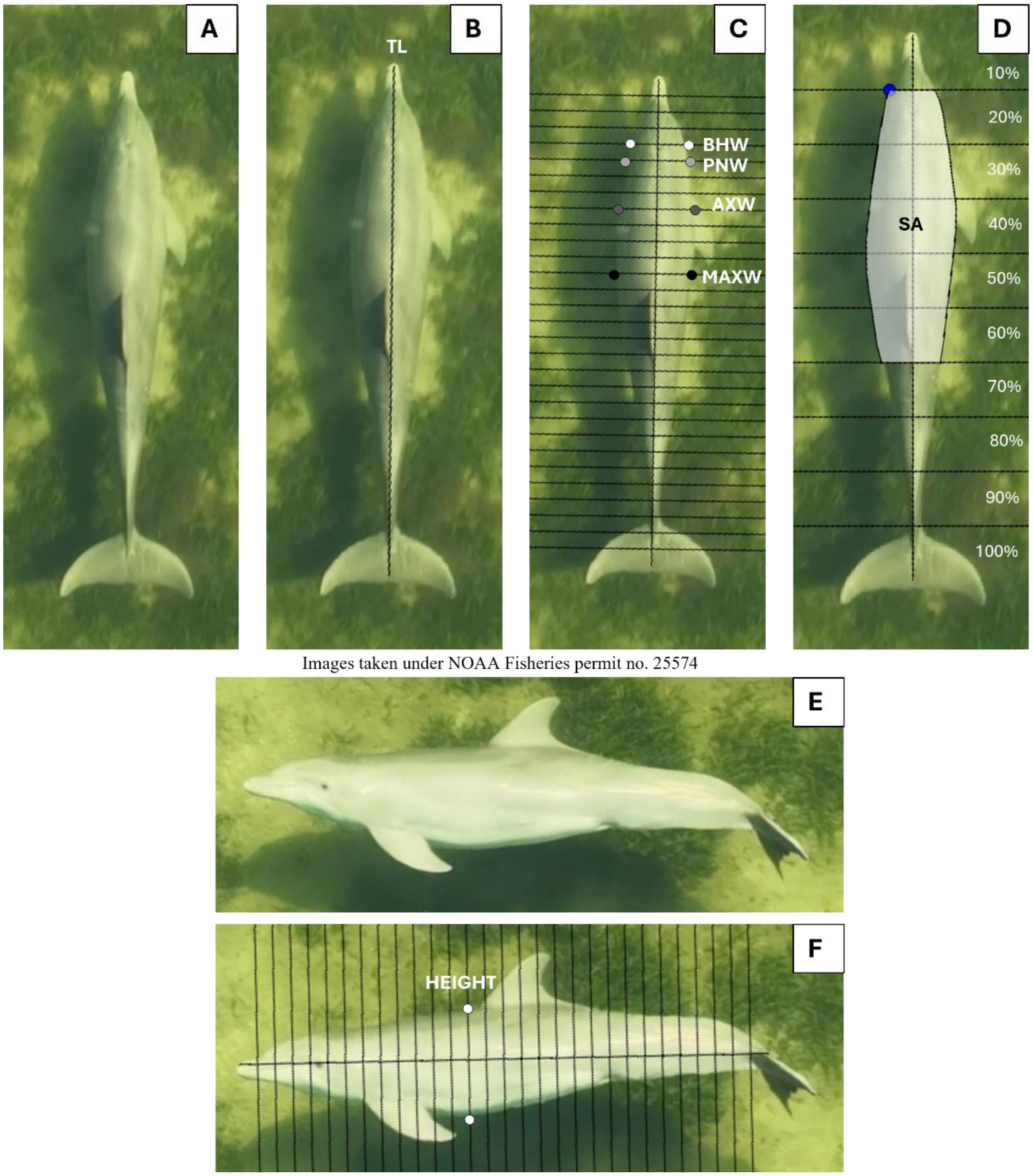

Figure 3: Examples of photogrammetry measurements in MorphoMetrix of the same individual. A and E are the original images from the UAS video. B. Total Length (TL) from tip of the rostrum to notch of the fluke. C. Width measurements from top to bottom: Blowhole Width (BHW; white dot), Post-nuchal Width (PNW; light grey dot), Axillary Width (AXW; dark grey dot), and Maximum Width (MAXW; black dot). D. Surface Area (SA) at 50% of TL, between 20% and 60%. F. Height just anterior to dorsal fin at the same location as MAXW. Images taken under NOAA Fisheries permit no. 25574.

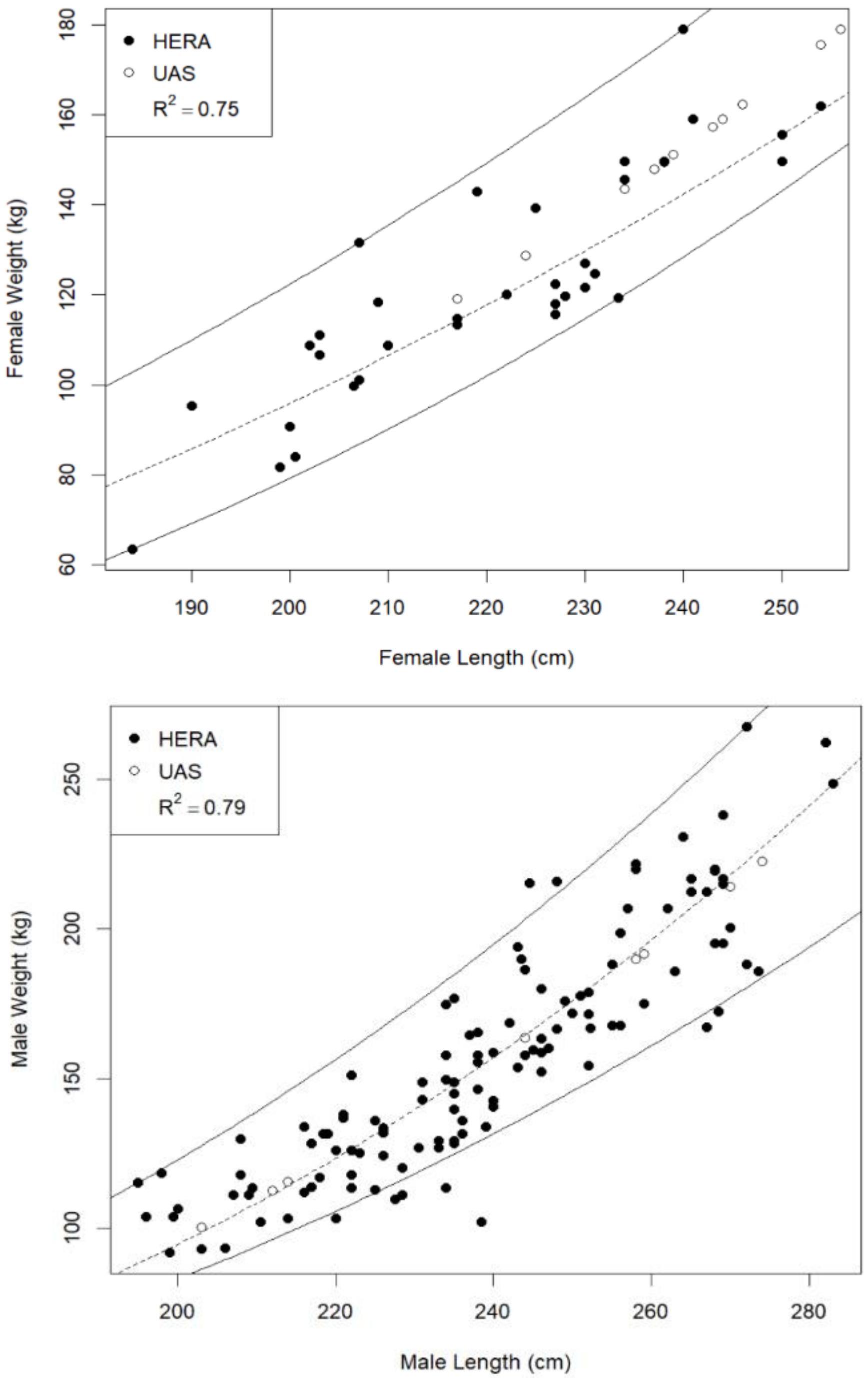

Figure 4: Nonlinear OLS regression models with 95% reference ranges for females and males based on historical HERA assessments used to estimate mass from total length UAS measurements for Indian River Lagoon dolphins (model 1). Closed circles: HERA dolphins (36 females, 119 males); Open circles: UAS dolphins (12 females, 13 males); Solid lines: upper and lower 95th quantiles; Dotted line: median quantile.

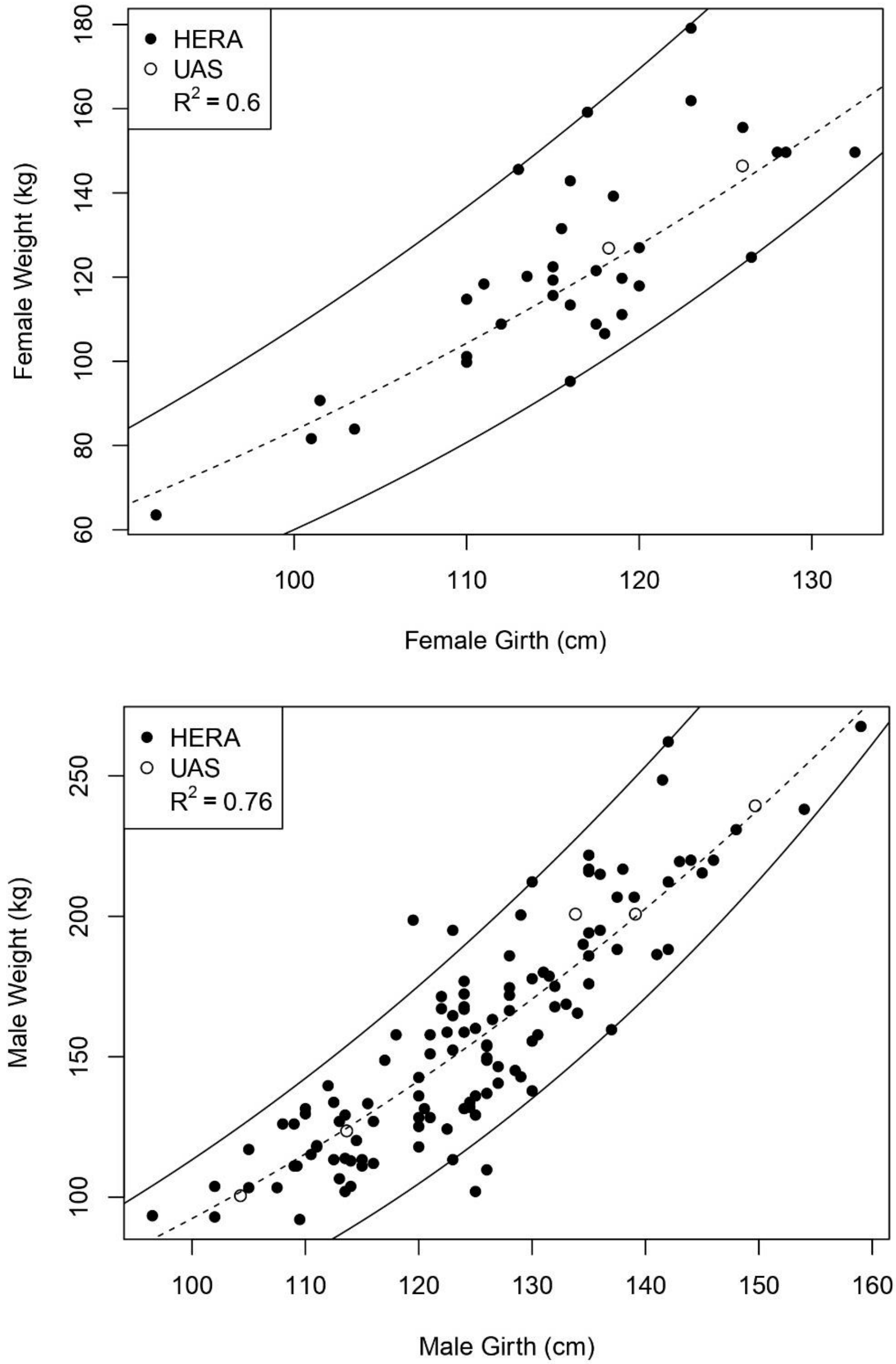

Figure 5: Nonlinear OLS regression models with 95% reference ranges for females and males based on historical HERA assessments used to estimate mass from estimated girth UAS measurements for Indian River Lagoon dolphins (Model 2). Closed circles: HERA dolphins (36 females, 119 males); Open circles: UAS dolphins (2 females, 5 males); Solid lines: upper and lower 95th quantiles; Dotted line: median quantile.

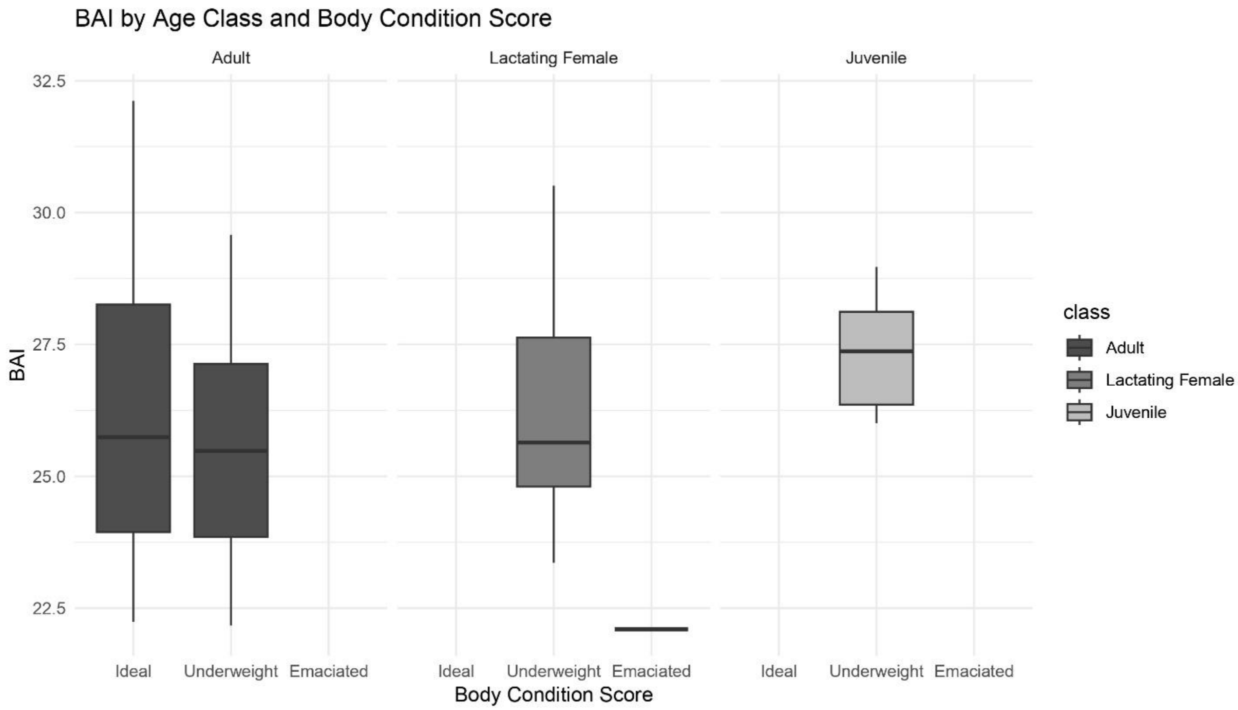

Figure 6: Body Area Index (BAI) for each Body Condition Score (Ideal, Underweight, Emaciated) by age class (Adult, Lactating Female, Juvenile) for Indian River Lagoon bottlenose dolphins (*n* = 29). BAI values did not differ significantly across age class (p = 0.314) or body condition score (p = 0.232) and did not differ among body condition score within each age class (Adult: p = 0.753; Lactating Female: p = 0.151).

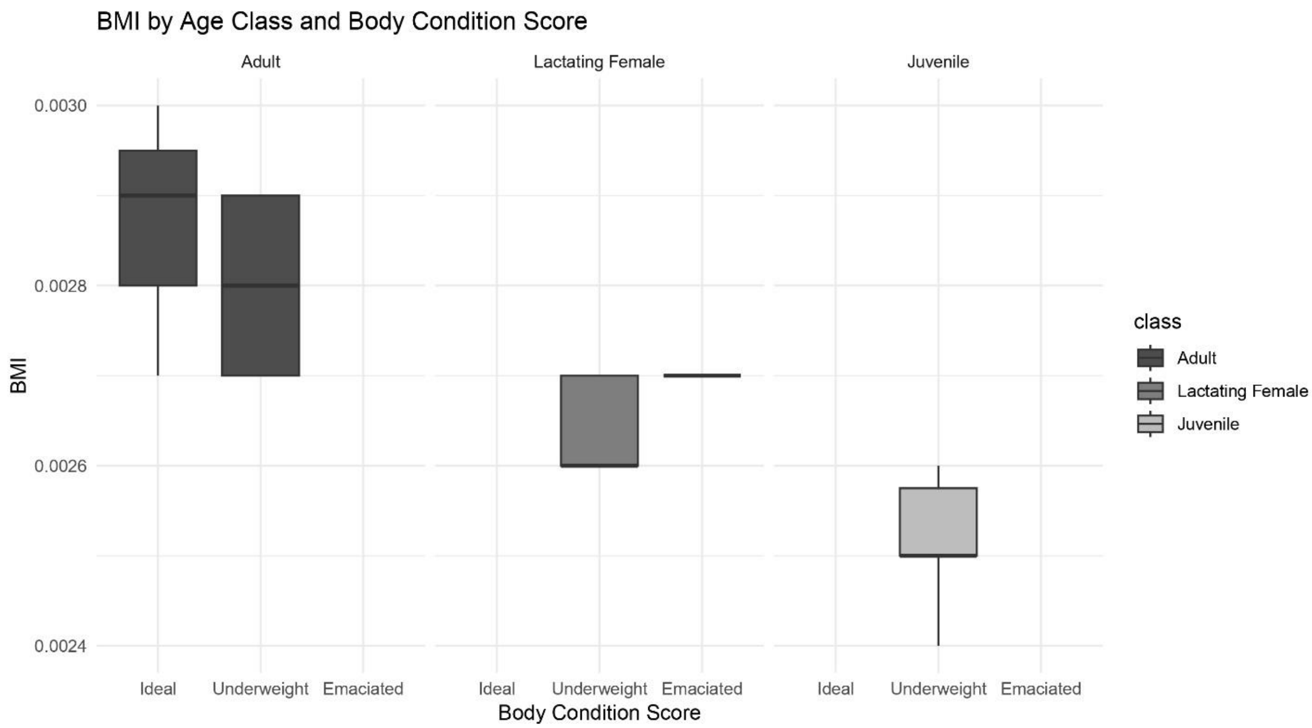

Figure 7: Estimated Body Mass Index (BMI) based on Model 1 mass estimates for each body condition score (Ideal, Underweight, Emaciated) within each age class (Adult, Lactating Female, Juvenile) for Indian River Lagoon bottlenose dolphins with determined sex (*n* = 25). Lactating females had significantly lower BMI compared to adults (adjusted p = 0.033) and significantly higher BMI compared to juveniles (adjusted p = 0.0072). Juveniles had significantly lower BMI compared to adults (p-adjusted = 0.0001). Body condition score did not differ significantly within each age class (Adult: p = 0.287.; Lactating Female: p = 0.935).

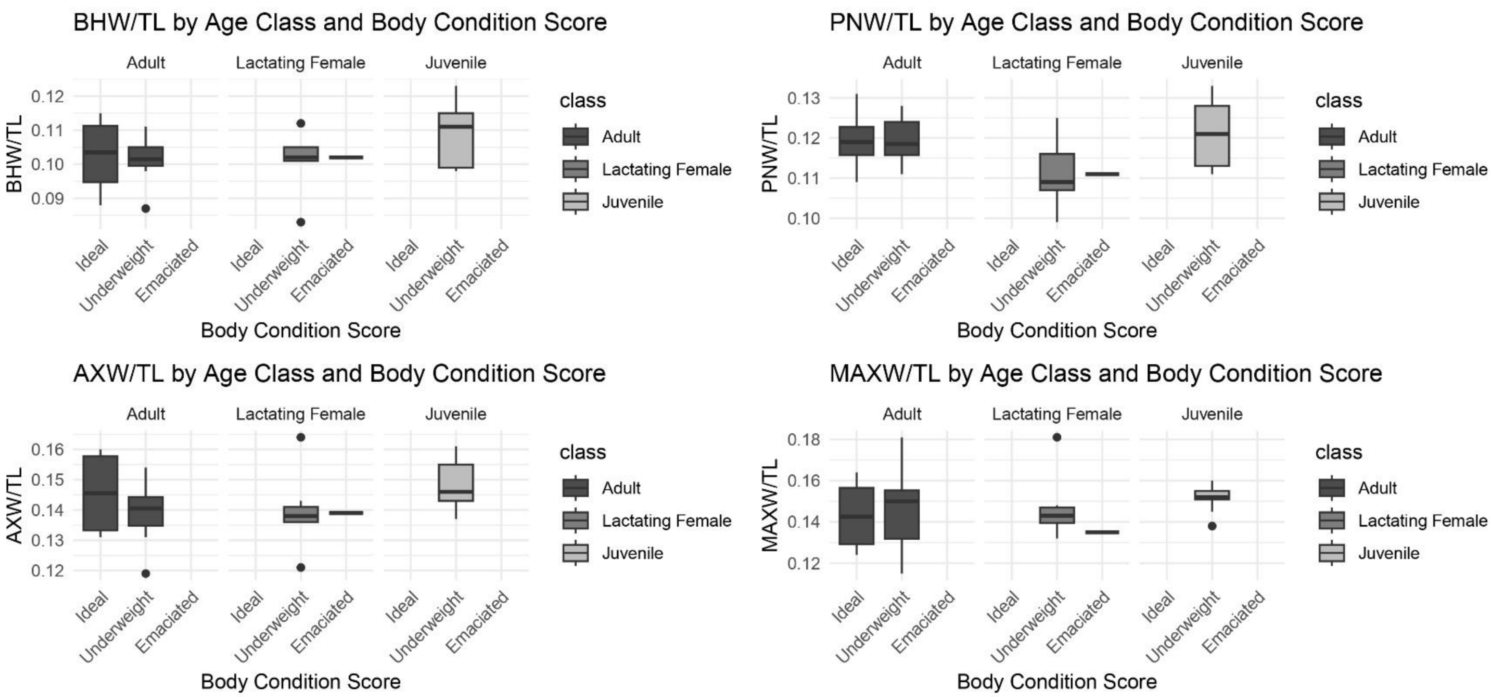

Figure 8: Width to total length ratios: Blowhole Width to Length (BHW/TL), Post-nuchal Width to Length (PNW/TL), Axillary Width to Length (AXW/TL), and Maximum Width to Length (MAXW/TL) for each body condition score (Ideal, Underweight, Emaciated) and within each age class (Adult, Lactating Female, Juvenile) for Indian River Lagoon bottlenose dolphins (n = 29). Ratios were not significantly different across age class or within body condition score except for PNW/TL which differed significantly only across age class ($p = 0.042$).

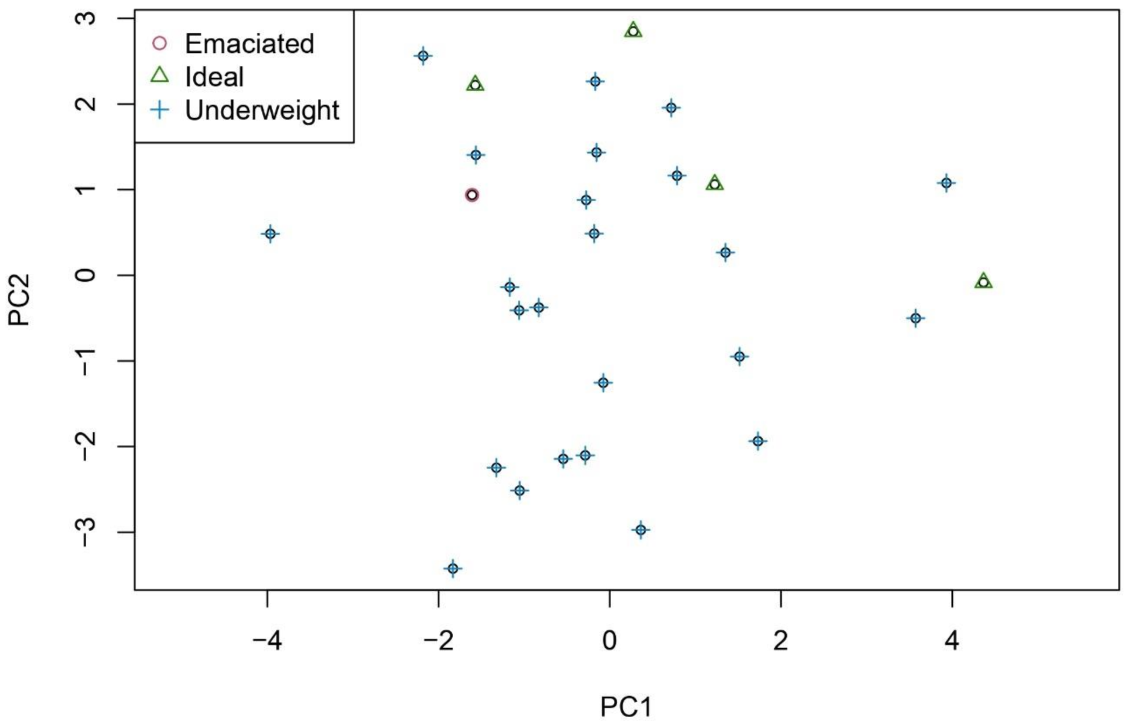

Figure 9: Output from the Principal Component Analysis (PCA) for PC1 and PC2 conducted on all measurements for 29 Indian River Lagoon Dolphins. Symbols represent lateral body condition scores; circles: emaciated, triangles: ideal, plus: underweight.

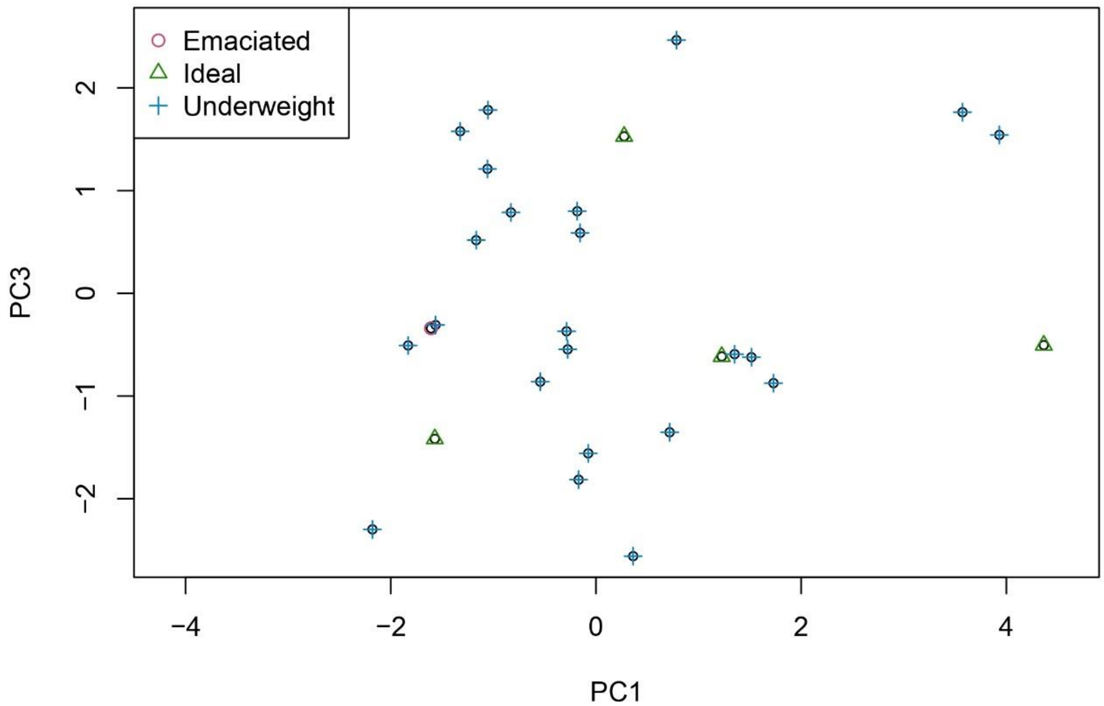

Figure 10: Output from the Principal Component Analysis (PCA) for PC1 and PC3 conducted on all measurements for 29 Indian River Lagoon Dolphins. Symbols represent lateral body condition scores; circles: emaciated, triangles: ideal, plus: underweight.

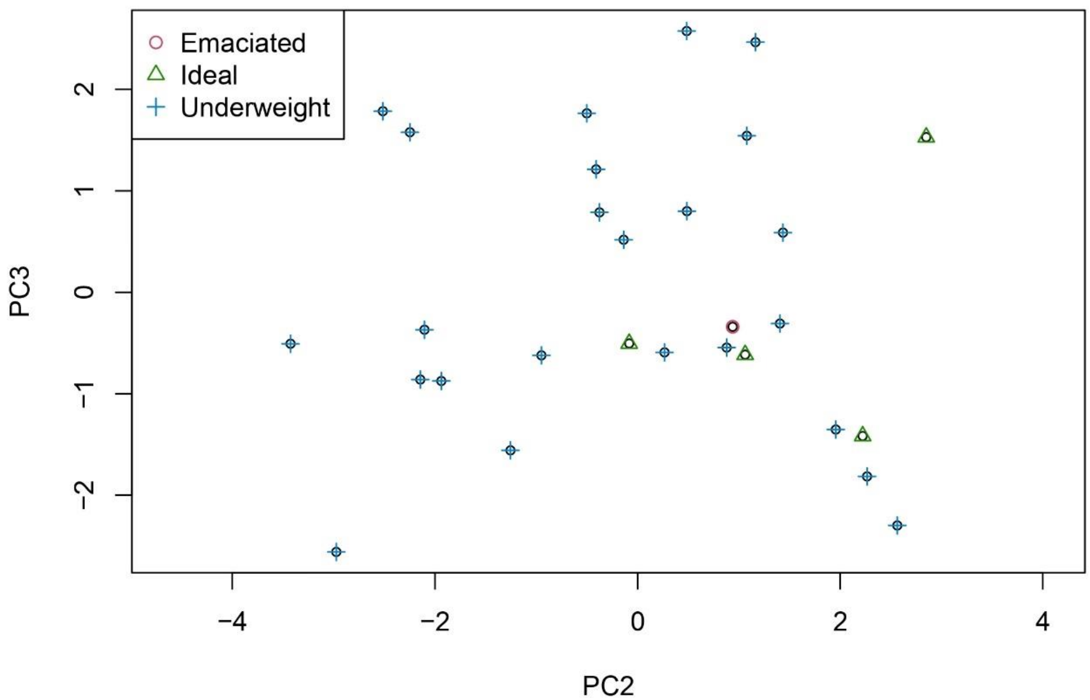

Figure 11: Output from the Principal Component Analysis (PCA) for PC2 and PC3 conducted on all measurements for 29 Indian River Lagoon Dolphins. Symbols represent lateral body condition scores; circles: emaciated, triangles: ideal, plus: underweight.