Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Evaluation of body condition of resident bottlenose dolphins (Tursiops truncatus) in the Sado region using Unmanned Aircraft Systems and photogrammetry

Angela Maria Garcia Castilla

Orientador(es) | Manuel Dos Santos
Eduardo Nuno Barata
Miguel Luca Augusto Grilo

Évora 2023

Esta dissertação não inclui as críticas e as sugestões feitas pelo júri.

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Table of Contents

Avaliação da condição corporal dos Golfinhos Roazes (*Tursiops truncatus*) na região do Sado utilizando Sistemas de Veículos Aéreos Não Tripulados (VANTs) e Fotogrametria

Resumo

A fotogrametria é uma tecnologia pela qual medições precisas podem ser obtidas a partir de fotografias. Pode ser uma técnica confiável para avaliar a condição corporal em cetáceos quando certos parâmetros da câmera são conhecidos, e os ângulos apropriados e as posições dos animais são capturados (Burnett et al., 2019). Em Portugal, uma população filopátrica de golfinhos-roazes (Tursiops truncatus) está estabelecida no estuário do Sado (Setúbal), onde medidas especiais de conservação estão em vigor. O estudo seguinte teve como objetivo avaliar a condição corporal dos indivíduos desta população através de fotografia de veículo aéreo não tripulado (VANT) e técnicas de fotogrametria com base nas metodologias de Christiansen et al. (2018, 2019). As imagens foram obtidas com um drone DJI Phantom 4 e um medidor de alcance a laser SF11/C voado a uma distância mínima de 10 m dos golfinhos. Os golfinhos foram categorizados em classes de idade (adultos, sub-adultos, juvenis, crias) e sexo para uma avaliação mais aprofundada do modelo. O modelo calculou o comprimento total (TL) e o volume corporal (BV) de cada sujeito medido durante o inverno e o verão. O presente estudo não encontrou evidências de dimorfismo sexual nesta população de golfinhos-roazes, enquanto diferenças significativas foram encontradas entre categorias de idade dos 18 animais amostrados. Inesperadamente, o volume corporal foi maior no verão do que no inverno, desafiando as expectativas de uma composição de gordura mais espessa durante a última estação para isolamento térmico, o que poderia indicar variabilidade na disponibilidade de alimentos. O presente estudo validou a eficácia do modelo (R2 = 0,9253) e destaca suas possíveis contribuições para futuras estratégias de conservação e empreendimentos de pesquisa.

Palavras-chave: *Fotogrametria, Condição Corporal, Tursiops truncatus, veículos aéreos não tripulados (VANT).*

Evaluation of body condition of resident bottlenose dolphins (Tursiops truncatus) in the Sado region using Unmanned Aircraft Systems and photogrammetry

Abstract

Photogrammetry is a technology through which precise measurements can be obtained from photographs. It can be a reliable technique to evaluate body condition in cetaceans when certain parameters of the camera are known, and the appropriate angles and positions of the animals are captured (Burnett et al., 2019). In Portugal, a philopatric population of bottlenose dolphins (Tursiops truncatus) is settled into the Sado estuary (Setubal) where special conservation measures are in place. The following study aimed to evaluate the body condition of the individuals in this population through unmanned aircraft systems (UAS) photography and photogrammetry techniques based on Christiansen et al. (2018, 2019) methodologies. Images were obtained with a DJI Phantom 4 drone and a SF11/C laser range finder flown at a minimum distance of 10 m from the dolphins. The dolphins were categorized in age (adults, sub-adults, juveniles, calf) and sex classes for further evaluation of the model. The model calculated total length (TL) and body volume (BV) of each subject measured during winter and summer. The present study found no evidence of sexual dimorphism in this bottlenose dolphin population, while significant differences were found among age categories of 18 animals sampled. Surprisingly, body volume was higher in summer than winter, defying expectations of thicker blubber composition during the latter season for thermal insulation, which could indicate variability in food availability. The present study validated the effectiveness of the model (R^2 = 0.9253) and highlights its possible contributions for future conservation strategies and research endeavors.

Keywords: *Photogrammetry, Body Condition, Tursiops truncatus, unmanned aerial systems (UAS).*

Preface and Background

CHAPTER 1 – Health monitoring in wild cetacean populations

1.1. The importance of wildlife health assessment in conservation

Globally, wildlife populations and ecosystems are facing different challenges due to climate change and anthropogenic pressures, including the effects of invasive species on trophic chains, pollution, and resource overexploitation. These could negatively affect wildlife health, increasing disease prevalence and interfering with the ecological roles of wildlife, hampering ecosystem functioning and conservation (Kophamel *et al.,* 2022). Therefore, comprehension of ecosystem health is necessary to restore wildlife populations, habitats, and the ecological processes involved (Buttke *t al.,* 2015). This could require engaging different disciplines and roles, according to the "One Health" concept. This concept recognizes that human health is linked to the health of animals and the environment (Buttke *et al.,* 2015), leading to a unified goal. Furthermore, health monitoring in wildlife populations and conservation medicine has been proposed as an area of great concern for conservation matters, in need of further strategic investigation and management frameworks (Delgado *et al.*, 2022). A population with decreased fitness can present altered parameters, such as reproductive performance, biologic development, and immunological response, which could lead to biodiversity loss and modification of ecological processes within the ecosystems due to inability to cope with alterations (Reyes *et al.,* 2006). In consequence, diligent action is required on the use and appliance of all the tools available in this field to ensure the effectiveness of the strategies. In terms of epidemiology, outputs generated by wildlife surveillance can improve the prediction of new disease events; inform about specific diseases or infections; and identify the distribution of diseases present in a population. Once a health problem is detected, further activities can be performed to handle the situation, including information management, analysis of the collected data and use of the surveillance information for decision-making and policy formulation (Ryser-Degiorgis, 2013).

However, infectious surveillance is not the only approach for wildlife health monitoring, and its definition should emphasize that health is the result of biological, social, and environmental interactions together with characteristics of the animals and their ecosystem that could affect their vulnerability and resilience (Decker, 2016). Conservation Medicine is a science that brings together the different points of view towards a health perspective and integrates an approach of species and ecosystems to further contribute to conservation health management. For this reason, it is crucial to evaluate the effects of ecosystems and environmental variables over the species as an aid for decision making in terms of wildlife conservation (Reyes *et al*., 2006).

Monitoring cetaceans' health is important not only for specific species, but to evaluate the overall health of marine ecosystems. Gathering information on the health parameters of sentinel species could help identify and measure the impact of pathogens and anthropogenic stressors such as pollutants in the marine environment (Fossi & Panti, 2017). Previous studies in this field have detected important infectious agents (e.g. morbilliviruses and *Brucella* spp) and their potential to impact cetacean populations (Bressem *et al.,* 2009). To conduct these evaluations, various common methodologies have been applied so far including blood analysis, body composition, blow samples, tissue biopsy, ultrasound, physical examination and fecal analysis. This stresses the importance of targeted, collaborative, and high-quality research efforts that could contribute to conservation endeavors (Moore *et al.,* 2021).

1.2. Monitoring live animals with minimum invasive methodologies to evaluate the health status of wild cetacean populations.

Multiple non-invasive parameters in free-range marine mammals can serve as indicators to determine the health status of cetacean populations. For example, reported signs of wounds, disintegrating skin, lumps in blubber and unusual odor in the abdomen are suggestive for infection (Castrillon & Bengtson, 2020), which could be a generalized pathology within a group. Moreover, health characterization of whale populations has been assessed based on their external appearance, and it has also been suggested that body condition may be useful as a viable indicator of food depletion or stress within a population, potentially leading to immune suppression in mammals (Burek *et al.* 2008). These are just a few examples of parameters that can provide us with crucial information about marine life and cetaceans' overall health.

Although it is essential to continue expanding our understanding in this field, it is important to be mindful of the potential environmental impacts that may arise from the research methodologies. Awareness on this matter could prevent harming the ecosystems and their animal populations. In consequence, the use of non-invasive methodologies is gaining popularity and should be applied to avoid disturbance of the subjects of study. Observation has been the method of choice for many years, and it has been used to evaluate many behavioral aspects of wild populations, and even to determine the composition of groups in cetaceans. With time, many methodologies have been developed to aid the researchers on a more detailed analysis, leading to strategies such as aerial photography and photo identification, which is now one of the most powerful non-invasive tools in wild cetacean populations (Augusto, 2007).

CHAPTER 2 – Evaluating body condition in cetaceans

2.1. Body condition

The concept of body condition holds significant relevance in different fields such as behavior, evolution, and conservation (Cooke *et al.,* 2023). It also comprises numerous variables related to health status, such as nutrition, immune response, and even hormonal balance. It is often represented or obtained through body mass, volume, and fat storage (Peig & Green, 2010). The measurement of blubber thickness is one of the many methodologies that have been considered suitable for body condition analysis and to infer the nutritional status of marine mammals (Siebert *et al.,* 2022). The variety of methodologies available to evaluate body condition reflects that this is a concept that should be seen on a broad perspective at the time of evaluating each case.

The body condition of an animal can be expressed by any variable that captures the energy reserves of the individual, independently from its structural body size (Christiansen *et al.* 2020). It has proven to be useful to analyze the drivers of a population change, and its indices (BCIs) have been used by ecologists to estimate the nutritional state of an animal including fitness traits as relative fat reserve (Stevenson & Woods, 2006), which plays an important role at physiological and metabolic processes. BCIs generally use a measure of body mass standardized by a linear measure of the body size to help differentiate individuals of the same length but different masses (Hodgson *et al.,* 2020). Although there are different BCIs, many of them can be obtained through external characteristics such as body mass, volume, girth, and width, which are often represented with a ratio of body length (Christiansen *et al.* 2020).

When assessing the body condition of mammals, it is taken into consideration the overall health of groups, dietary patterns, and level of physical fitness. It could be inferred that a mammal with a healthy body condition is one that receives an adequate supply of nutrients and maintains a desirable physical fitness. Conversely, an animal with a poor body condition might be reflecting impairments such as malnutrition, diseases, or other underlying health problems (Cunningham *et al*., 2021). Environmental conditions can also be inferred from body condition evaluation. Assessments of nutritional status have been used in marine mammals as a proxy to understand the influence of environmental fluctuations on energy reserves of each animal. This has been considered a suitable tool to identify changes in the food supply (Siebert *et al.,* 2022). Therefore, an understanding and monitoring of this field is of paramount importance for conservation efforts and enables researchers to assess not only the animal's overall health, but the general status of a population and its surrounding environment.

2.2. Body condition as a health indicator in cetaceans

The evaluation of body condition in wild cetaceans is of great value for their conservation because it reflects their fitness (Castrillon & Bengtson, 2020). It is believed that the mass of an animal can influence aspects such as metabolic rate, food requirements, growth, fasting endurance, thermoregulation, and foraging capacity, among others (Christiansen *et al.,* 2018). According to Pettis *et al.* (2004), body condition is an optimum health indicator because it is related not only to the wellbeing of the individuals, but also to their reproductive success, suggesting that body condition is linked to marine mammals' survival. Likewise, there are many other parameters that could be evidenced by body condition research. For instance, since lactation represents a high energetic demand, studies in this area can demonstrate that the body condition can be compromised during the early months of lactation when the endogenous nutrient reserves are required to support the energy expenditure (Claridge *et al.,* 2015).

It has also been shown that the comparison of trends in body condition between different reproductive, gender and age classes can provide valuable information regarding energy acquisition and expenditure at different time periods (Christiansen *et al.,* 2016). These trends can be represented as changes in body condition between the different members of a group. For example, in cetaceans, immature individuals allocate a significant amount of energy towards growth rather than building fat reserves, which is the case for adults (Castrillon & Bengtson, 2020). In consequence, understanding about the variation of body condition among the individuals of a population, and how it can influence the demographic processes, is extremely important to predict the population's fitness (Kershaw *et al.,* 2017).

Furthermore, body condition is a parameter that can supply information about the environment's status and stressors. The use of unmanned aircraft systems (UAS) for monitoring the body condition and growth of beaked whales has been reported to facilitate an understanding of the effects of disturbance of this species in navy operation areas (Claridge *et al.,* 2015). Also, the Japanese Whale Research Program, under Special Permit in the Antarctic (JARPA), has evidenced an apparent decline in body condition of harvested animals over two decades, attributed to reduced krill availability (Konishi *et al.,* 2008). Some other studies suggest that the manifestation of poor body condition could be related to scarce alimentary resources and anthropogenic stressors such as fishing gear pollution (Hart *et al.,* 2013). This shows an intricate relation between an animal's mass, its dietary resources, and its overall condition that can be reflected in its blubber structure. The blubber is a vascularized tissue composed by adipocytes and collagen that serves as an insulation to surrounding water of cold-water temperature and influences the dolphin's buoyancy. The blubber is metabolically active and can be mobilized during nutritional stress, which is one of the reasons why this structure represents a good part of body composition. It is very relevant at the time of evaluating body condition (Adamczak *et al.* 2021).

2.3. Cetacean body condition analysis through non-invasive methodologies

Invasive wildlife sampling often compromises the welfare of the animals being studied. To alleviate this situation, the development and application of non-invasive methodologies is required (Zemanova, 2020).

Body condition can be evaluated through a variety of non-invasive methodologies including stranding research, observation, photographic analysis, and photogrammetry.

In cetaceans, physical body condition evaluation is possible through observation of external characteristics including shape of the whale; presence of rolls or lateral folds; scars; wounds; markings; and color (Harrington *et al.,* 2005). Wasting of epaxial musculature, determined by the concavity or convexity ventrolateral to the dorsal fin, has also been analyzed in common dolphins by visual observation (*Delphinus delphis*) (Joblon *et al.,* 2014). Observation, however, might not provide sufficient data, which is why marine mammals have also been studied through the analysis of stranding data, including morphological measurements (Castrillon & Bengtson, 2020).

To further contribute towards a more profound analysis of the information gathered, some technologies are being applied for the understanding of body condition in wild animals. Ultrasound, for example, has provided an alternative way to measure blubber thickness, which has been shown to correlate with direct measurements and that allows to assess live animals. In stranded animals, it provides fast and valuable information on the distribution and structure of fat according to the species (Zeng *et al.,* 2015). Nevertheless, novel technologies are required for a more profound analysis in wild populations and live animals in their natural environment. Drone photography analysis gives nowadays another image perspective that couldn´t be obtained from deceased animals or to be accessed through traditional photography. Aerial drone imaging can also lead to other procedures such as photogrammetry, a technique that can provide useful and precise information to evaluate the animal's body condition, and even allowing the determination of parameters such as growth rates. For example, Christiansen *et al.* (2018) used repeated photogrammetry measurements of southern right whales via UAS to identify growth rates of calves in relation to the body size and condition of their mother. Due to this and other applications, drone photography and photogrammetry have started to grow as a non-invasive methodology and seems promising for wildlife monitoring and body condition assessment (Dimauro et al., 2022).

While providing new research opportunities, it is possible that drones (UAS) may disrupt the natural behaviors of marine wildlife and have negative impacts on individuals. Consequently, regulations to control their use have been legally established and it is important to evaluate the preventive measures to avoid any collateral damage to the study populations (Mulero-Pázmány *et al*., 2017). In the case of dolphins, UAS presence and noise can alert the animals when near the water surface (Christiansen e*t al*., 2016). Therefore, to avoid behavioral distress and to ensure success in quality image collection, it is important to set minimum distances for the UAS to be flown above the animals. A 10m altitude distance has been established as appropriate for bottlenose dolphins, although they might show response at altitudes between 11 and 30m when alone or in small groups (Ramos *et al.*, 2018).

CHAPTER 3 - Photogrammetry

3.1. General aspects of photogrammetry

Photogrammetry has been defined as the science of measuring in photos and it is an engineering discipline heavily influenced by developments in computer science and electronics which are in constant change (Linder, 2009). Nowadays, it is a computerized process that produces spatial accuracy from photographs. Data acquisition with photogrammetry is obtained through reliable information from surfaces and objects, which is accomplished without physical contact with the objects or organisms, making it easier and less invasive for research in wildlife fauna. Once the relevant data is obtained, it can be grouped into categories, where geometric information involves the spatial position and the shape of the objects (Schenk, 2005). Information collected through photogrammetry is obtained in pixel size and needs to be transformed from pixels to the actual size of the photographed object. The pixel is the smallest unit of an image and its value in terms of data is a shade of a particular image location. These units are often converted to meters through different formulas (Schenk, 2005).

Additionally, when working with photogrammetry with living beings, information obtained from the same object in different periods of time requires an analysis related to its changes in a specific time lapse, which is denominated temporal information. Once the measurements are obtained, the temporal information data can be analyzed through allometry. Allometry evaluates the changes in relative dimensions of parts of the body that are correlated with changes in overall size and often evaluates changes through time and growth (Gayon, 2000).

These concepts are relevant and important for the understanding of the photogrammetry methodologies and the analysis of body condition.

3.2. Photogrammetry systems and methodologies

There are three general types of photogrammetry: aerial (including plans, maps, and models); field photogrammetry (used *in-situ* objects, among others); and lab photogrammetry (to measure simple objects and artifacts). The basic task of any of the methodologies is the mathematical collection of information from the images of a scene, followed by the inspection of single-perspective images, image pairs, image triplets and blocks of several images (Hellwich, 2008). What normally happens is that information of 2D images from frame cameras is converted to 3D objects' measurements given in pixels (Hellwich, 2008). Since most photogrammetry methods will result in pixel measurements, a conversion from pixels to ground distance can be performed having the values of the focal length of the lens and the pixel width. These can be calculated from the camera sensor size and the sensor resolution. This is possible because a straight-line distance of an object in a photograph can be calculated if the lens's focal length and the distance from the lens to the object are known (Allan, 2019). It is then assumed that a single projection ray of the camera can lead to a measurable image point to infer 3D information (Allan, 2019) (figure 1). To do so, a geometric model of projection for image points should be formulated through specific programs, according to the shape of the object and characteristics of the image. This allows the projection process to infer spatial direction to 3D points from their observed images.

In whales, this conversion has been performed by estimating a proportion of the relative size of the animal in the photographs obtained by UAS, based on the known resolution of the image and the size of the camera sensor (Christiansen et al., 2018).

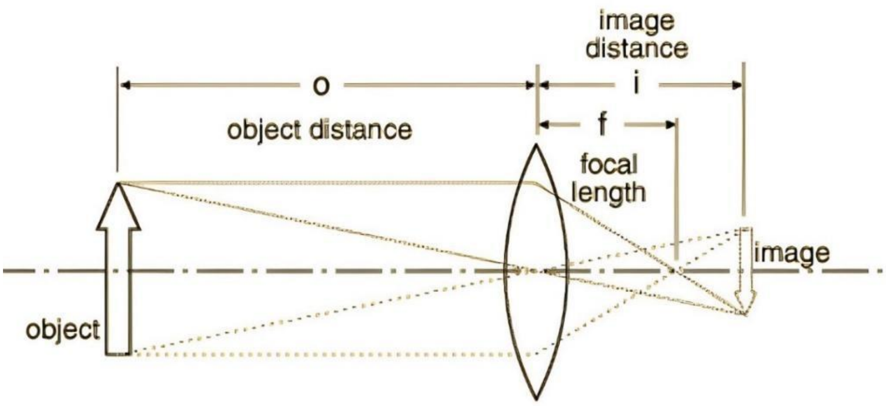


Figure 1. Ray projection in a camera (Allan, 2019).

When applied to live animals like cetaceans, the photogrammetry data is first obtained two-dimensionally, as explained previously. This means that to be able to perform morphometric analysis and assessment of body volume or mass, additional mathematical actions and measurements are required, including width measurements along the body axis (Burnett *et al.,* 2018). Morphometry is a numerical expression of an animal's morphological characteristics. Body condition indices derived from these characteristics can help predict body volume and mass in cetaceans, assuming that the individual has an ellipsoid shape (Castrillon & Bengtson, 2020). This can be performed by using the body girth in relation to the body total length, which has also been applied in a variety of ways to predict the body volume (Castrillon & Bengtson, 2020). But in order to employ an appropriate method, it is important to choose the correct image processing software and follow the appropriate guidelines (Hellwich, 2008). For instance, Lemos et al. (2020), generated an output table with whales' morphometric attributes such as whale length and width at different body length percentage points between 20% and 60% of the body axis, to which a program fitted parabolas along the body edges (image 2) to evaluate the body condition. To do so, programs such as MATLAB (Burnett *et al.,* 2018) or R Studio were required. R studio allows the creation of mathematical codes to estimate more complex measurements. MorphoMetriX is another flexible photogrammetry graphical user interface used to make efficient manual morphometric measurements of wild animals via aerial imagery (Torres & Bierlich, 2020).

3.3. Photogrammetry using UAS

Photogrammetry has been previously applied from vessel and aerial surveys. Nowadays, these are considered to be time-consuming methods that can be demanding and expensive (Giles, 2019). To mitigate those conditions, other technologies such as bio-logging, radio tracking and satellite tracking have contributed to data collection on marine vertebrates, mainly in the behavioral area. But in search of better techniques with a wider research spectrum, UAS have shown to provide new opportunities for the collection of important information on marine wildlife as a non-invasive methodology (Giles, 2019). For example, drones have been used to determine the extent of climate-based bias in the sex ratios of sea turtles (*Caretta caretta*) (Schofield *et al.*, 2017); and UAS-derived photogrammetry images have been used to determine body condition of humpback (Christiansen *et al*., 2016) and right whales (Christiansen *et al.,* 2018). It has been suggested that models based on drone photogrammetry can lead to determine crucial dynamics of cetacean's populations, such as the importance of feeding areas, or to represent morphological differences between individuals within a group and accurately classifying members of a population or community (Castrillon & Bengston, 2020). Furthermore, reproductive analysis can also be assessed in whales. This was evidenced by Christiansen *et al.* (2018) through relationships obtained from growth rate in calf, body volume, rate of decline in maternal body volume, and relationship between rate of change in calf body volume and maternal body volume.

Another advantage of the UAS is that the sampling can be performed by a small team, and it has been suggested that the UAS-based approach significantly reduces disturbances to animals, making it a much safer approach for research, at the same time as it reduces the costs of sampling (Hart *et al.,* 2013). After sampling, it is possible to obtain morphometric measurements through photogrammetry that can be later approached through a custom-written script in R (R Core Team 2014; free download available from Christiansen *et al.* 2016) or similar programs. Christiansen *et al*. (2016) designed an R script through which length measurements of humpback whales (in pixels) were obtained. These included the distance from the tip of the rostrum to the notch of the fluke; the distance from the tip of the rostrum to the position of the eyes (measured along the body axis of the whale); and the distance from the tip of the rostrum to the end of the tail stock. These measurements along with the body width of the whales (in pixels) at 5% intervals along the entire body of the animals, perpendicular to the body axis, were then assessed to calculate each whale's body condition (figure 2).

To obtain precise results in photogrammetry research by UAS, calibration of the images taken by the drone is required as part of the process of dimension determination, and to convert pixel measurements to the real size of the animals sampled. For this purpose, an object of known size can be photographed at different altitudes, or a rangefinder can be adapted to the UAS to measure precise altitudes. Altitude values are necessary for pixel to meter conversion through mathematical formulas (Christiansen et al., 2016).

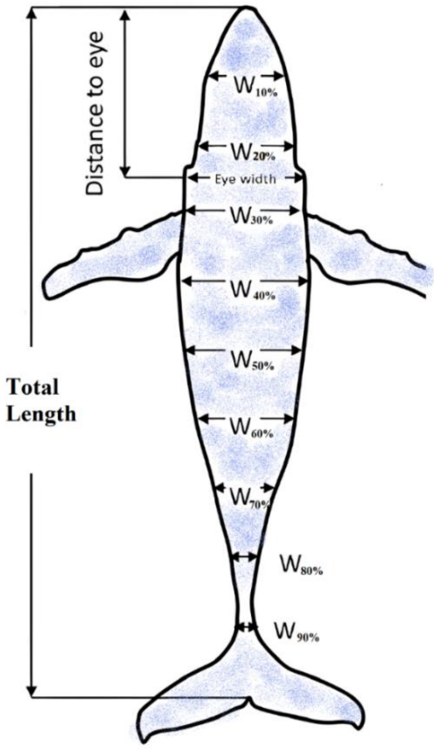


Figure 2. Diagram of width measurements along every 5% of the body axis of humpback whales (Christiansen *et al*., (2016).

CHAPTER 4 - The bottlenose dolphin (*Tursiops truncatus)*

4.1. General characteristics and distribution

The bottlenose dolphin is a marine mammal of the order Certartiodactyla, infraorder Odontoceti and family Delphinidae. This species generally presents two ecotypes, the coast and the pelagic dolphin, which differ from one another by its nutritional strategies. The coastal variation is usually represented by resident animals occupying a restricted area, while the others navigate open waters. Bottlenose dolphins are easily recognizable due to its dorsal blue-gray homogenous color and its white ventral portion. These animals are well adapted to aquatic locomotion due to their pisiform bodies and hairless smooth skin without dermic glands, and although they ascend to the surface every five minutes in average to be able to breath, they can dive up to 15 minutes, although usually less than four minutes and they maintain an approximate body temperature of 36ºC (Dimaura *et al.* 2022).

In terms of reproduction, bottlenose dolphins usually breed during the whole year in certain areas, or during specific months when the nutritional resources are predicted to be better. The gestation occurs during 11 to 12 months, with females giving birth to one calf only that lactate until 18 months of age. In the wild, the life span of these animals can reach up to 45 years of age, although females live longer than males. Bottlenose dolphins have a large distribution in temperate and tropical water bodies around the world, with a preference for coastal areas. Coastal populations are frequently found in bays, lakes, estuaries, and river currents, being residents of delimited coastline areas with water temperature ranging between 10ºC and 32ºC. Some of these populations perform seasonal migrations towards better conditions and better resource supply during the reproductive season. Sexual maturity is reached between the five and 13 years of age in the case of females, and from eight to 13 in the case of males (Mann *et al.,* 2000).

Anatomically speaking, the most anterior structure of the dolphin is the mouth, which is denominated the rostrum, or the tip of the rostrum. Right after the head, the body trunk is fused by the cervical vertebrae, lacking a structure comparable to a neck. "The pectoral fins are located latero-ventral and caudal to the head, and the dorsal fin can be located on the dorsal middle line of the animal. The posterior extremity is composed by the caudal fin, which ends in the denominated "tail fluke". In males, at the ventrocaudal portion, two longitudinal slits protect their reproductive organs and anus, while on females three slits are found; one protecting the reproductive tract and anus, and two laterally where the mammary glands are located (Lopes *et al.* 2019).

In Portugal, bottlenose dolphins can be found throughout the Portuguese continental coast and at the archipelagos of Azores and Madeira (Harzen, 1998). These dolphins can also be found at the Sado estuary, which is an area near Setubal that shelters a small bottlenose population that is unique in the country because it is the only resident community in mainland Portugal. This population, with an approximate of 29 members, inhabits the region of the estuary and moves through the sea and the local river. Although iconic, this population is aging and therefore decreasing in numbers because it is mostly composed of adults. Females are capable of giving birth to a new calf every three or four years due to its characteristically long lactation period (Giménez *Et al.* 2017).

4.2. The bottlenose dolphin diet

The general diet of bottlenose dolphins is characterized by a diverse range of preys, including pelagic fish like mullets and bream, mollusks such as octopus and squid, and crustaceans like crabs and shrimps. When examining the diet of Mediterranean bottlenose dolphins through stomach analysis, researchers have identified nine fish species and seven cephalopod species. Among these, fish constitute the most important prey category in terms of quantity and frequency, although it is worth noting that females exhibit a stronger preference for cephalopods. The most frequently encountered fish species in the stomach contents include *Cepola rubescens*, *Conger conger*, *Ophidion sp*., and *Pagellus erythrinus*. Regarding benthic cephalopods, *Octopus vulgaris* and *Eledone moschata* are notable prey items (Blanco *et al.,* 2001). Isotope diet analysis has also revealed the consumption of crustaceans, *Sparidae* species, European hake, and mackerels by bottlenose dolphins (Giménez *et al.*, 2017).

Regional differences in their diet have been observed, and at the Sado estuary in Portugal, dolphins exhibit a particular preference for squid and mullets (Dos Santos *et al*., 2007), although they incorporate flounders and cuttlefish into their diet (RNES, 2021). *Anguilla anguilla* has also been confirmed as prey of the population (Dos Santos *et al*., 2007). Bottlenose dolphins have a substantial daily consumption rate estimated at approximately 20 kilograms, and they allocate a significant portion of their time to foraging, employing various hunting strategies both individually and in groups (RNES, 2021). In the Sado estuary, foraging behavior predominantly occurs in shallow waters (Dos Santos *et al*., 2007).

4.3. Morphometric data in Tursiops Truncatus

Morphometric data is essential for the understanding of population ecology, including key aspects such as growth rates and reproductive status. Data collection of free-range bottlenose dolphins is scarce due to the difficulty in obtaining measurements in the wild, however morphometrics derived from stranded animals or live captures have been assessed (Englund *et al*., 2008). Registry of morphometric data can provide us with valuable information about the different species and the members of a population. For example, in *Tursiops truncatus* it can indicate sexual dimorphism. According to Tolley *et al*. (1995), males are significantly larger than females, not only regarding the total length, but also in specific measures such as fluke's width or length of the rostrum-dorsal fin, as well as in body girths. Age can also be defined in bottlenose dolphins based on their size (Read *et al,* 1993) and it has been suggested that cetacean's body composition is significantly influenced by age and reproductive status (Adamczak *et al.*, 2021).

In general terms, it has been reported that during the first month of age each bottlenose individual can reach up to 131 cm in length and 86 cm in girth (Englund *et al*., 2008). In adulthood, a bottlenose dolphin can measure up from 1.8 to 3.9 meters in length with a range of 136 to 600 kg of weight (NOAA, 2023). However, measurement data in bottlenose dolphins may vary significantly according to the different regions of distribution (Table 1).

Reference	*Location*	*Girth Base of dorsal fin (cm)*	*Girth anterior to dorsal fin (cm)*	*Girth at anus (cm)*	*Total length (cm)*
Tolley (1955)	USA	34-44 (males) 30-41 (females)	140-164 (males) 126-154 (females)	82-100 (males) 68-87 (females)	252-283 (males) 231-265 (females)
Vivier (2023)	USA	-	-	-	166–285
NOAA (2023)	USA	-	-	-	180-396
Fish (1993)	USA	-	-	-	251-270 (females) 254-270 (males)
Godall et al. (2011)		164-168 71 (Fetus)	174-184 77 (Fetus)	86-100	-
ARROJAL (2023)	Portugal	77-169	70-180	50-149	138-374
RALVT (2022)	Portugal	140	140	76	321

Table 1. List of available girth and length measurements for bottlenose dolphins from different references.

5.1. The Sado estuary geography and climate conditions

The Sado estuary, part of which was designated as a Portuguese natural reserve (RESERVA NATURAL DO ESTUÁRIO DO SADO (RNES)), is located 40km south from Lisbon with approximately 20km long reaching intertidal mudflats and salt marshes that occupy about one third of the estuary (Martins et al., 2001), additional to an important hydrographic basin within the country (ICNF, 2020). Originating from an altitude of 230 meters at "Serra da Vigia," the Sado river extends for 180 km south to north before reaching the Atlantic Ocean at the Sétubal bay, with an average depth of 10 m. RNES encompasses four distinct countries: Setúbal, Palmela, Alcácer do Sal, and Grândola. Geographically, the estuary consists of two primary regions. A northern region covering approximately 140 square kilometers in the Setubal Bay, and a southern region housing Marateca’s and Comporta’s channel in the Troia Peninsula area (Brito 2023).

The RNES has Mediterranean weather with Atlantic influence dependent on the ocean’s proximity and its orography. A very important characteristic of the Sado estuary is that the oceanic currents play a crucial role for the hydrodynamic and hydrologic conditions of the region due to its reduced local depth. Altitudes are also reduced within the Sado basin which adjudicate some of the areas with dry conditions and elevated salinity. Average temperatures and dryness in the region are elevated with temperatures ranging around 23ºC during summer and between 8ºC and 9ºC during the winter (Alves & Machado, 2021).

5.3. The estuary resources

RNES is a vast biodiverse area where the Sado estuary is considered a humid region of international interest due to the biodiversity it protects, especially in ichthyological, malacological and ornithological terms. It not only has the riverine and oceanic ecosystems, but it also has a diverse variety of freshwater resources in forms of lakes or lagoons, and agricultural-rural landscapes. These characteristics make the estuary one of the most productive ecosystems and awards it with great richness of flora and fauna. Within its natural areas, a lot of different types of ecosystems can be found including dunes, coastlines, marshes, lakes and reeds, being a crucial spot for avian nidification and hibernation, and of spawn and development for many fishes. As the river and the oceans are connected, it allows canals, creeks and marshes to house a variety of mammals including the bottlenose dolphin, being one of the representative species of the area, near to a hundred bird species, and a whole spectrum of mollusks, crustaceans and pelagic fishes (Alves & Machado, 2021). Being RNES such a productive area, it is considered of important economic and cultural value, and for that its worth to be protected from the pollution and anthropogenic stressors and to be preserved for future generations. The location is also recognized by its varied activities such as salterns, cork production, resin production, and it constitutes an important fishery location (Ferreira, 2012). Due to its economic importance, the Sado estuary is not immune to the impacts of human activities, including boat traffic, fishing, tourism, and diverse sources of pollution (Caeiro *et al*, 2002).

5.4. The philopatric bottlenose dolphin community at the Sado estuary

Because the Sado estuary provides a rich feeding ground of lower competition (Augusto, 2007), a bottlenose dolphin community inhabits the area as a philopatric group. This community is composed of 29 animals, of which the majority are adults and subadults, and where only nine females and six males have been categorized by sex (~50% of the population with uncertain sex ratio in 2021) (ICNF, 2023). This group of dolphins is of economic importance for the region, and it is of great interest for the tourism industry. Their social structure with tight bonds and the restricted contact with other populations makes them a vulnerable population that needs to be monitored and protected. The community is described in figure 3.

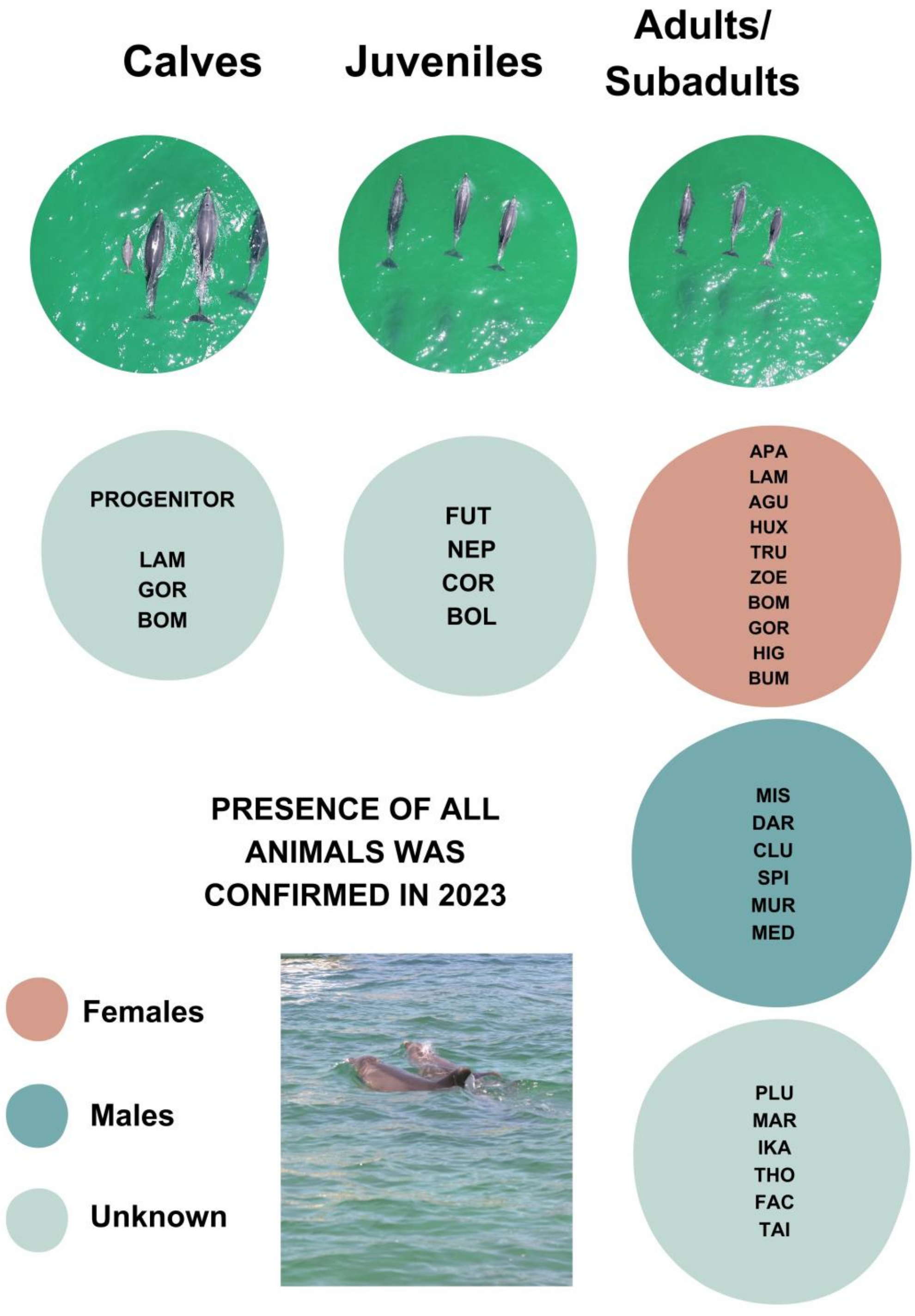


Figure 3. Description of the bottlenose dolphin population in the Sado estuary based on ICNF records, ISPA dolphin codes and present study observations (2023).

Scientific Article

The use of an unmanned aircraft systems and photogrammetry techniques for the assessment of body condition of bottlenose dolphins in the Sado region

Authors: Angela Maria Garcia Castilla

Abstract

Photogrammetry is a technology through which precise measurements can be obtained from photographs. It can be a reliable technique to evaluate body condition in cetaceans when certain parameters of the camera are known, and the appropriate angles and positions of the animals are captured (Burnett et al., 2019). In Portugal, a philopatric population of bottlenose dolphins (Tursiops truncatus) is settled into the Sado estuary (Setubal) where special conservation measures are in place. The following study aimed to evaluate the body condition of the individuals in this population through unmanned aircraft systems (UAS) photography and photogrammetry techniques based on Christiansen et al. (2018, 2019) methodologies. Images were obtained with a DJI Phantom 4 drone and a SF11/C laser range finder flown at a minimum distance of 10 m from the dolphins. The dolphins were categorized in age (adults, sub-adults, juveniles, calf) and sex classes for further evaluation of the model. The model calculated total length (TL) and body volume (BV) of each subject measured during winter and summer. The present study found no evidence of sexual dimorphism in this bottlenose dolphin population, while significant differences were found among age categories of 18 animals sampled. Surprisingly, body volume was higher in summer than winter, defying expectations of thicker blubber composition during the latter season for thermal insulation, which could indicate variability in food availability. The present study validated the effectiveness of the model (R^2 = 0.9253) and highlights its possible contributions for future conservation strategies and research endeavors.

Keywords: *Photogrammetry, Body Condition, Tursiops truncatus, unmanned aerial vehicles (UAS).*

Introduction

An accurate evaluation of animals' body condition within a population is essential for its conservation. As body condition is linked to the ecosystem productivity and trophic mechanisms, it provides crucial data on features such as reproductive status, growth rate, energetic requirements and phenotypic differences between species or populations (Lemos *et al*., 2020). Furthermore, other associated factors and indicators of health status such as injury, diet limitations or anthropogenic impact, have the potential to be analyzed from physical condition (Gray *et al*., 2019). In marine mammals, decreased body conditions that may affect the animals' fitness have been used as proxies to assess individual and population health conditions (Guiles *et al*., 2019). Consequently, understanding the variation of the body index in cetaceans in response to environmental conditions is of great contribution to the field of Conservation Medicine (National Academies of Sciences, 2017).

Although conservation medicine is rapidly evolving, this field still faces many limitations due to the restricted access to wildlife in their environments. Morphometric data in cetaceans has been collected through measurements performed in necropsies of animals that have been found in bycatch incidents and strandings, or from live individuals in captivity. Although these approaches have provided significant data for taxonomic revisions, small sample sizes could affect demographic studies at population scales (Christie *et al*., 2021). Other techniques have been applied for wildlife fitness evaluation such as capture-release studies. Nevertheless, these are invasive approaches that could lead to a behavioral distress (among others), altering the dynamics and wellbeing of the populations. Hence, it is important to develop and implement non-invasive methodologies. To mitigate these negative effects, recent advances in aerial photography by unmanned aircraft systems (UAS) including aerial drones, represent the possibility to better understand the biology, ecology, behavior and welfare of marine wildlife (Guiles *et al*., 2019). In the marine environment, UAS can be used with a less invasive perspective for marine mammal's research and population survey. Although these could generate behavioral changes in wildlife communities when flown at low altitudes, they represent a viable alternative for collecting data of aquatic fauna at discrete altitudes (Castro *et al*. 2021).

Photogrammetry is a technology through which precise measurements can be obtained from photographs. It can be a reliable technique to evaluate body condition in cetaceans when certain parameters of the camera are known, and the appropriate angles and positions of the animals are captured (Burnett *et al*., 2019). Body condition has been evaluated in gray whales under the recommended parameters using drone photogrammetry and applying a body area index (BAI) (Lemos *et al*., 2020). Additionally, in humpback, minke, and blue whales, morphometric parameters were achieved through photogrammetry, allowing to establish size classes of whales automatically (Gray *et al*., 2019). Regarding dolphins, Christie *et al*. (2021) demonstrated that the use of UAS is feasible and effective to collect morphometric measurements of Australian snubfin and humpback dolphins and that photogrammetry provides a powerful noninvasive resource to monitor the health status of small dolphin species of conservation concern.

In Portugal, a population of bottlenose dolphins (*Tursiops truncatus*) settled into the Sado estuary located in the region of Setubal and adjacent coastal waters. It is now the only philopatric population of the species within the country, establishing rare contact with groups from neighboring coastal waters (Augusto, 2007). This population has a strong established social structure with very stable associations within the group (Augusto, 2007). According to Augusto *et al*. (2012), the dolphins' resident community at the Sado estuary is a decreasing population with a low offspring survival record and the group is considered to be aging as most of its members are adults (being the lifespan of a bottlenose dolphin between 40 and 50 years). Moreover, the philopatric characteristic of this single community makes it vulnerable due to lack of immigration exchanges with other groups, and therefore the disappearance of any member of the community could deeply affect the population (Augusto, 2007). Consequently, conservation measures are required, and it is critical to evaluate the current status of the group through non-invasive methods and Precision Medicine, which might be a promising strategy to evaluate the biological and ecological challenges that this community might be facing. Although the community has been studied since 1980, a population health approach has not been performed yet, and the use of photogrammetry through drone photography and recording might represent a suitable methodology. Consequently, the aim of this study was to evaluate the size, volume and body condition of the population of dolphins of the Sado estuary, through the application of UAS photography and photogrammetry modeling.

Materials and Methods

Study site and Population:

The Sado's estuary is a natural reserve located in the Setubal bay (38°27′20″N 8°45′30″W), delimited by the basins of Tejo (north), Guadiana (east), and Mira (west) (R. N. D. E, 2021). It is one of the largest estuaries in Europe with an area of approximately 180 km^2. Due to its hydric characteristics and multiple landscapes, this is a very rich ecosystem highly diverse in flora and fauna that allows the establishment of many resident species such as the bottlenose dolphin (Cabral, 2000). The Sado's bottlenose dolphin community is composed of 29 animals of which 18 are adults, three are subadults, four are juveniles. Four newborn calves were seen for the first time during this project, in July 2023. The presence of six previously identified members of the group was not confirmed during 2021, including two females that were found stranded in 2022 (ICNF, 2021; RALVT, 2022).

Drone flights and image Collection:

Drone flights were performed in compliance with the Portuguese aerial regulations. In order to collect good quality images and to avoid behavioral disturb in the Sado's bottlenose dolphins' community, a series of parameters required delineation and were assessed as follows:

Altitude: According to Castro et al. (2021), there are no alterations generated when drones are flown over dolphin populations at a minimum distance of 5 m. Although other studies have reported behavioral responses under 30 m sampling (Ramos *et. al.,* 2018), data has been obtained for Australian and Snubfin dolphins with an approaching distance of 15 m and a maximum distance in altitude of 60 m; which is considered appropriate to take morphometric sampling (Christie *et al*., 2021). Following the recommendations on previous studies, the images were obtained at a minimum distance of 10 m. The maximum distance to obtain measurable photographs was tested with captive life animals at the "Zoomarine" water park in Albufeiras, Portugal. The best recording distance was set in between 10 m and 20 m, in terms of image quality. Above 30 m, the quality of the images was not adequate for measurement because elements such as blow hole, and dorsal fin were not visible. These are required parameters for further assessment of measurements.

Maritime conditions: In accordance with Guiles *et al.* (2020), the drone flights were programmed and performed considering meteorologic predictions as follows: wind current under 12 km/h, low cloud cover, and appropriate water clarity (1 to 5 scale, being 1 very poor and 5 optimum; 3 was the lowest result in the scale acceptable for appropriate water clarity). Climatological conditions were analyzed and defined through IPMA (Instituto Português do Mar e da Atmosfera) prior to every flight and registered afterwards for project documentation.

Drone usage and position over the animals: The UAS was launched from the bow of the vessel once the dolphin group was visually located. Then, the UAS was directly positioned perpendicular above the dolphin group to record the video and then followed on the telemetry screen with the camera position straight down.

Image collection and selection: still images were captured every two seconds while simultaneously recording. Video recordings were performed with a resolution width between 1920 and 5472 pixels per inch. Many of the images were extracted manually through VLC media player and filed. Every still frame and image collected were registered with metadata including time, altitude, and image resolution.

Images were selected for measurement according to quality and position of the animals. Image quality was classified from 1 to 5, being 5 the best quality and 1 the poorest, based on Guiles *et al*. (2020) methodologies. Only images with quality above 3 were used for the study, others were discarded (figure 4). Evaluation parameters included, horizontal straight position, image resolution clarity, blowhole and caudal fin visibility, and water clarity.

Figure 4. Photograph with quality classification No. 2. considered adequate to be measured.

Dolphin identification and class identification: Throughout the study, the members of the community were also photographed from the research boat and identified through dorsal fin photo identification. Time of capture of the dorsal fin photographs and UAS images were matched to differentiate males from females or adults from juveniles and calves. However, due to the number of animals observed in a single image, most of the identifications were performed with the help of the staff of the RNES. The dolphins were identified from the vessel, recording the exact times the animals were seen, along with the locations of the animals within the group, or in the image of the drone screen frame. These records were matched later with the drone photography time records. Marks in the dolphin's dorsal fins were not visible in the drone's images taken at a horizontal perspective (figure 5). For this reason identification of individuals through UAS images was not achieved.

To evaluate the body condition variation within the population, the identified individuals in the group were categorized as follows: males, females, adults, sub-adults, and juveniles.

Figure 5. Drone photograph of dolphins in Sado's population.

Photogrammetry

Photogrammetry can be defined as the act of defining precision measurements from photographs. Because body condition is an animal's overall mass and volume relative to its length, its evaluation was achieved through different photogrammetry and modeling assessments that led to the calculation of the animal's Body Condition (BC). Methodologies for BC were addressed based on the following methods:

Calibration and scaling: For post-processing evaluations and scaling of the photographed dolphins to meters, precise altitude values are required. Altitude can be measured with exactitude with the aid of a laser range finder adapted to a UAS (Christiansen *et al*., 2018). For altitude calculation a Lightware SF11/C laser range finder was installed to the UAS (DJI Phantom 4 pro V.2.0. drone). The altitude measured by the device was divided by the focal length of the camera, then multiplied by the sensor size and known resolution of the image. This, along with the total length of the photographed dolphins in pixels, allowed the setting of the ground sampling distance or scale of each photo to convert pixels (*pix*) to the animal's real size in meters in each photograph. Scaling was performed following the methodologies of Christiansen *et al*. (2018).

The calibration references in the present study were obtained through live captive animals of known measurements at the Zoomarine Park, Albufeira, Portugal. Five bottlenose dolphins were recorded and photographed by the UAS selected for this study to serve as reference specimens for model validation. The dolphins were positioned by their trainers at a horizontal line to comply with the quality image standards (figure 6).

Figure 6. Horizontal photograph collected for method's at Zoomarine.

Data comprised measurements obtained from two female adults, two male adults and one male juvenile. These were employed in the photogrammetry model to assess the accuracy of measurements in wildlife subjects. During our filming sessions at the Zoomarine park, 10 meters emerged as the preferred and most frequently employed height throughout the duration of the study.

Data collection/sampling methods: 4K videos were obtained in four different dates through short period recordings during morning and noon sessions, in February and July. Initially, the study was designed to evaluate body condition variation along the different seasons of the year. However, due to temporary military restrictions enforced in the area, the approach was narrowed to evaluations during winter and summer.

The drone was launched from a semi-rigid, inflatable boat. Data specific to each drone flight was documented, including the start and finish time of each video taken within the flight, and notes regarding the group size and composition. Each video was visually processed to extract still images of the dolphins lying flat at the surface in a straight body axis (dorsal side facing up), with the least degree of arch in the dolphin's body position as per Christiansen *et al*. methodologies (2018). The visibility of the tip of the rostrum and fluke notch of the animals at water surface was an important parameter for photo selection, as per Currie *et al*. (2021). Each photograph was assessed to ensure to be in-focus, well lit, and not affected by glare, angle, or distance. An imaging score was determined and categorized as good or poor, according to attributes relative to the dolphin's body position (edge certainty, straightness and arching; Christiansen *et al*., 2018).

Videos were assessed at VLC media player® and Adobe premiere®. Images were extracted while watching the videos or extracting frames from a single video automatically using Premiere Pro (Adobe)®. Marks were placed at the timeline of the video to estimate the time of each frame, which was then calculated by recording its time lapse and adding it to the initial hour of the video (figure 7).

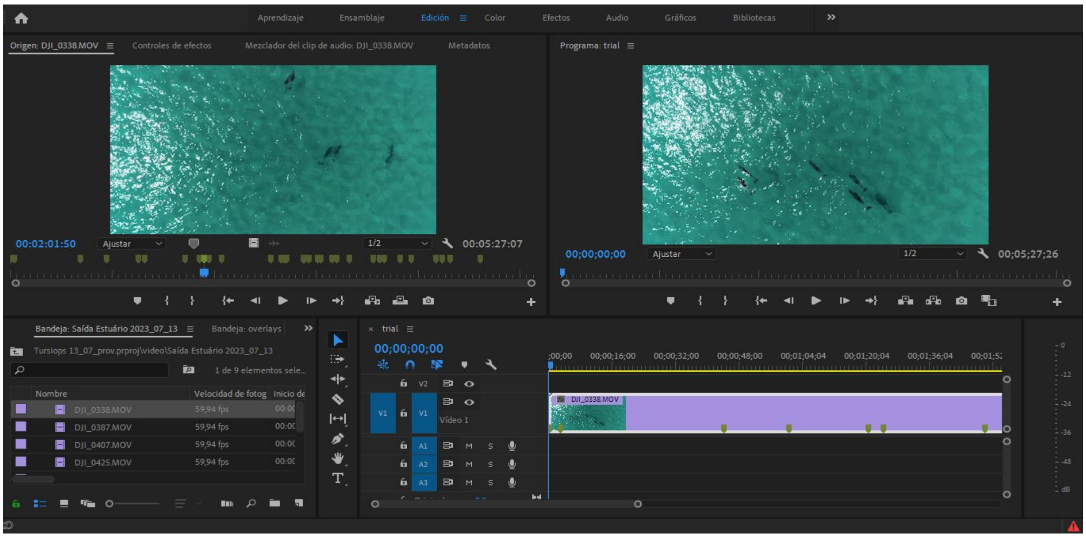


Figure 7. Video post processing methodologies.

Morphometric measurements: The total length (TL) of the dolphin (in pixels) (figure 8), including the distance from the tip of the rostrum to the blowhole; the end of the dorsal fin; the width between the position of the eyes; and the beginning of the tail fluke, were measured manually in each image through the R script of Christiansen *et al*. (2018) with minor modifications (figure 9). Following these measurements, the width (W) of the animal was established as a cross section perpendicular through 5 to 95% of the body axis at 5% intervals between each measurement point (similar to Christiansen *et al*., 2018) (figure 10). Height (HT) was also obtained in dorso-ventral and lateral views at each measurement site per Christiansen *et al*. (2019) methods (figure 11). This was performed with the aim of obtaining a height/width ratio by dividing the animal's height by the animal's width, which was later used as part of the photogrammetry methodologies to obtain the dolphins' body volume and body mass.

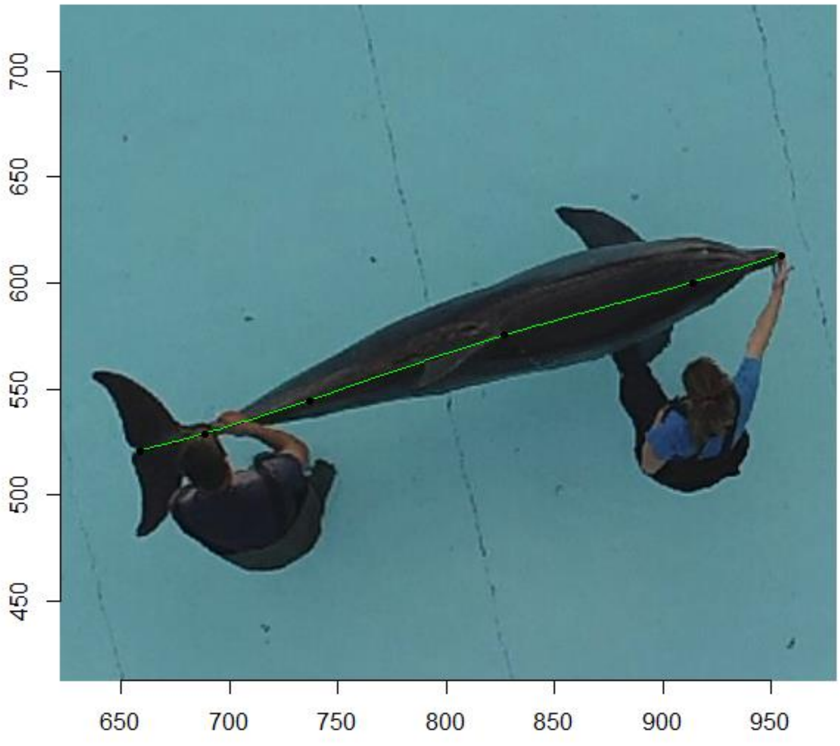


Figure 8. Total length.

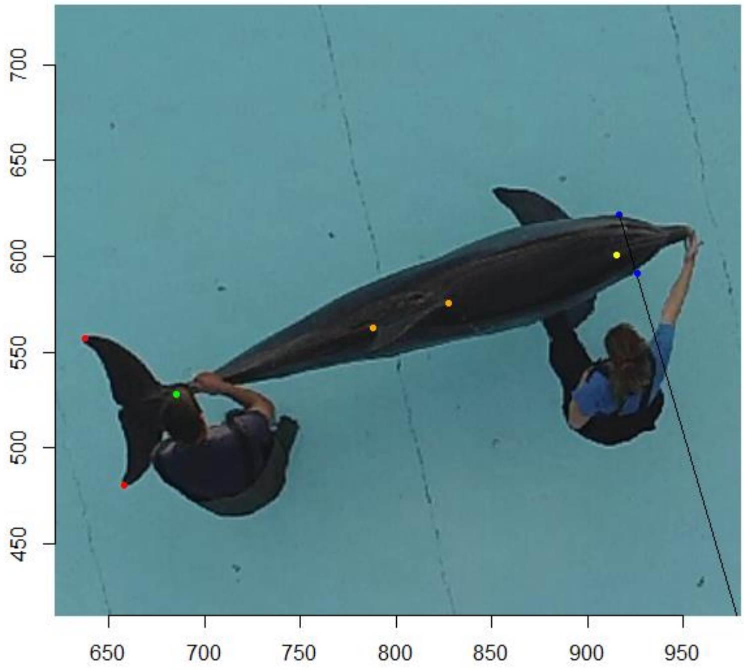


Figure 9. Distance from the tip of the rostrum to the blowhole; the end of the dorsal fin; the width between the position of the eyes; and the beginning of the tail fluke.

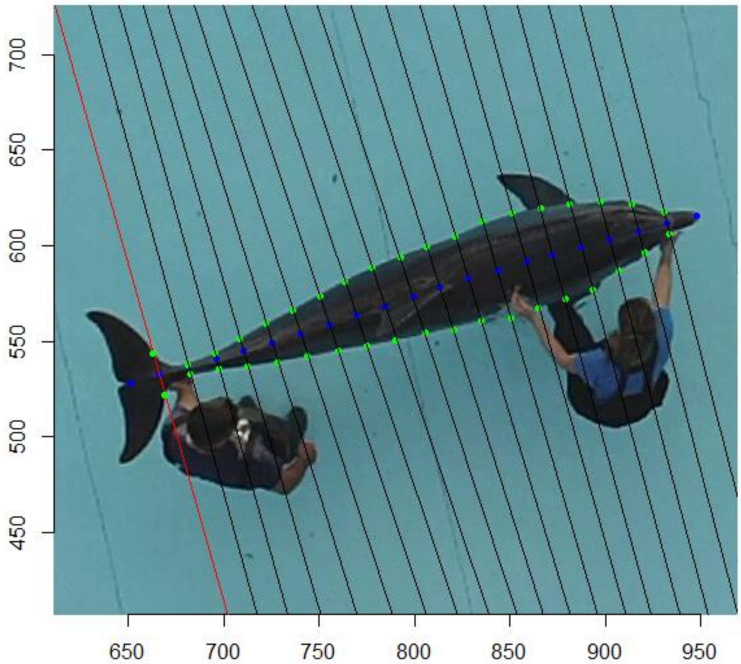


Figure 10. Width measurements along 5 to 95% of the body axis.

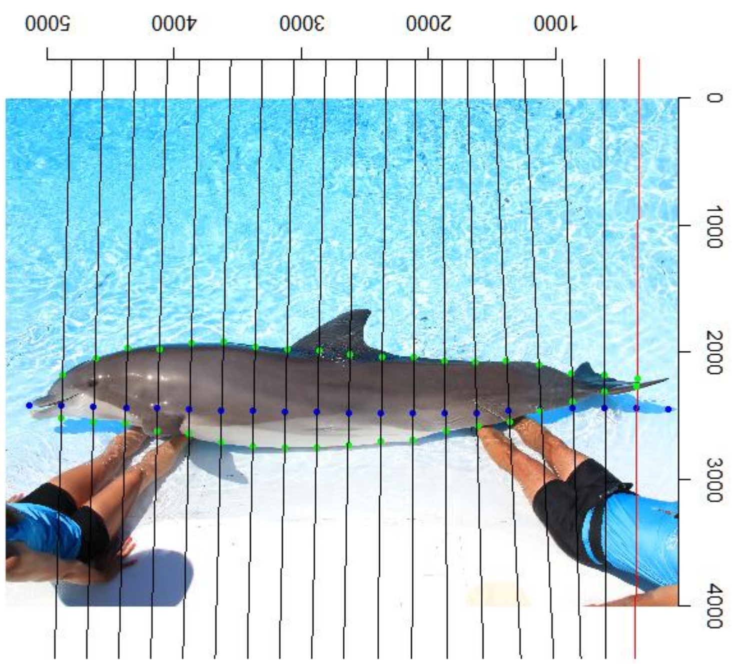


Figure 11. Height measurements

Body Volume (BV): To quantify the body condition of the free living dolphins in the Sado region, *BV* of the animals was calculated based on the previously obtained measurements; body *W, HT* and *TL* data. Evaluating this parameter is desirable because cetaceans metabolize their blubber all around the body and not from a specific surface. To be able to identify variation across the body length, for each dolphin, height-width (*HW*) ratios need to be incorporated assuming that the cross-section of the dolphins is circular to calculate a body girth for each width measurement site. This incorporation was performed by modeling the cross section as an infinite number of ellipses in between the two adjacent *W/HT* measurement body segments (*bs*) for each 5% increment of the cross section, following Christiansen *et al*. (2019) methodologies. To be able to capture the decrease in widths and heights towards the tail of the dolphin, the end points of the dolphins representing 0 and 100% in the cross section (rostrum and tail fluke) were represented with a value of 0 m. Dolphins' head, fins and tail fluke represent low blubber reserves, for this reason and to avoid including the fluke details within the calculations, the values for W and H from 90-95% of each dolphin's cross section, were calculated based on a linear interpolation between 85-100% of the TL measurement sites. The aforementioned data allows the total body volume (*TBV*) to be calculated for each dolphin by the sum of volumes of all segments (Christiansen *et al.*, 2019).

Body Condition: Body condition calculations acquired through known measures of the bottlenose dolphins are required to test the performance of the *BV* model. Based on Christiansen *et al*. (2019) methods, the body condition of Sado's bottlenose dolphins was calculated based on the linear relationship between body volume and total body length.

The blubber is a subcutaneous thick layer of adipose tissue and collagen present in marine mammals that can metabolically change in response to environmental changes. Because the blubber serves as an efficient thermal insulator to protect the body from cold water temperature (Adamczak *et al.* 2021), it was expected to obtain increased body volume measurements during winter in comparison to the summer season.

Other Statistical methods

To ensure that assumptions were met for the model validation statistical tests were conducted within R studio. Homoscedasticity or homogeneity of variances, was verified through scatter plots examining the relationship between residuals and fitted values. This confirmed the assumption of consistent variances across data points. Normality of residuals was confirmed through residual histograms, quantile-quantile (Q-Q) plots, and linear regression models. To assess the distribution of the studied population, we employed a residues histogram to depict the frequency of recorded mean for TLs. Prior to any evaluation, collinearity tests were performed via variance inflation factor (VIF) assessments, all below a VIF threshold of 3, signifying no collinearity among variables.

Given the non-normal distribution of the data ($p < 0.001$) and consistent variance across groups *($p = 0.230$)*, non-parametric tests implementing SPSS statistics (v. 17, SPSS Inc, Chicago, IL) were also conducted for further analyses. The Mann-Whitney U test was employed for sex class comparisons as an independent sampling method to evaluate the distribution differences in TL and BV between captive (zoo) and wildlife bottlenose dolphins (wild) across age and sex classes. Also, Kruskal-Wallis test, followed by pairwise comparisons, was employed for age class analysis.

A t-Student test was determined to be more suitable for evaluating differences in BV and TL between winter and summer due to the small sample size. Significance was determined based on p-values less than 0.05. Also, the accuracy and suitability of the linear regression model were assessed to characterize the relationship between the BV of the Sado's bottlenose dolphins (Volume.m3) and their TL (Total.length.m).

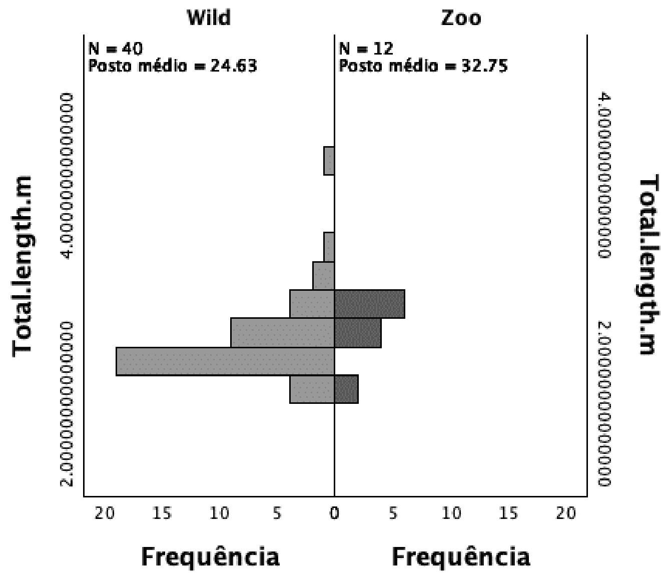
Results

Initially, it was intended to capture video footage of the animals across all seasons of the year. However, we were able to conduct video recording only on four occasions. The four field trips were conducted during winter and summer periods, with the first two sessions occurring in February, and the subsequent two sessions in July of 2023. During winter, the entire group of dolphins was observed traveling together, while in summer, the group exhibited divisions into smaller subgroups. Throughout the field work, all members of the Sado´s bottlenose community were located and observed at least once. However, not all individuals could be conclusively identified in photographs in every session. While the majority of the animals were photographed, a subset of these images was not selected for measuring due to quality concerns based on selected inclusion parameters. For these reasons an average of the number of times an animal was measured was not calculated.

Calibration with captive animals and model accuracy

Reference calculations of the Height-Width (HW) ratio were conducted based on the Zoomarine's captive animals. This was feasible due to the availability to obtain lateral photographs with the assistance of the dolphin's trainers at the Zoomarine park. The measurements of the male adults *(TL mean (M) = 256.5cm)*; female adults *(TL M = 267cm)*; and the male juvenile *(TL M = 224cm)* were considered for this purpose.

The Mann-Whitney U test analysis revealed no significant difference between captive and wildlife animals concerning measurement distribution *(P=0.103 (TL), P=0.158 (BV))* (Figure 12) considering as null hypothesis that the distribution of BV and TL are equal within the categories.



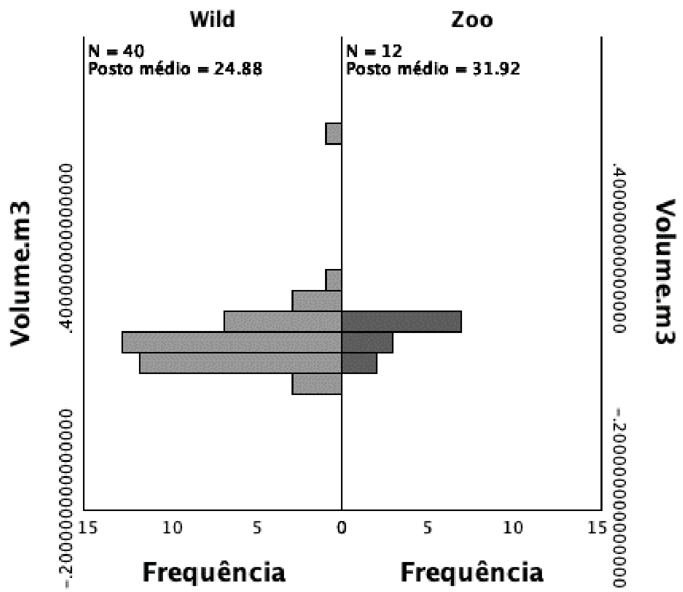


Figure 12. Distribution of age and sex classes in Captive and wildlife animals (U=305, W=383, P<0.05, n-wild=40, n-zoo=12)

Regarding the Sado's bottlenose dolphin's population, predominantly, the most frequently occurring mean for total lengths ranged between two and three meters *(M = 2.4607 meters)*. Given that a significant portion of the population consists mainly of adults, followed by sub-adults and juveniles, the distribution of mean for TLs closely aligns with the age categories of the animals within the population. Lower values are indicative of calves. This distribution appears to be statistically well-suited for representing the population (Figure 13).

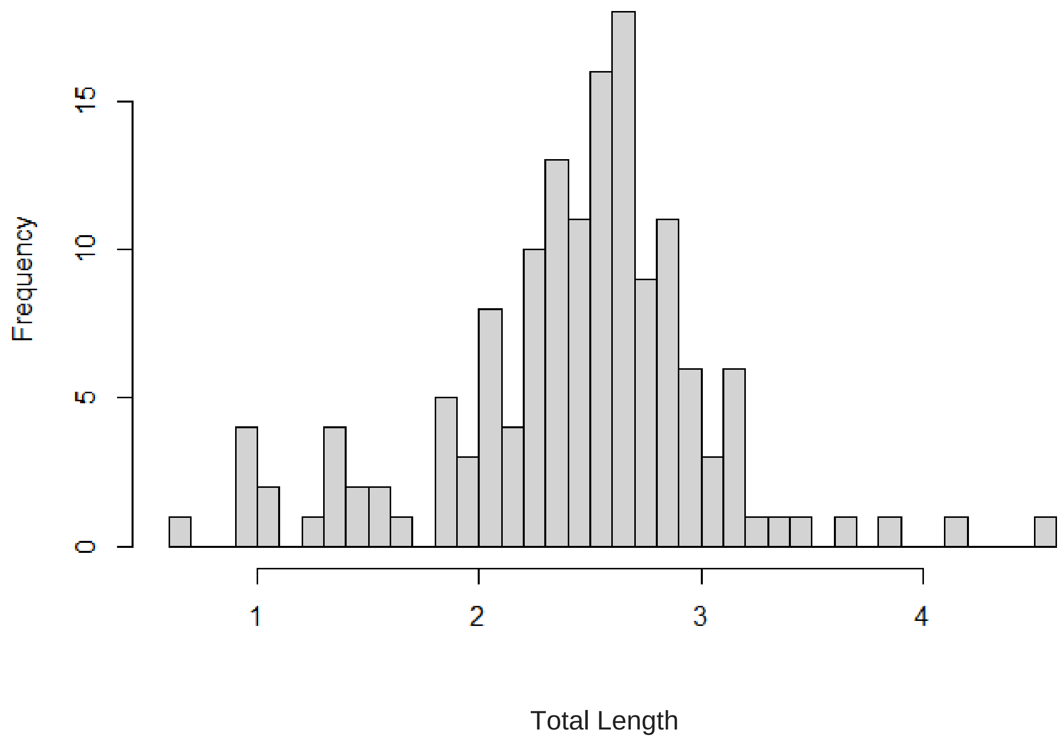


Figure 13. Distribution of the total length (mean = 2.4607m) of the photographed bottlenose population in the Sado Region.

Moreover, the estimated coefficients of the linear regression model revealed a strong positive association, where each unit increase in the TL corresponded to an approximate 2.54-fold increase in BV. The model demonstrated high statistical significance *($p < 0.001$)* associated with both the intercept and the logarithmic total length coefficient. Goodness-of-fit by R-square-value *($R^2 = 0.9253$)* was also exhibited indicating that an estimate of *92.53%* of the variance in body volume can be attributed to variations in total length, reflecting a significant relationship between body volume and total length among the subjects, and validating the efficacy of the model (Figure 14).

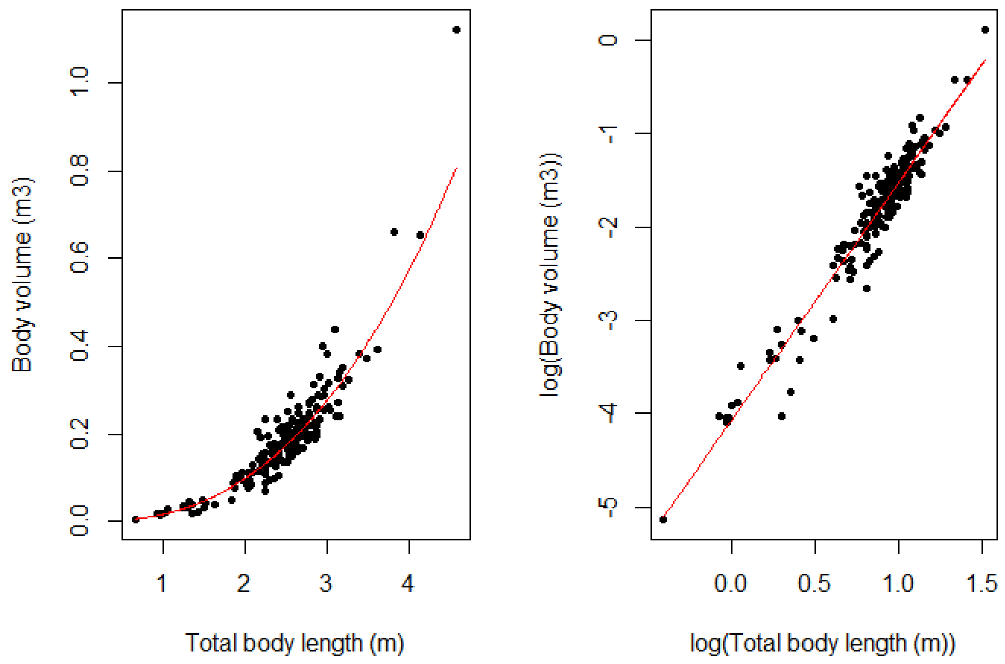


Figure 14. Influence of TL over BV. Red lines representing fitted values (R^2 = 0.9253, SD = 0.2099)

Aerial Image Collection and Sample Size

The UAS flights were conducted in four sessions under favorable weather conditions at an altitude between 10 and 30 meters. The drone footages resulted in the collection of 168 selected images with the animals in horizontal positions on the surface of the water. Between three and four battery changes were required during each session, which is a factor of important consideration for future projects. As per methodologies, the intention was to record precise altitude details during the flights. Regrettably, we encountered inaccuracies in our range finder equipment, and due to the limited number of sessions conducted, some of the altitude details had to be estimated rather than measured with the expected level of precision.

To determine the age and gender of the animals, it was imperative for them to be individually identified. Unfortunately, our initial attempts to synchronize precise timestamps between conventional photographs and drone-captured images was not effective. This challenge primarily arose from the presence of multiple individuals in each image. Notably, no dolphins were successfully identified during the winter recording sessions. During the summer, we were able to identify 20 individual dolphins through direct observation at the time of recording. These individuals were categorized by classes. Sex class included 2 males *(TL M: 2.894 m)* and 6 females *(TL M: 2.893 m)* (Table 2). Gender class was comprised by 4 juveniles *(TL M: 2.459 m)*, 2 sub-adults *(TL M: 2.407 m)*, 11 adults *(TL M: 2.861)*, and 3 calves *(TL M: 1.304 m)* (Table 3). It is worth noting that from the total 168 samples, 148 of these remained unidentified *(TL M: 2.485 m)*.

AGE CLASS	***MEAN***	***INFERIOR LIMIT***	***MAX LIMIT***	***P-VALUE***
FEMALE	2.893	2.721	3.064	0.066
MALE	2.894	1.433	4.355	0.114

Table 2. Descriptive statistics for sex class of the identified dolphins in Sado´s population.

AGE CLASS	***MEAN***	***INFERIOR LIMIT***	***MAX LIMIT***	***P-VALUE***
CALF	*1.304m*	*0.898*	*1.709*	*0.127*
JUVENILE	*2.459m*	*2.226*	*2.693*	*0.073*
SUB-ADULT	*2.407m*	*2.255*	*2.559*	*0.011*
ADULT	*2.861m*	*2.729*	*2.992*	*0.571*

Table 3. Descriptive statistics for age class of the identified dolphins in Sado´s population.

Testing Body Volume values

The BV was estimated assuming a cylindrical body shape that gradually transitions from the rostrum to a flattened posterior axis. We subjected the HW ratio modeling to examination, where it was postulated that a 1:1 ratio would represent a circular body shape. Figure 1 explains the HW ratio's trend. It notably approaches a value of 1 within the range of 30% to 40% along the body length, implying the presence of a circular body shape that progressively flattens towards the posterior portion, as it deviates from this value.

Variations in HW ratio from the tip of the rostrum to 15% of the body axis are evidenced, as well as from 60% of the body and towards the posterior end. These variations within the model can be attributed to variations in TL between the different classes. It may also reflect the challenges of measuring the caudal fin due to positioning of the dolphins, or the effects of water when the caudal fin is either shallower or submerged. Nevertheless, the overall model appears to exhibit a satisfactory level of accuracy (Figure 15).

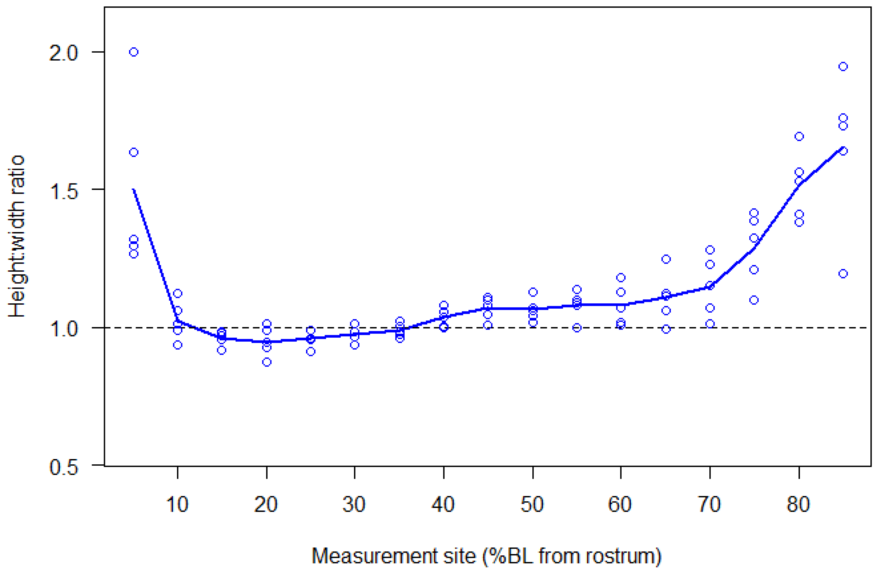


Figure 15. Body shape evaluation of bottlenose dolphins through HW ratio across 5% and 85% of the body length, from the tip from the rostrum. The blue line represents the average HW ratio of the group of the Sado community. The dash line represents a ratio of 1:1 equivalent to a circular body shape.

Variation of Body Condition in age and sex classes

According to the Wilcoxon-Mann-Whitney test for independent samples *(females = 6, males = 2)*, there are no differences between males and females in BV *(p = 0.429)* or TL *(p = 1)* in Sado's bottlenose dolphin community (figure 16 and 17, respectively).

Differently from sex class, BV and TL varied significantly among the categories of age class ($p < 0.001$), except for sub-adults *(BV M = 0.1689m³)* and juveniles *(BV M = 0.1618 m³)* that revealed similar means (figure 18 and 19, respectively). Body Volume estimates of Sado's bottlenose dolphins ranged between 0.1673-0.3161m3 in adults *(TL M = 2.861)*; 0.1219-0.1944m3 in sub-adults and juveniles *(TL M = 2.459m, 2.407m, subsequently)*; and 0.0174-0.0439m3 in calves *(TL M = 1.304).*

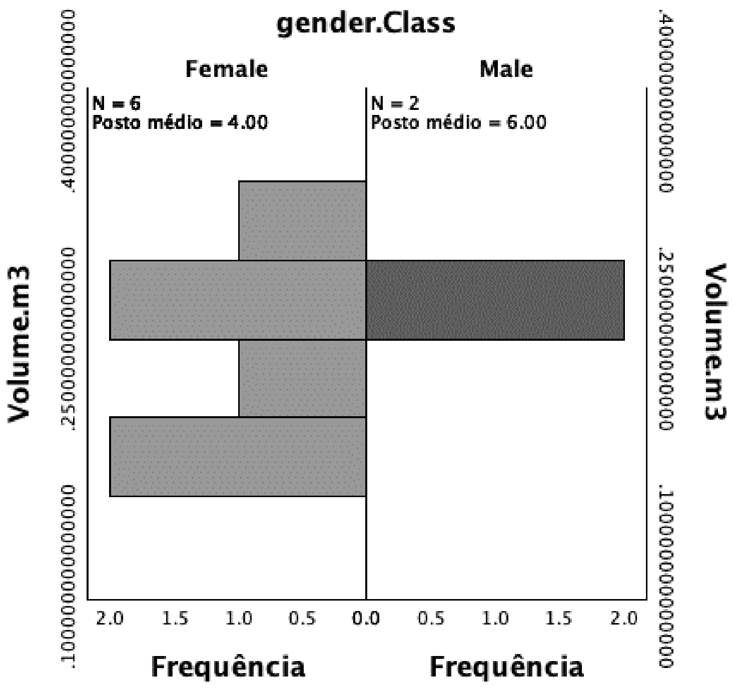


Figure 16. Distribution of BV to evaluate significance in differences between males and females of the bottlenose dolphins in the Sado region. U=6, W=9, n=8.

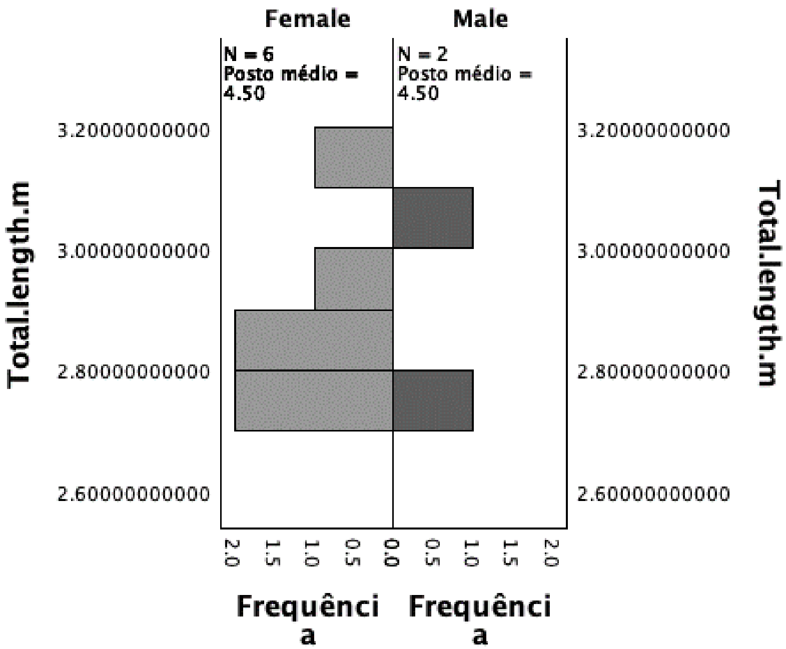


Figure 17. Distribution of TL to evaluate significance in differences between males and females of the bottlenose dolphins in the Sado region. U=6, W=9, n=8.

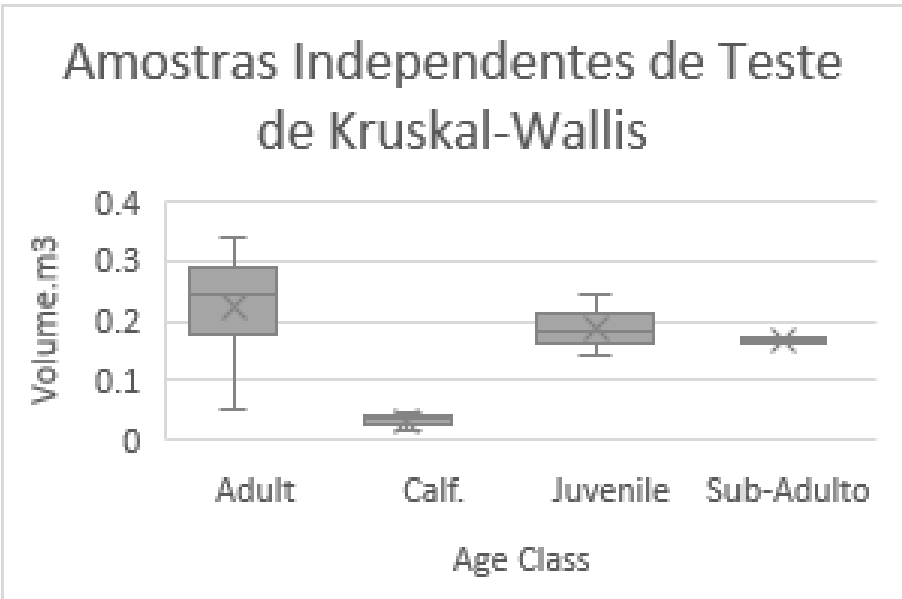


Figure 18. Distribution of BV ($X^2_{kw}(4)=9113$; $p=0.028$; $n=18$) in age classes.

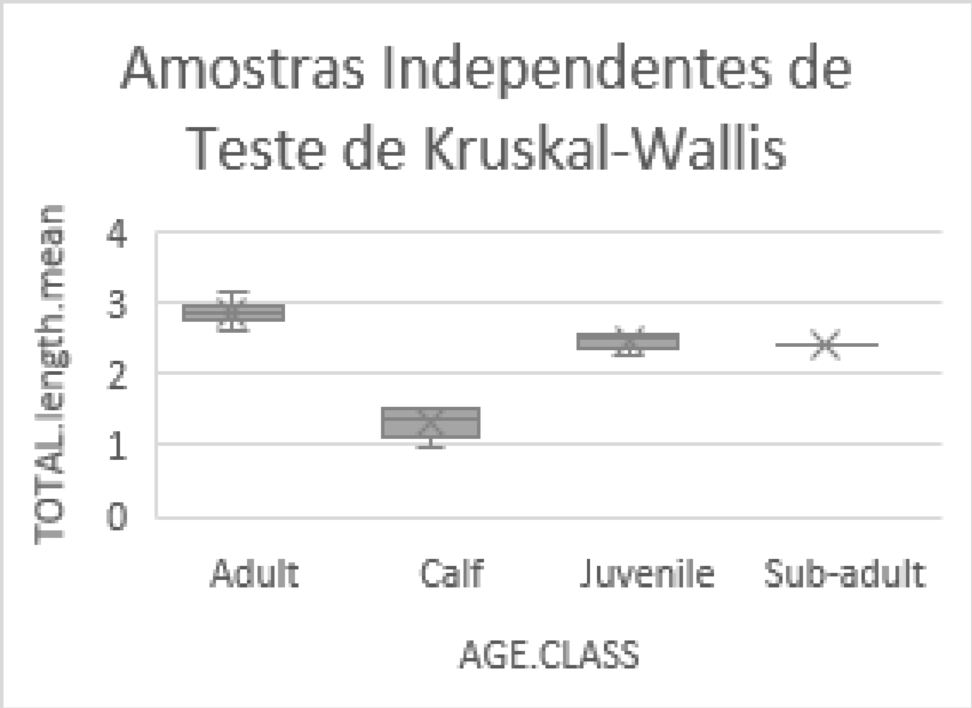


Figure 19. Distribution of TL ($X^2_{kw}(4)=15.489$; P=0.001; n=19) in age classes.

Body condition variability between winter and summer

The effect of seasonal variation during winter and summer over TL and BV was subjected to analysis using a two-sample T-test. Prior to conducting this test, homoscedasticity was confirmed through a Levene's test *(df = 1.457; p = 0.230)*.

As anticipated, the TL of the dolphins did not exhibit significant variation between winter *(M = 2.55, SD = 0.38, n = 40)* and summer *(M = 2.68, SD = 0.409, n = 70)* as determined by p-value *(p = 0.101)* with a 95% confidence interval (I.C). Consequently, it can be inferred that any potential increase in TL may be attributed to factors other than the season of the year or other variables (Figure 20).

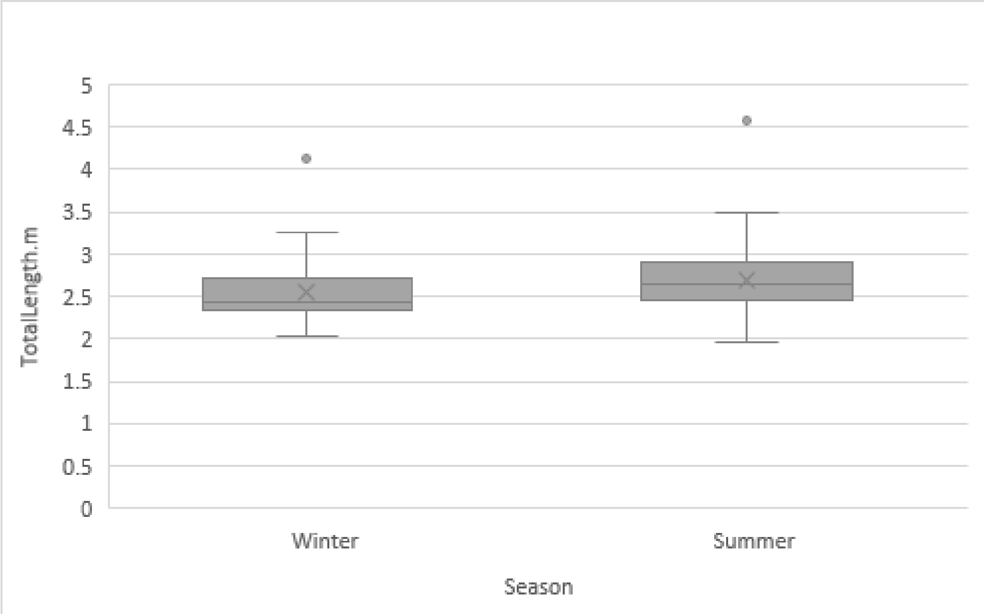


Figure 20. TL mean values (±SEM) of Sado´s bottlenose dolphins in winter and summer.

Regarding BV, a significant *(bilateral p <0.05; I.C. 95%)* higher mean was evidenced during the summer *(M: .233 SD: 0.1356)* in comparison to the winter samples *(M: 0.18 SD: 0.093)* (figure 21).

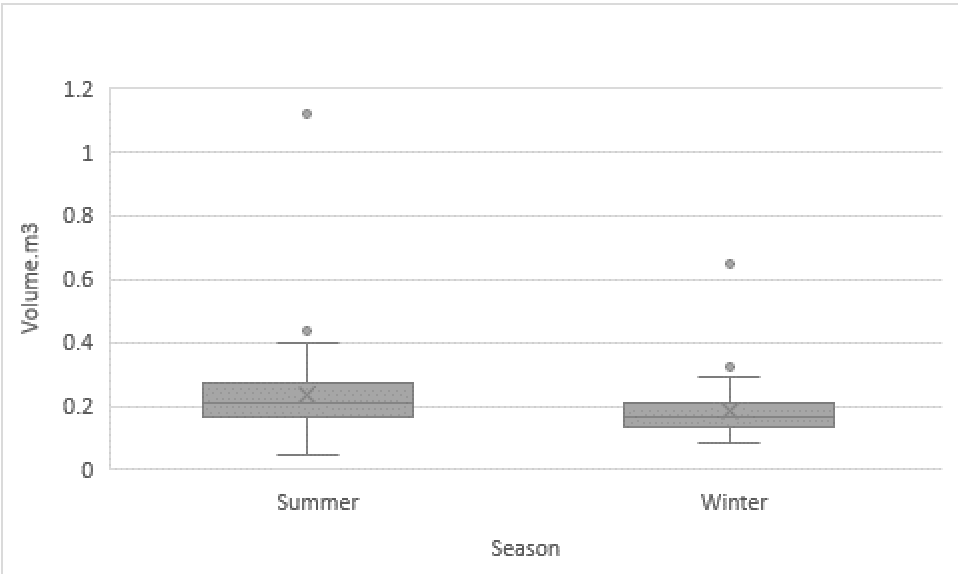


Figure 21. Mean values of BV in summer and in winter (bp= 0.46)

Discussion

Testing the Model

Our primary objective was to evaluate the applicability of a model originally tailored for whales as described by Christiansen *et al*. (2018, 2019), when applied to the distinctive morphological features of bottlenose dolphins. The statistical analysis employed by the Mann-Whitney U test indicated that the assumption of equal distribution for captive and wild animals served as validation of the model. Despite the differences in anatomy and body structure between these groups, our investigation yielded evidence supporting the effectiveness of the model. Our measurements by photogrammetry aligned closely with those physically obtained at the Zoomarine park, and statistical validation was achieved. While minor variations among individual measurements were observed, our dataset confirmed the model's capacity for accurate measurement. Deviations or outliers in comparison to the actual measurements obtained at the Zoomarine aquarium were attributed to inherent challenges associated with altitude estimations of the laser range finder.

Furthermore, we compared our measurements with data documented in the existing literature and sourced from local stranding networks, notably ARROJAL and RALVT (2023). The results obtained in our study exhibited concordance with the mean of values reported and falling within the established range of minimum and maximum total length (TL) measurements documented in various references (Table 4). This alignment encourages the model's utilization in the calculation of measurements of other bottlenose dolphin’s populations. It also extends the possibility of examining new models to estimate BV based on known girths derived from available literature and other stranding networks, while comparing it to BV calculated through this model. This expanded approach holds the promise of enriching the species dataset over time and potentially facilitating comprehensive comparisons of BC in free-range dolphin populations. Given the consistent cylindrical conical body shape characteristic of bottlenose dolphins, we forecast that this model could be successfully tested on other odontocete species with similar body structure. This validation further extends to the application of UAS photography and its potential as a reliable tool for the monitoring of cetaceans.

The possibilities for further research encompass refining the model, evaluating behavioral aspects, exploring variations in different regions, monitoring protected populations, tracking calf growth, and calculating body weight. In essence, this investigation opens the door to a spectrum of opportunities, both in terms of research and conservation, emphasizing the importance of non-invasive techniques like UAS photography in advancing our understanding of cetaceans and fostering their protection.

AGE CLASS	***MEAN***	***INFERIOR LIMIT***	***MAX LIMIT***
SADO (2023)	2.893	2.721	3.064
ARROJAL/RALVT (2023)	2.74	1.38	3.74
NOAA (2023)	-	1.80	3.96
Tolley (1955)	-	2.31	2.83
Fish (1993)	-	2.51	2.70
Vivier (2023)	-	1.66	2.85

Table 4. TL length records of bottlenose dolphins according to academic literature, local stranding networks, and the present study (SADO).

Body Condition Analysis

To evaluate BC results a reference point of comparison is essential, because we need to have an established parameter of what represents a positive or negative BC. Regrettably, the scarcity of BC records for wild dolphins hinder a comparative assessment of the results.

Nevertheless, the present study represents an opportunity to collect records within populations and contribute to a greater database. Such an assessment has the potential to categorize BC outcomes as either negative or positive in the future, leading to the comprehension of BCIs and their potential applications. The determination of BCIs systems constitute an opportunity to further investigate this model or similar. Estimations can be obtained through external characteristics such as body mass, volume, girth, and width, often represented with a ratio of body length (Christiansen *et al.* 2020).

Sexual Dimorphism

While existing literature alludes to sexual dimorphism determined by TL, our study did not show significant differences in average TL between male and female dolphins. This leads to the inference that TL alone may not reliably indicate the sex of dolphins within this population. Our results notably diverge from earlier studies, such as Tolley *et al*. (1995), which reported marked differences in size between male and female dolphins. However, it should be noted that our sample size was reduced and could have affected the outcome's accuracy.

Differences in measurements within a specific sex class may also be linked to the reproductive status of females within a group (Christiansen *et al*., 2018). Conducting further research in this area could contribute to the understanding of variations in girth and BV, driven by the distinct physiology and metabolic changes experienced by females during pregnancy and lactation.

Additionally, it would be intriguing to investigate whether there exists variation in the body condition of male dolphins concerning their activity levels, behavior, hunting strategies, dietary expenditure, and travel patterns. Such investigations could offer a deeper understanding of the dynamics within the dolphin population and their responses to various ecological factors.

Due to the limitations of our study, particularly the inability to identify individual animals during the winter months, we could not conduct a comparative analysis involving the females that gave birth to new calves within the community. Nevertheless, our model might be promising in predicting pregnancy in females in future projects.

Age Class Variation

Significant differences in BV and TL were revealed across the different age classes within this project, demonstrating the insights documented by Read et al. (1993). Notably, the lack of pronounced differences between juveniles and subadults may require merging them into a single category.

Outcomes suggest that age can potentially be estimated in bottlenose dolphins based on their size, allowing the assessment of group structures. These assessments could significantly contribute to conservation projects by providing essential insights into population dynamics. The distribution of TL within the group is particularly noteworthy, as it predominantly consists of adults. The alignment with the broader population profile provides a reasonable level of confidence in the model's validity.

Because it has been suggested that cetacean's body composition is significantly influenced by age (Adamczak *et al.*, 2021), there are many applications of this model for further evaluations such as energetic expenditure between the age classes.

Season influence over Sado's bottlenose dolphins body condition

Despite our initial expectations, our analysis of seasonal variations in Sado's bottlenose dolphins' body condition yielded some unexpected results. We initially approached the study considering body condition in terms of energy reserves and morphological features, particularly relative fat reserves. Consequently, we anticipated detecting differences between summer and winter related to blubber thickness, which would likely influence body volume due to temperature variations (Stevenson & Woods, 2006). Contrary to this prediction, we observed higher body volumes during the summer. Several factors could contribute to these findings, potentially impacting physiological and metabolic processes, such as dietary patterns, food availability (Siebert et al., 2022), and growth dynamics (Castrillon & Bengtson, 2020).

This disparity might be attributed to the relatively mild regional climate in Setubal, where water temperatures during winter rarely drop below 8°C, avoiding the extreme cold temperatures experienced in other regions. Consequently, dolphins in this area may not need to develop excessively thick blubber layers for insulation. However, variations were detected, possibly linked to some females being pregnant and giving birth during the summer, indicating a preparation for lactation. While we cannot definitively confirm this hypothesis, it presents an interesting possibility for further research. Future investigations could explore differences in body condition associated with reproduction, assessing variations in female dolphins during pregnancy, lactation, and non-pregnant phases, as well as differences between young and older females.

Drawing from the work of Claridge *et al*. (2015), which highlights the substantial energetic demands of lactation, our study raises questions about the potential connections between body condition and pregnancy. At the time of our measurements, the dolphin calves were no more than two weeks old, suggesting that body condition might be linked to pregnancy and not yet influenced by lactation. Further research could delve into potential changes over time, clarifying on whether body condition undergoes alterations during the early stages of lactation, when endogenous nutrient reserves are crucial to support increased energy expenditure (Claridge *et al*., 2015).

The use of the Model to monitor the Sado's bottlenose dolphin community and other cetacean populations

The present study contributes to new perspectives for the monitoring of the bottlenose dolphin community in the Sado region. Our dataset provides valuable information about the overall community and also specific data that can be allocated to some individuals (table 5). Further exploring on the application of our methodologies, offers the opportunity to begin monitoring the dolphins within the community over time. This could enable the detection of anomalies related to diseases, growth rate, weight fluctuations, pregnancies, and lactation, among others. The potential is vast, and further research in this direction could yield invaluable contributions to the community's preservation. Furthermore, the potential to calculate body weight based on body mass and body density introduces yet another layer of possibilities. The data collected for BV values provides a strong foundation for the development of mathematical models to estimate the body weight of this community and track variations over time. Such data can unravel crucial insights into ecosystem dynamics, animal health, and can act as indicators of anthropogenic stressors.

We anticipate that this model can unlock further possibilities for research, such as exploring differences between regional dolphin populations, monitoring of protected groups, and tracking the growth of calves and juveniles. Findings within this project represent an important step forward in non-invasive methodologies for monitoring cetacean populations.

DOLPHIN ID	**AGE CLASS**	**TOTAL LENGTH MEAN (m)**	**BODY VOLUME MEAN (m3)**
Calf (unk)	Calf	**1.47**	**-**
Calf (GOR)	Calf	**1.24**	**0.03372**
Calf (LAM)	Calf	**0.96**	**0.095375**
Calf (BOM)	Calf	**1.52**	**0.04395**
FUT	Juvenile	**2.52**	**0.180435**
BOL	Juvenile	**2.54**	**0.245635**
COR	Juvenile	**2.52**	-
NEP	Juvenile	**2.24**	**0.131595221**
BOL	Sub-adult	**2.39**	**0.163474056**
MAR	Sub-adult	**2.41**	**0.174440332**
LAM	Adult	**2.70**	**0.167341031**
THO	Adult	**2.60**	**0.171137715**
APA	Adult	**2.95**	**0.288296525**
MED	Adult	**2.77**	**0.270794041**
AGU	Adult	**2.78**	**0.267383493**
HUX	Adult	**2.86**	**0.194791377**

DAR	Adult	**3.00**	**0.288489828**
BOM	Adult	**3.17**	**0.35191275**
GOR	Adult	**2.87**	**0.218748457**

Table 5. TL and BV obtained from some individuals of the Sado's bottlenose dolphin community.

Other Applications of UAS in cetacean Conservation

Despite not being the primary focus of this study, our observations during video analysis revealed intriguing patterns of movement, group division, and composition among the dolphins. These behavioral characteristics could be invaluable for future research projects, shedding light on activities, hunting strategies, and social interactions among dolphins. The non-invasive nature of UAS photography and videography could potentially provide an unprecedented window into these aspects of dolphin behavior. While we did not conduct a formal statistical analysis of the dolphins' responses to the UAS, our anecdotal observations suggest that drones hold substantial potential for monitoring cetacean populations beyond morphometry and body condition assessments. These aerial tools offer a multifaceted approach to gathering valuable information.

Recommendations for future research projects

Based on the present research results and challenges we suggest the following recommendations for future projects following similar methodologies:

- Precise altitude values are required to obtain accurate measurements. In consequence, the adaptation of laser ranger finder equipment is of great importance as part of the methodologies. To avoid failure in the equipment, make sure to perform regular maintenance to the device and guarantee local repair service.
- Climatological conditions, animal behaviour and other external factors may influence the possibility to comply with all the scheduled field activities. We

suggest planning of a timeline calendar with sufficient time ahead, including supplementary available dates to mitigate contingency events. The number of suitable images to be measured may be limited, therefore, we suggest to allow the scheduling of as much sessions as possible.

- Collection of metadata including date and time recording is important at the time calibration and subject identification, among others. If possible, use drone video recording systems that accurately captures image and video frame metadata, this will facilitate the postprocessing procedures.
- When working with dolphins be mindful of their behaviour. Dolphin's swimming speed can be quite challenging at the time of photographing the animals, therefore, video recording and still frame extraction is preferrable over image capturing. Additionally, when the drone is flown at lower altitudes the subjects might increase it's diving depth. Consider this when choosing the locations of video recording. Deep water may represent a challenge for the collection of images with satisfactory requirements for photogrammetry purposes.

In conclusion, despite the challenges associated with altitude estimations, our findings align closely with existing literature and local stranding networks, affirming the model's efficacy in accurately measuring body volume (BV) and total length (TL). This validation not only enhances the potential of this model for other odontocete species with similar body structures, but also underscores the utility of UAS photography as a reliable tool for cetacean monitoring.

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