

## ORIGINAL RESEARCH

# Spy in the sky: a method to identify pregnant small cetaceans

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## Abstract

Data on sex ratios, age classes, reproductive success and health status are key metrics to manage populations, yet can be difficult to collect in wild cetacean populations. Long-term individual-based studies provide a unique opportunity to apply unoccupied aerial system (UAS) photogrammetry to non-invasively measure body morphometrics of individuals with known life history information. The aims of this study were (1) to compare length measurements from UAS photogrammetry with laser photogrammetry and (2) to explore whether UAS measurements of body width could be used to remotely determine pregnancy status, sex or age class in a well-studied bottlenose dolphin population in Scotland. We carried out five boat-based surveys in July and August 2017, with concurrent photo-identification, UAS and laser photogrammetry. Photographs were measured using bespoke programmes, MorphMetriX for UAS photos and a Zooniverse project for laser photos. In total 64 dolphins were identified using photo-ID, 54 of which had concurrent UAS body length and 47 with laser body length measurements. We also measured body widths at 10% increments from 10% to 90% of body length for 48 individuals of known sex, age class and/or pregnancy status. There was no significant difference in the length of individuals measured with UAS and laser photogrammetry. Discriminant analyses of the body width–length (WL) ratios expected to change during pregnancy, correctly assigned pregnancy status for 14 of the 15 females of known pregnancy status. Only one pregnant female was incorrectly assigned as not pregnant. However, our results showed that length and body width cannot accurately allocate these bottlenose dolphins to sex or age class using photogrammetry techniques alone. The present study illustrates that UAS and laser photogrammetry measurements are comparable for small cetaceans and demonstrates that UAS measurements of body WL ratio can accurately assign pregnancy status in bottlenose dolphins.

## Introduction

Population management and conservation benefits greatly from accurate information on individuals. Anthropogenic stressors may have effects on individuals before they impact population processes, and monitoring individuals' reproductive success, health status or sex and age class structure can identify drivers of change before they affect populations (Clements et al., 2017). However, these data are not always easy to collect and commonly require long-term individual based studies with extensive field effort, often over decades (Albon et al., 1987; Clutton-

Brock & Sheldon, 2010; Wells, 2014). More recently unoccupied aerial systems (UAS) have been adopted to overcome some of these challenges, in particular utilizing photogrammetry to measure size and links to individual and population health (Durban et al., 2021; Johnston, 2019).

Reproductive success can be key to population growth (Wasser et al., 2017) but is challenging to measure in wild mammal populations when there are a lack of, or ambiguous, visual clues or characteristics (Beehner et al., 2006; Holekamp et al., 1996; Wells et al., 2014). As a result, reproductive success is often based on the

number of young that are born to mature females or the number of young that survive to a specified age (Brough et al., 2016; Festa-Bianchet et al., 2017). Although this approach is limited to populations where extensive field effort is undertaken (Beehner et al., 2006). Also, only using data on successful births where young are observed can underestimate pregnancy and birth rates, and overestimate early survival, age of sexual maturity, interbirth intervals and senescence (Burgess et al., 2012; Cheney et al., 2019; Henderson et al., 2014; Mann et al., 2000). Quantifying the number of unsuccessful pregnancies provides information on female and population health (Burgess et al., 2012; Wells et al., 2014) and can indicate the drivers of reproductive failure (Brodie et al., 2006). For example, reproductive failure has been linked to harsh environmental conditions (Wasser & Barash, 1983), decline in prey availability (Wasser et al., 2017), pollution (Kellar et al., 2017; Schwacke et al., 2002) and naturally occurring toxins (Brodie et al., 2006). Yet often reproductive failure can only be estimated in populations where animals are easy to capture or remotely sample (Beehner et al., 2006; Bergfelt et al., 2013; Pallin et al., 2018; Wells et al., 2014).

Ecological information can be especially challenging to collect for cetaceans (whales, dolphins and porpoises) that spend most of their time underwater, are long lived, highly mobile and wide ranging. Vital rates and health status have been investigated using stranded animals (Mattson et al., 2006); biopsy sampling (Kershaw et al., 2021; Pallin et al., 2018); capture and release including blood samples and ultrasounds (Bergfelt et al., 2013; Wells et al., 2004); photo-identification (Wells, 2014) and/or faecal samples (Rolland et al., 2006). However, these techniques may not be possible for all species and can be logistically difficult or present too high a risk, especially for populations of conservation concern. In addition, these methods are often limited by sample size which can result in variability in estimates and reduced power to detect trends (Kershaw et al., 2021). Long-term photo-identification studies can help to determine sex, age and reproductive rates in cetaceans (Würsig & Jefferson, 1990), but limitations exist. Age can only be determined accurately if animals are seen in their first few years of life and in species with no observable sexual dimorphism (Ralls & Mesnick, 2009) assigning sex requires genital photographs or association with a calf (Cheney et al., 2019; Cheney, Wells, et al., 2018; Mann et al., 2000). Determining pregnancy status is hampered by a lack of obvious external characteristics and as females are not seen on every occasion photo-identification can only identify successful pregnancies if the calf survives for a number of weeks, months or even years (Cheney et al., 2019; Henderson et al., 2014).

Although if a calf survives it can remain with its mother from 3 to 6 years (Wells, 2014), which can allow the identification of females and previous pregnancies. Body condition provides a proxy for individuals' health (Schick et al., 2013) but the collection of morphometric data to accurately measure size, mass and morphology of cetaceans at sea has required capturing individuals or observing them for continuous periods at the surface.

UAS have been utilized in many aspects of marine science and conservation as a non-invasive method for sampling over the large scales often needed to collect data on mobile vertebrates (Brooke et al., 2016; Fiori et al., 2017; Johnston, 2019; Seymour et al., 2017). Small, portable and affordable UAS have been applied to study abundance, distribution, movement and behaviour (Durban et al., 2015; Fiori et al., 2017; Hodgson et al., 2013; Raoult et al., 2020). UAS also allow the collection of whole-body measurements and can be calibrated with data on altitude to convert to useful body measurements (Bierlich et al., 2021; Burnett et al., 2019; Dawson et al., 2017). Measurements taken at increments along the body axis from the lateral edges of the animals' body have been shown to be a reliable measure of body width (Christiansen et al., 2016; Miller et al., 2012) and this approach has been successfully applied to study body condition, nutritional status and health (Allan et al., 2019; Christiansen et al., 2018; Christiansen et al., 2019; Durban et al., 2016, 2021). UAS techniques also provide the potential to remotely measure pregnancy, with photographs of pregnant killer whales (*Orcinus orca*) being visually wider (NMFS, 2014). Manned aerial surveys of grey whales (*Eschrichtius robustus*) revealed suspected near-term pregnancies in southward travelling whales that were wider, relative to length, than others (Perryman & Lynn, 2002) and reproductive status was determined by body shape in known adult female right whales (*Eubalaena* sp.) (Miller et al., 2012). For small cetacean populations, using metrics such as body width, that are more sensitive to changes in reproductive success or nutritional stress, enables identification of potential population level changes when animals are still alive, enabling the implementation of adaptive management approaches.

Long-term individual-based studies provide a unique opportunity to apply UAS techniques to non-invasively measure body morphometrics and monitor pregnancy status as life history information, including age, sex and reproductive status, are readily available. We utilized data from a 30-year photo-identification research study on a bottlenose dolphin (*Tursiops truncatus*) population on the east coast of Scotland (Cheney et al., 2014; Wilson et al., 1997, 1999). This population is increasing (Cheney et al., 2014), at least in part due to increasing

reproductive rates, early calf and juvenile survival and stable adult survival (Arso Civil et al., 2019; Cheney et al., 2019). Little is known about the drivers of these changes or the health of individuals in what remains a small population (Arso Civil et al., 2021) whose habitat is close to many anthropogenic activities (Graham et al., 2017; Pirota et al., 2015). Also, although reproduction is increasing there is interannual variability in the number of calves identified. Whether this variability is driven by low pregnancy rates or poor early calf survival is unknown.

The aims of this study were to compare length measurements from UAS photogrammetry with existing laser photogrammetry methods regularly used to measure body length in this protected population (Cheney, Wells, et al., 2018). Then to explore whether aerial measurements of body width and/or length enable remote determination of pregnancy status and sex or age class within this well-studied bottlenose dolphin population.

## Materials and Methods

### Study population

This study was conducted on a population of 224 (2015–2019 weighted mean estimate, with lognormal 95% confidence interval 214–234; Arso Civil et al. [2021]) bottlenose dolphins using the Moray Firth Special Area of Conservation (SAC) (92/43/EEC) (Cheney et al., 2014; Cheney, Graham, et al., 2018; Wilson et al., 1997, 1999) (Fig. 1). Photo-identification surveys are carried out annually from May to September, under NatureScot Animal Scientific Licences. Individual dolphins are matched to the existing Universities of Aberdeen and St Andrews catalogue of known individuals from the east coast of Scotland. Sex is determined using available genital photographs or if adults are seen in repeat associations with a known calf. The year of birth of calves, up to 2 years old, is estimated from photographs, based on their colour, size, foetal folds and behaviour (Grellier et al., 2003). When the year of birth is unknown adults are identified as individuals who have been photographed for at least 10 years. Reproductive females are identified if they are observed with the same calf on more than one occasion. In the last decade, 226 individual dolphins were identified within the SAC, with sex, age and reproductive status identified for 59%, 64% and 34% respectively. Of these 226 individuals 80% have repeat annual sightings.

Laser photogrammetry photographs are taken concurrently with photo-identification on every survey (Fig. 1). This technique involves two laser sights fixed horizontally to a camera, 10 cm apart and parallel, which are projected onto the dolphin during photo-identification,

providing a scale on the image (for full details see Cheney, Wells, et al., 2018). Using this scale the distance from the dolphins' blowhole to dorsal fin (BH-DF) is measured using a bespoke Zooniverse project (<https://www.zooniverse.org/projects/bjcheney/laser-photogrammetry>). Body lengths are estimated using the relationship between the BH-DF and body length derived from measurements of stranded individuals (for full details see Cheney, Wells, et al., 2018).

### Data collection

During 24 July to 1 August 2017, five surveys were carried out from our 5.8 m photo-identification rigid inflatable boat when the Beaufort Sea State was  $\leq 3$ . During these surveys, high-resolution aerial photographs were taken using a LemHex-44 UAS (long endurance marine hexacopter) fitted with a Sony A5100 24-megapixel CMOS mirrorless camera, Sony E 50 mm F1.8 OSS fixed focal length lens and Lightware SF11/C laser altimeter. Designed and built by Duke University's Marine Robotics and Remote Sensing (MaRRS) laboratory the camera and laser altimeter are co-located on a two-axis gimbal, which provides the functionality to tilt the camera to locate and position over the animals, and then hold nadir when taking images. This negates any post-processing compensation for tilt in the image or laser altitude measurement. The UAS was launched and recovered from our research vessel by hand and flown at c. 30 m above groups of dolphins for a maximum of 25 min (Fig. 1). Due to the fast surfacing sequence of these small cetaceans, images were taken at a burst rate (up to six frame per second) to ensure a useable image of each individual was collected. UAS flights were conducted under UK Civil Aviation Authority permit ref.: 20170711DukeUniversityMarineRoboticsAndRemoteSensingLabPAndEUAV4313 by an experienced pilot (>1000 flights) who has conducted photogrammetry operations over a range of cetacean and pinniped species worldwide. Individual dolphins were tracked underwater using the first person view live feed from the photogrammetry camera and image bursts taken as the individuals began to surface. The pilot communicated these events to the boat-based photographer to synchronize the aerial photogrammetry and photo-identification (Fig. 2).

Matches were made between the photo-identification and UAS images using calibrated image times (GPS time synchronization), the location of identifiable marks, such as tooth rakes and skin lesions on the body of each dolphin, and the position of the dolphin in the group (Fig. 2). Good quality UAS photogrammetry images were selected based on (1) the individual had just broken or was just below the surface; (2) the animal was straight in



**Figure 1.** UAS photogrammetry surveys from a small RIB using a LexHex-44 hexacopter, including manual hand launch and recovery, operation ~30 m above a group of bottlenose dolphins with first-person view screen for the operator and concurrent photo-identification and laser photogrammetry. Inset map of the study area, the Moray Firth Special Area of Conservation, Scotland. UAS, unoccupied aerial system.

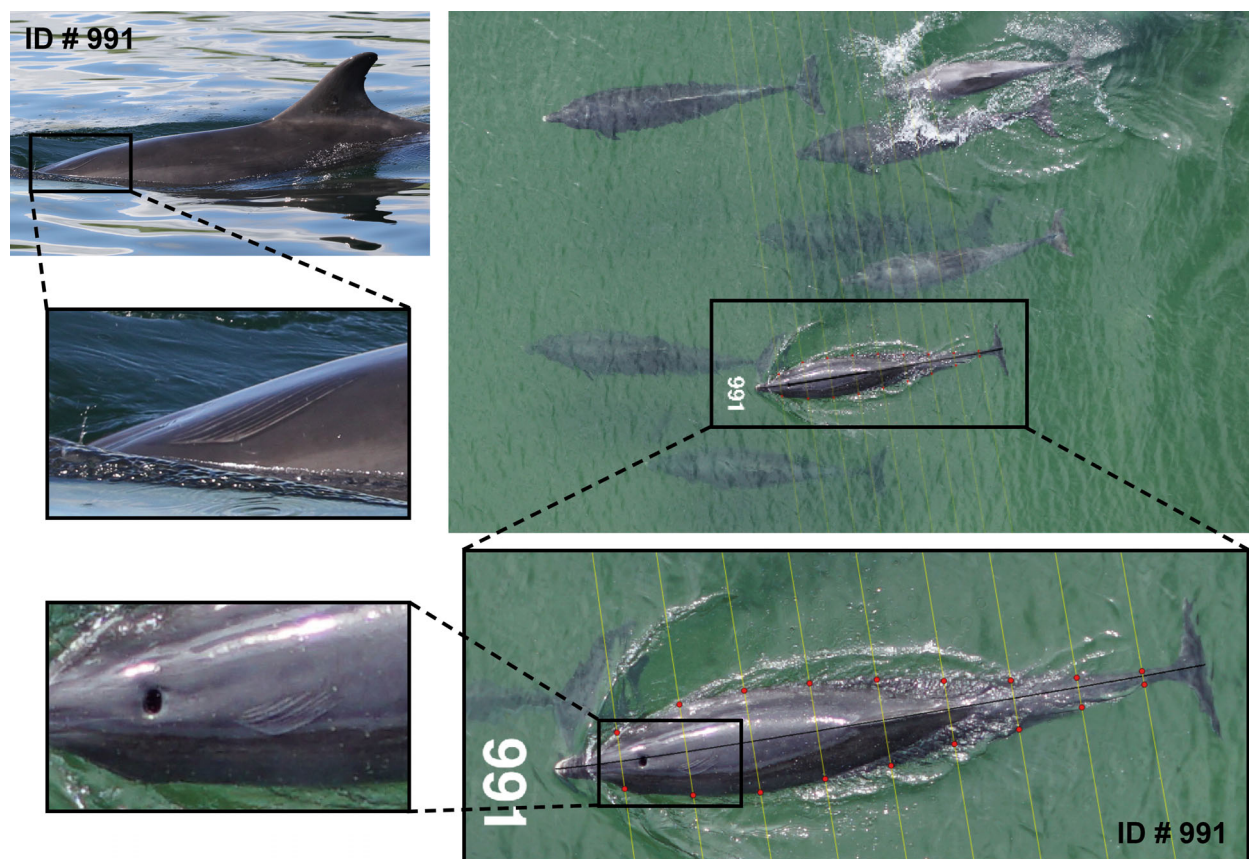
the horizontal axis; (3) the fluke was close to horizontal by interpretation of the width to length ratio of the fluke and (4) the contours of the animal could be identified and were not significantly obstructed by water distortion or glare. The lengths and widths of individual dolphins in each good quality photograph were measured once using the programme MorphMetriX (Torres & Bierlich, 2020) (Fig. 2). To measure length the tip of the rostrum and the end of the tail notch were marked and a Bezier fit line function was ‘drawn’ along the spine using the blowhole, dorsal ridge and peduncle ridge as a guide. To measure width an inbuilt ‘width segments’ function was used to provide a guide, perpendicular to the length line, at 10% increments along the body axis. Images were selected and measured by a researcher experienced in aerial photogrammetry and the measurement programme. A similar approach has been used to measure body width in other marine mammal studies (Miller et al., 2012).

Pregnant females were identified when repeatedly seen with a newborn calf in 2017 (after the UAS flights) or with a 1-year-old calf in 2018. The majority of females in this population give birth when their current calf is at least 3 years old (Cheney et al., 2019), with an average interbirth interval of 4.5 years (Arso Civil et al., 2017), and there is no evidence that females in this population give birth in consecutive years (Cheney et al., 2019). Therefore, females were identified as not pregnant if they were seen repeatedly with a newborn calf in 2016, 2018 or before the UAS flights in 2017, or if they were seen with a 2-year-old calf in 2017.

## Analyses

To investigate the precision of the UAS measurements the coefficient of variation (CV) was calculated from measurements of length and width of the same individual dolphin across different photographs. Model II ANOVAs were performed to establish if variance within measurements of both length and widths of individuals in different photographs was less than measurements between different individuals (Webster et al., 2010). Wilcoxon signed-rank test and linear regression were used to compare length measurements estimated using both UAS and laser photogrammetry. All statistical analyses were carried out in R version 4.0 (R Core Team, 2019).

To investigate differences in body width–length (WL) ratio between pregnant and not pregnant females, we used a beta regression model using the *betareg* package (Cribari-Neto & Zeileis, 2010) with the WL ratio as the dependant variable. Beta regression models were used as WL ratios are a continuous proportion between zero and one, ensure the proportions remain at their original scale and allows simpler statistical inferences (Douma & Weedon, 2019). To explore WL ratio as a predictor of pregnancy in female dolphins, a linear discriminant analysis (LDA) with jackknifed prediction was performed in the ‘MASS’ package (Venables & Ripley, 2002). This discriminant analysis used the body widths expected to change during pregnancy (20–60% widths/increments along the body axis) (Fig. 2). The 10% width (dolphins’ head) and 70–90% widths (peduncle/tail stock) were excluded based



**Figure 2.** Matching and measuring bottlenose dolphin UAS photogrammetry. Photo-identification picture of ID#991 (top left), and rakes near the blowhole provided match to UAS photo on 30 July 2017 (top right). ID#991 measured in UAS photo (right) using MorphMetriX software with length (straight black line) and 10–90% body widths (red dots on yellow line). UAS, unoccupied aerial system.

on the assumption that pregnancy would not be reflected in the head or tail regions. Shapiro–Wilk tests were carried out in R and the WL ratios used showed multivariate normality (Shapiro & Wilk, 1965). The LDA also determined if pregnancy could be predicted for four females of unknown pregnancy status (i.e. not seen with a calf in 2016, 2017 or 2018).

Linear models were used to assess differences in length and body width in dolphins of different sex or age class (newborn <6 months, calf = 1–3 years, juvenile = 4–5 years, sub-adult = 6–9 years, adult  $\geq 10$  years old). To investigate differences in WL ratio between male and female dolphins, we applied a beta regression model (Cribari-Neto & Zeileis, 2010) with the 10–90% WL ratio as the dependant variable. To explore differences in WL ratio across age class individual beta regression models for each WL ratio were used. To determine which interaction terms between age classes were significantly different, post-hoc pairwise comparisons were run using the ‘em-means’ package (Lenth, 2020). Shapiro–Wilks tests

showed that for each age class most mean length and width measurements were normally distributed. Therefore the ability to assign age class based on length and/or body width was explored with a LDA with jackknifed prediction in the ‘MASS’ package (Venables & Ripley, 2002).

## Results

### Accuracy and repeatability of UAS photogrammetry

UAS photographs were taken during 11 flights on five surveys including 12 encounters with groups of bottlenose dolphins from 24 July to 1 August 2017. A total of 64 dolphins were identified via boat-based photo-identification images and matched to individuals in the existing catalogue. Sixty of these dolphins were also identifiable in UAS photographs with 249 UAS photographs of good quality for measurement of 54 individuals (Table 1). Therefore, 84% of the dolphins identified using

**Table 1.** The number of bottlenose dolphins for which UAS and laser photogrammetry measurement data were obtained, by sex, age class (new-born <6 months, calf = 1–3 years, juvenile = 4–5 years, sub-adult = 6–9 years, adult ≥10 years old) and pregnant females.

Technique	Number of individuals	Sex			Known age class					
		Males	Females	Unknown	Newborn	Calves	Juveniles	Sub-adults	Adults	Known pregnant
UAS	54	15	27	12	1	10	8	6	29	8
Laser	47	14	25	8	0	8	6	6	27	7

UAS, unoccupied aerial system.

photo-identification were measured for length and body width. The number of photographs available for measurement varied across individuals (mean = 5, range 1–12) (Table S1). Length measurements were made for all 54 dolphins and all width measurements of 10–90% increments were possible for 48 dolphins. Due to image clarity no width measurements were possible for four dolphins and only partial width increment measurements were possible for two dolphins (50% and/or 60% increments missing) (Table S2). On each survey slightly more individuals were identified from boat-based images compared to UAS images, with 44 dolphins photographed and identified on only one UAS survey and 10 individuals photographed on two or more UAS surveys (Table S3).

Laser photogrammetry photographs were obtained for 47 of the 54 individuals measured in UAS photographs (Table 1). Laser photogrammetry photographs taken during this study were used to measure 46 individuals, and photographs from 2007 to 2018 were used to aid measurements for one adult, and for additional measurements of nine other adults. The number of photographs available for laser measurement varied across individuals (mean = 8, range 2–17) (Table S1).

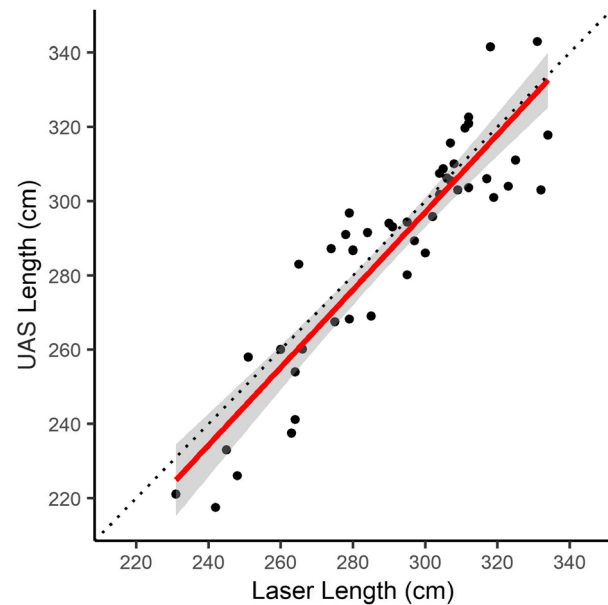
The mean CV for length of the same individual in different UAS photographs was 3.8% (range 0–10.85%) (Table S1). The CV of width measurements of different photographs of the same individual ranged from 0.12% to 37.46%, with the biggest variability seen on the narrow areas of the body (10% and 70–90% body width increments), range of 1.12–37.76%. For the 20–60% body width values the CV ranged from 0.12% to 14.92% (Table S2). There was significantly less variation in length measurements from multiple photographs for a single individual compared to length measurements between individuals (model II ANOVA,  $F_{45,195} = 55.43$ ,  $p < 0.0001$ ). There was also significantly less variation in the width measurements from multiple photographs of the same individual than between individuals (model II ANOVA,  $p < 0.05$ , Table S4).

There was no significant difference between length measurements made using UAS and laser photogrammetry for the same individual (Wilcoxon test,  $V = 692.5$ ,  $p > 0.05$ ) and there was a significant linear relationship

between the lengths obtained from the two different techniques (adjusted  $R^2 = 0.81$ ,  $F_{1,45} = 202.7$ ,  $p < 0.0001$ , Fig. 3). On average length measurements using UAS photogrammetry were 3.4 cm smaller than those estimated using laser photogrammetry (range = –29.0 to 23.6 cm) (Tables 2 and 3; Table S1). Average length measurements of each age class were also similar between UAS and laser photogrammetry, with only 2–3 cm differences for juveniles, sub-adults and adults, and 8 cm for calves (Table 3). Accuracy error was calculated at <1% from a controlled scale experiment for UAS photogrammetry and for laser photogrammetry estimates of length just over 2% of the physical length of dolphins measured during health assessments (Cheney, Wells, et al., 2018).

### Determining pregnancy status

Using body WL ratios showed that pregnant females ( $n = 6$ ) were significantly wider at 40% and 50% body

**Figure 3.** Relationship between UAS and laser photogrammetry measurements of length (red line), with 95% confidence limits (grey shading) and 1 to 1 line (dotted). UAS, unoccupied aerial system.

**Table 2.** Mean (and standard error) UAS measurements of length (cm) and width increments (cm) and laser measurements of length (cm) of pregnant and not pregnant females, adult male and adult female bottlenose dolphins.

	Pregnant	Not pregnant	Adult male	Adult female
UAS length	306 (4.16) (n = 8)	295 (4.44) (n = 10)	313 (5.43) (n = 8)	304 (3.30) (n = 21)
Laser length	306 (4.98) (n = 7)	297 (4.25) (n = 9)	316 (5.66) (n = 8)	305 (2.83) (n = 19)
10% width	25.2 (0.39) (n = 7)	25.0 (0.60) (n = 9)	26.5 (0.69) (n = 8)	25.4 (0.32) (n = 19)
20% width	43.0 (0.72)	41.9 (0.40)	44.3 (0.60)	42.5 (0.40)
30% width	54.1 (0.96)	50.5 (0.68)	52.7 (0.47)	52.0 (0.67)
40% width	56.4 (1.93)	49.6 (0.81)	51.6 (0.47)	52.3 (1.05)
50% width	50.9 (1.35)	43.8 (0.77)	47.1 (0.58)	46.5 (1.16)
60% width	38.4 (1.34) (n = 6)	35.3 (0.53)	39.0 (0.58)	37.0 (0.80) (n = 18)
70% width	25.4 (0.76)	23.6 (0.36)	27.0 (0.64)	25.1 (0.59)
80% width	14.2 (0.52)	13.6 (0.23)	15.6 (0.63)	14.6 (0.62)
90% width	6.6 (0.28)	6.4 (0.31)	7.0 (0.22)	6.7 (0.20)

Note: The samples sizes for the body width increment measurements are the same as the 10% width unless otherwise noted. UAS, unoccupied aerial system.

**Table 3.** Mean (and standard error) UAS measurements of length (cm) and width increments (cm) and laser measurements of length (cm) of bottlenose dolphin age classes (newborn <6 months, calf = 1–3 years, juvenile = 4–5 years, sub-adult = 6–9 years, adult ≥10 years old).

	Newborn	Calf	Juvenile	Sub-adult	Adult
UAS length	139 (n = 1)	243 (6.34) (n = 10)	275 (5.14) (n = 8)	286 (3.79) (n = 6)	306 (2.88) (n = 29)
Laser length	–	251 (4.05) (n = 8)	273 (4.17) (n = 6)	284 (3.41) (n = 6)	309 (2.72) (n = 27)
10% width	13.3 (n = 1)	21.4 (0.61) (n = 9)	22.9 (0.71) (n = 8)	23.7 (0.34) (n = 5)	25.7 (0.31) (n = 27)
20% width	20.7	34.7 (0.89)	37.8 (0.94)	40.0 (1.21)	43.0 (0.36)
30% width	24.1	41.3 (0.82)	46.8 (1.38)	50.0 (1.36)	52.2 (0.49)
40% width	24.1	41.8 (0.81)	46.1 (1.48)	49.3 (0.71)	52.1 (0.74)
50% width	19.8	37.3 (0.86)	39.8 (1.23) (n = 7)	44.0 (0.63)	46.7 (0.82)
60% width	15.0	29.8 (0.58)	32.0 (1.15) (n = 7)	35.0 (0.64)	37.6 (0.60) (n = 26)
70% width	10.1	19.9 (0.64)	21.7 (0.78)	23.7 (0.56)	25.7 (0.48)
80% width	6.6	11.5 (0.53)	12.6 (0.40)	14.1 (0.51)	14.9 (0.48)
90% width	7.9	6.1 (0.16)	6.6 (0.47)	6.6 (0.40)	6.8 (0.16)

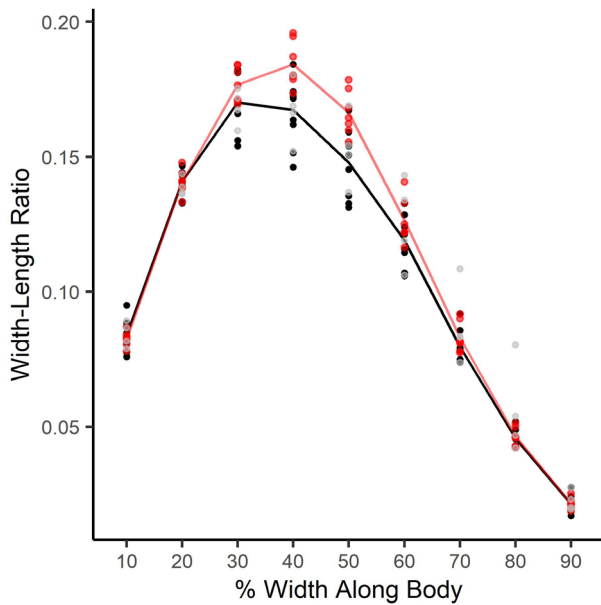
Note: the samples sizes for the body width increment measurements are the same as the 10% width unless otherwise noted. UAS, unoccupied aerial system.

widths than not pregnant females (n = 9) (beta regression,  $z = 8.454$ ,  $p < 0.001$ , Fig. 4, Table 2, Table S5, Fig. S1). The discriminant analyses assessed the ability to classify adult female dolphins as pregnant or not based on their body WL ratios expected to change during pregnancy (20–60% body width increments). The 10% and 70–90% body widths were not included as dolphins head and tail regions were not expected to change during pregnancy. We correctly assigned pregnancy status for 14 of the 15 females. One female was incorrectly assigned as not pregnant although she was seen with a ~1-year-old calf in 2018. Of the four reproductive females that could have been pregnant (i.e. not seen with a calf in 2016, 2017 or 2018) all were assigned with a not pregnant status (3 at >99% and 1 at 65% certainty) by the discriminate analyses.

### Determining sex and age class

UAS measurements produced similar results to laser photogrammetry for this population (Cheney, Wells, et al., 2018) suggesting adult females (mean = 304 cm, range 277–323 cm) were on average smaller than adult males (mean = 313 cm, range 292–342 cm), but this was not significant (linear model,  $F_{1,26} = 4.03$ ,  $p > 0.05$ ). WL ratio also showed no significant difference between adult males and females (beta regression,  $z = 10.79$ , interaction  $p > 0.05$ , Fig. 5A, Table S6).

There were significant differences in length (Table 3) between all age classes except juveniles and sub-adults (linear model,  $F_{4,49} = 52.59$ ,  $p < 0.05$ , Table S7). There was also a significant difference in width measurements between the age classes that are further apart, but not



**Figure 4.** Comparison of width-length ratio at 10% increments along the body of adult females. Pregnant individuals (red dots) and not pregnant individuals (black dots) with the black and red lines representing the average for each. Grey dots indicate the WL ratio of four females of unknown pregnancy status. WL, width-length.

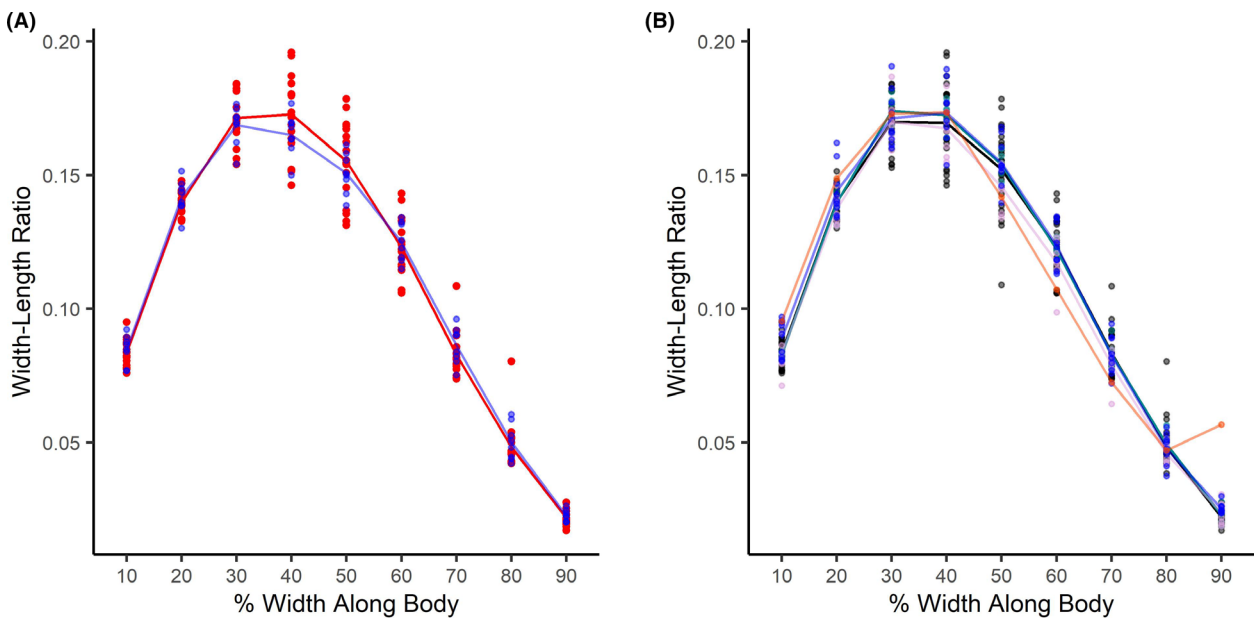
between sub-adults and adults or juveniles (linear model,  $F_{44,402} = 339.6$ , Table S8). However, the discriminate analyses suggested that neither length nor body width are

completely reliable in determining age class as approximately one third of individuals were assigned to the incorrect age class (Table S9). There were no significant differences in WL ratio between the age classes (Fig. 5B, Table S10) suggesting dolphins retain similar body dimensions throughout their lives.

## Discussion

This study demonstrates that morphometric measurements from UAS photographs are repeatable and comparable with laser photogrammetry. Using UAS photographs we demonstrate accurate assignment of pregnancy status for females in a bottlenose dolphin population. Our results also show that length and body width calculated from UAS images cannot accurately allocate these bottlenose dolphins to sex or age class.

Our results showed that the variation in length and width measurements of the same individual in multiple photographs was less than the variation between individuals. Our mean CVs of length and width were comparable, although generally higher than found in right whales (1.3–11.7% [Miller et al., 2012]). This is perhaps unsurprising as the body widths of the small cetaceans in our study are  $\leq 50$  cm compared to  $\geq 1$  m for these larger whales and lengths were  $< 4$  m compared to  $\sim 13$  m. Similar to other studies of larger cetaceans (Miller et al., 2012; Perryman & Lynn, 2002) the CVs increased as the size of the body width being measured decreased. Our length measurements



**Figure 5.** Comparison of width-length ratio at 10% increments along the body of (A) adults males (blue) and adult females (red) with lines representing the average for each sex and (B) different age classes, adults (black), sub-adults (green), juveniles (purple), calves (blue) and newborn calf (orange) with lines representing the average for each age class.



from UAS photogrammetry were in line with the robust laser photogrammetry estimates that have been used to measure the length of dolphins in this population since 2007 (Cheney, Wells, et al., 2018). To the best of our knowledge this is the first study to compare boat-based laser and UAS photogrammetry length measurements and show they are comparable in small cetaceans.

Data on pregnancy rates and reproductive success are important to assess both the health of the population and to monitor individual fitness. Reproductive failure is often high and undetected in mammals (Wasser & Barash, 1983) and can be a key constraint in population growth (Wasser et al., 2017). Our results show that body width measurements are wider at 40% and 50% for pregnant females, although it is debateable whether pregnancy is as visually apparent in bottlenose dolphins (Fig. S1) as it is in killer whales (NMFS, 2014). In addition, a discriminant analysis using multiple body WL ratios correctly assigned pregnancy status to all not pregnant females and only one pregnant female was incorrectly assigned. It is possible that this female was in an earlier stage of pregnancy, however, she was not seen again in 2017 and was next photographed in 2018 with a ~1-year-old calf. Four of the other pregnant females were also not seen with a calf until 2018. Yet this method could help estimate how many calves are lost *in utero*, often an unknown factor in wild populations, potentially providing information on female health. High reproductive failure is a good indicator of nutritional stress (Kershaw et al., 2021; Wasser et al., 2017) as females are not able to accumulate sufficient energy reserves to successfully complete pregnancy and produce a live calf (Kershaw et al., 2021). Females may reduce investment in reproduction in the short term when conditions are not favourable for their longer-term survival (Kershaw et al., 2021; Wasser & Barash, 1983). Identifying whether pregnancy failure or calf survival are influencing populations' reproductive success could help to establish the intrinsic and/or extrinsic drivers and determine conservation interventions. For example, early calf survival can be influenced by a number of other factors including female aptitude (Henderson et al., 2014), predation (Fearnbach et al., 2012; Mann et al., 2000), boat strikes and entanglements (Steiner & Bossley, 2008) or infanticide (Patterson et al., 1998).

Of the four females that could have been pregnant in 2017 (i.e. they were not seen with a calf in 2016, 2017 or 2018) all were predicted as not pregnant using the discriminant analyses. This implies no females in our study lost their calf during pregnancy or before the calf was observed. Although our sample size is small ( $n = 19$ ) this reproductive success is higher than in other bottlenose dolphin populations where reproductive success determined using blood samples and ultrasounds during health

assessments ranged from 50% ( $n = 20$ ) (Bergfelt et al., 2013) to 83% ( $n = 12$ ) (Wells et al., 2014). This suggestion of a low level of reproductive failure aligns with previous studies that have shown the east coast of Scotland population is increasing (Cheney et al., 2014) at least in part as a result of increasing reproduction (Cheney et al., 2019). It is important to note that our study was conducted late in the dolphins' pregnancy as the majority of calves in this population are born during August (Cheney et al., 2019), although newborn calves have been identified as late as October (Cheney et al., 2019; Grellier, 2000). Female right whales width measurements were most variable at different stages of the reproductive cycle (Miller et al., 2012). However, in a killer whale population where pregnancies were detected using faeces 33% of the unsuccessful pregnancies failed late in gestation or immediately post-partum (Wasser et al., 2017). To determine if pregnancy status can be accurately determined earlier repeat UAS surveys could be conducted over several months during Spring and Summer. In addition, the success of identifying pregnancy opens up the feasibility of investigating the unusual life history strategy of reproductive senescence by identifying older adult females that are no longer reproductive or determining the more common decline in reproduction as a result of age (Ellis et al., 2018; Karniski et al., 2018).

Sex ratio and age structure of a population are useful to increase the accuracy of state-structured population models by providing information on population growth rate, but both can be difficult to obtain without capture or biopsy sampling (Booth et al., 2020; Wells, 2014). Our results suggest that allocating bottlenose dolphins to either sex or age class from length or body width is challenging due to individual variability, and we found the body shape of dolphins that were not pregnant was consistent irrespective of age or sex. This study does provide baseline information on widths of individuals of different age, sex and reproductive status. In the future this could allow us to explore changes in body condition and corresponding health status (Durban et al., 2021; Schick et al., 2013) in response to environmental or anthropogenic effects.

With an experienced UAS pilot, 84% of the dolphins identified using photo-identification were measured for length and body width via UAS photogrammetry. This equated to 45% of the dolphins identified in this area in 2017 and approximately one quarter of the entire population. Small cetacean photo-identification catalogues tend to focus on marks on the dorsal fin. We found concurrent photo-identification with calibrated time was essential to allow matching of dolphins to our existing dorsal fin catalogue and facilitate matching to the UAS photographs. Photo-identification also provided up-to-date photographs to match new tooth rake and skin lesion

marks. With the development of a UAS photo catalogue concurrent photo-identification should become less necessary. In addition, more useable photographs were collected when cohesive groups, especially those travelling, were targeted. In the murky temperate waters of the Moray Firth we found Beaufort Sea State (<3) and water depth (<10 m) an important determinant of both individual identification (tooth rakes and skin lesions were visible) and sharpness of the dolphins' body outline for accurate measurement (Fig. S2). Due to the fast surfacing sequence of these small cetaceans a high frame rate photogrammetry camera also proved highly beneficial.

This study provides a snapshot of known individuals in a protected population showing the utility of UAS photogrammetry combined with life history information to routinely monitor not only reproductive failure but also body condition in small cetacean populations. Laser and UAS photogrammetry measurements were consistent, body width measurements (especially at the wider widths) were repeatable, and a high proportion of the total population was photographed and measured with only five boat trips. UAS photogrammetry has already been used to investigate body condition in larger cetaceans (Christiansen et al., 2018; Durban et al., 2016, 2021; Miller et al., 2012; Schick et al., 2013) and seals (Allan et al., 2019), and has the capability to estimate body mass (Christiansen et al., 2019; Noren, 2011). Long-term photo-identification studies provide the potential to obtain repeat measurements of individuals over time, allowing monitoring of changes in both body condition and reproductive success/failure. Data on body condition and failed pregnancies provide information on individuals' nutritional health and help identify the drivers of changes in vital rates and population dynamics (Christiansen et al., 2016; Nowacek et al., 2016). For example, body condition in grey whales was linked to temporal variation in prey availability and/or quality (Soledade Lemos et al., 2020), and in humpback whales (*Megaptera novaeangliae*) the probability of seeing a female with a calf was related to prey availability (Kershaw et al., 2021). All of which could facilitate our understanding of energetic and prey requirements for this and other bottlenose dolphin populations.

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## Data Accessibility

All the measurement data presented here and the R code used for the analyses are accessible on Dryad Digital Repository doi:10.5061/dryad.f7m0cfxz2.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** UAS and laser photogrammetry measurements of the blowhole to dorsal fin distance and length (with standard error and coefficient of variation) of 54 dolphins from 2017 with their age in 2017 (AD = adult if seen for at least 10 years), sex (1 = male, 2 = female) and pregnancy status (see Methods) and the number of photographs measured.

**Table S2.** UAS photogrammetry measurements of widths (standard error and coefficient of variation) along the body of 54 bottlenose dolphins from 2017 with their sex, age and pregnancy status (NA where width measurement could not be made due to image clarity).

**Table S3.** Number of dolphins identified using photo-identification and measured in good quality UAS photogrammetry images on each of five boat-based surveys.

**Table S4.** Results of model II ANOVA investigating where there are measurements of multiple photographs that variance between individuals is greater than variance within individuals.

**Table S5.** Parameter values from the beta regression comparing width–length (WL) ratios and pregnancy status of adult female bottlenose dolphins at width measurement sites between 10% and 90% body lengths (BLs) from the rostrum. The *p*-value is also provided, and significant relationships are indicated in bold.

**Table S6.** Parameter values from the beta regression comparing width–length (WL) ratios for adult male and female bottlenose dolphins at width measurement sites between 10% and 90% lengths from the rostrum. The *p*-value is also provided.

**Table S7.** Results from the linear model with post-hoc pairwise comparison investigating difference in lengths of bottlenose dolphin between the age classes (newborns, calves, juveniles, sub-adults and adults). The *p*-value is also provided, and significant relationships are indicated in bold.

**Table S8.** Results from the linear model post-hoc pairwise comparison, comparing widths of bottlenose dolphin newborns, calves, juveniles, sub-adults and adults at width measurement sites between 10% and 90% lengths from the rostrum. The *p*-value is also provided, and significant relationships are indicated in bold.

**Table S9.** Classification results from discriminant analyses to assess whether age class can be determined using length and body widths in bottlenose dolphins, (A) length and (B) length and all body widths.

**Table S10.** Results from the beta regression post-hoc pairwise comparison, comparing width–length (WL) ratios of bottlenose dolphin newborns, calves, juveniles, sub-adults and adults at width measurement sites between 10% and 90% lengths from the rostrum. The *p*-value is also provided, and significant relationships are indicated in bold.

**Figure S1.** Aerial photographs taken in the Moray Firth of (A) pregnant and (B) not pregnant females.

**Figure S2.** Aerial photographs taken in the Moray Firth from 24 and 30 July 2017 at different depths and Beaufort Sea states, (A) 4.8 m, sea state = 1, (B) 7 m, sea state = 2, (C) 17.9 m, sea state = 3.