

Variation in female reproductive tract morphology of the common bottlenose dolphin (*Tursiops truncatus*)

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Abstract:

Cetaceans exhibit vaginal folds, unusual protrusions of the vaginal wall into the vaginal lumen. Inconsistent terminology and a lack of anatomical landmarks in the literature have hindered comparative studies of the form and function of vaginal folds. Our objectives are to: 1) develop a standardized measurement protocol for the reproductive tracts of female cetaceans, 2) assess variation in morphometrics within the common bottlenose dolphin (*Tursiops truncatus*), and 3) determine if vaginal muscle is skeletal, and therefore of somatic origin in this species. We selected 15 measurements to characterize female reproductive tracts and evaluated variability using fresh or frozen-thawed specimens from southeastern USA representing a range of sexual maturity states and reproductive states (n = 18 specimens). Presence of skeletal muscle and variation in the density of muscle banding were assessed using 90 histological samples (n = 5 specimens). Analyses of the gross morphological data revealed that the dolphins generally had one large vaginal fold that bisected the vaginal lumen. Vaginal morphology was similar for sexually mature and immature specimens and across reproductive states. The histological data revealed that the vaginal musculature consisted of smooth muscle, consistent with other mammals, leading us to conclude that vaginal contractions are likely under autonomic rather than somatic control. No differences were found in the density of smooth muscle banding among vaginal regions or sexual maturity states. Our systematic protocol lays the foundation for evaluating the function (e.g., sexual selection, natural selection) and evolution of vaginal folds.

Keywords: genital diversity, reproductive tract morphology, reproductive biology, vaginal fold, common bottlenose dolphin

Introduction:

In many taxonomic groups, male genitalia have been preferentially described as compared to female genitalia (Ah-King et al., 2014). Most existing publications on the female genitalia of cetaceans (whales, dolphins, and porpoises) have focused on the ovaries that can be used to infer life history characteristics (Sljper, 1966; Harrison and Ridgway, 1971; Perrin et al., 1984; Marsh and Kasuya, 1986; Plön and Bernard, 2007; Dabin et al., 2008), or genital diseases and anomalies (Woodhouse and Rennie, 1991; Van Bresseem et al., 2000; Van Elk et al., 2009). Few internal measurements of cetacean vaginal morphology have been routinely collected during necropsies over the past few decades.

The cetacean vagina contains unusual transverse fold(s), first described by Hunter (1787). These protrusions of the vaginal wall often occur in the cranial portion of the vagina, caudal to the cervix (Schroeder, 1990). Vaginal folds are most prominent on the dorsal vaginal wall and project into the lumen of the vagina, with the distal ends directed towards the vulva (Pycraft, 1932). Various terms have been used to designate these cylindrical vaginal structures, including transverse rugae (Jackson, 1845), valvular folds (Murie, 1873), spermathecal folds (Meek, 1918), pseudo-cervices (Pycraft, 1932; Schroeder, 1990), circular folds (Ommaney, 1932; Green, 1977; Tarpley and Hillman, 1999), vaginal folds (Morejohn and Baltz, 1972; Clarke et al., 1994), and rings of transverse folds (Chen et al., 1984). Across multiple species, these transverse vaginal folds have also been described as pleated, triangular-shaped, saucer-shaped, rugose, deep, leaf-like, crisscrossed, and puckered (Murie, 1873; Pycraft, 1932; Chen et al., 1984). Existing publications on the presence, numbers, shapes, and sizes of these folds are dated and inconsistent (Scott and Parker, 1889; Meek, 1918; Pycraft, 1932; Harrison, 1949; Morejohn and Baltz, 1972;

Harrison and McBrearty, 1973; Schroeder, 1990; Woodhouse and Rennie, 1991; Clarke et al., 1994). For example, Meek (1918) reported two vaginal folds in the harbor porpoise (*Phocoena phocoena*), while Daudt (1898) reported nine to twelve folds in the same species. Morejohn and Baltz (1972) reported an absence of vaginal folds in immature Dall's porpoises (*Phocoenoides dalli*), because vaginal structures were not funnel-shaped, while the corresponding image showed numerous circular vaginal folds. When measurements have been provided, accompanying images and descriptions of anatomical landmarks have often been missing, which makes it difficult to reproduce these data (e.g., Murie, 1873). While some of the incongruence is likely due to species-specific diversity, standardized terminology and a protocol of measurements with clearly defined anatomical landmarks are necessary precursors to explore diversity and functionality.

The functions of cetacean vaginal folds are unclear and alternative functional hypotheses are not mutually exclusive (Clarke et al., 1994). It is most commonly hypothesized that the vaginal folds relate to mating in water, although their exact function is not clear and empirical tests of mechanisms are lacking. Vaginal folds may be adaptations to prevent the incursion of seawater into the upper reproductive tract once the penis is withdrawn (Slijper, 1962; Green, 1972; Green, 1977; Chen et al., 1984; Schroeder, 1990; Robeck et al., 1994). For example, the vaginal folds could “squeegee” seawater off the penis as it penetrates the cranial vagina (T. Robeck, pers. comm.). This hypothesis is supported by evidence that seawater is lethal to cetacean sperm (Schroeder and Keller, 1989). Vaginal folds have also been hypothesized to serve as vaginal plugs to prevent the loss of semen (Meek, 1918; Harrison, 1969). Retention of semen inside the female reproductive tract could be particularly important as male cetaceans lack

the seminal vesicles and bulbourethral glands that typically facilitate coagulation of semen (Slijper, 1966; Harrison, 1969). Alternatively, the contact of vaginal folds with the penis could stimulate ejaculation (Meek, 1918; Harrison, 1969). The function of vaginal folds could also be associated with pregnancy rather than mating. For example, the folds could prevent miscarriages during pressure changes while diving (Kellogg, 1938) or distend during parturition and ease the birthing process (Meek, 1918; Slijper, 1962). Similar folds have been reported in some closely related terrestrial artiodactyls (e.g., domestic pigs, *Sus domesticus*; Dyce et al., 2010), potentially indicating that vaginal folds are not an adaptation to an aquatic environment and could be inherited through a common ancestor (Pabst et al., 1998).

Based on what little evidence exists, cetacean vaginal folds are muscular and capable of contractile movement (Harrison, 1949; Chen et al., 1984). The ability to contract may help elucidate a potential function. Harrison (1949) hypothesized that vaginal folds engage in a pumping action that directs seminal fluids cranially towards the uterine horns where fertilization occurs. Alternatively, the pumping action of vaginal muscles could shunt sperm caudally away from the uterine horns. For example, in both rats and cats, uterine contractions are propagated in both cranial and caudal directions, with caudal-oriented peristalsis expected to carry sperm away from the uterine horns (Crane and Martin, 1991; Chatdarong et al., 2002). The vaginal folds of cetaceans project caudally towards the vaginal opening, potentially facilitating the caudal direction of sperm expulsion. In diverse terrestrial taxonomic groups, females can selectively reject sperm by shunting it within their reproductive tracts away from sites of fertilization (Eberhard, 1996; Dean et al., 2011; feral domestic fowl, *Gallus gallus domesticus*; Pizzari and Birkhead, 2000; arctiid moths, *Utetheisa ornatrix*; Curril and LaMunyon, 2006; Grevy's zebras,

Equus grevyi; Ginsberg and Rubenstein, 1990). Sexual selection by cryptic female choice entails females biasing paternity after copulation that can favor particular mates (e.g., those with genital designs that elicit more effective stimulation during copulation), and is particularly prevalent in species in which females mate with multiple males and have complex reproductive morphology (Eberhard, 1996, 2010). Anatomical analyses of vaginal gross morphology and musculature are necessary first steps to test the potential function(s) of vaginal folds in sexual selection and natural selection before distinguishing between active uptake or expulsion of semen, or no active semen movement.

The mammalian vaginal wall consists of the internal mucosal/submucosal layer (epithelium/connective tissue), an intermediate muscularis layer (smooth muscle), and an external adventitial layer (connective tissue) (Coleman, 2001). Skeletal muscles (e.g., *M. bulbospongiosus*) are located near the vaginal wall and are part of the urogenital diaphragm (Oelrich, 1983). Unlike smooth muscle, which is under autonomic control, skeletal muscle is under somatic control. Skeletal muscle can be distinguished from smooth muscle by the presence of striations. If skeletal musculature is present in the vaginal folds of cetaceans, unlike most other mammals that lack vaginal folds, the musculature could provide females with a mechanism to pump the sperm of select males within their cranial vaginal tract. However, to our knowledge, skeletal muscle has not been described in the muscularis region of any mammalian vaginal wall. Past studies of the microstructure of cetacean vaginal folds are sparse and have focused on the mucosal layer (Tarpley and Hillman, 1999) rather than the muscularis layer. Microstructural modifications within the female reproductive tract can also reflect functions, such as expansion during parturition or copulation (e.g., spotted hyenas, *Crocuta crocuta*; Cunha et al., 2003).

Among cetaceans, Harrison (1949) reported that long-finned pilot whales (*Globicephala melas*) had smaller, more numerous, and closely-spaced muscle bundles within their caudal vaginal walls compared to their cranial vaginal folds.

Our study was designed to characterize the gross morphology of reproductive tracts of female common bottlenose dolphins (*Tursiops truncatus*) and assess whether vaginal muscle tissue is skeletal. Our approach to addressing these questions included development of a standardized protocol with anatomical landmarks to collect measurements of reproductive tract morphology. We validated the reliability of the protocol by exploring variation across sexual maturity states, reproductive states, and geographic areas. We present the protocol in detail here to facilitate future comparative studies. We also characterized muscle types and the density of muscle bands in vaginal tissue.

Materials and Methods:

Specimen Collection

Female reproductive tracts of common bottlenose dolphins were collected from opportunistic beach strandings along the southeastern U.S. coastline. Specimens were provided by marine mammal stranding networks located in Galveston, Texas, Morehead City, North Carolina, Jacksonville, Florida, and Virginia Beach, Virginia. Tissues were collected from fresh (< 24 hours postmortem) or moderately decomposed deceased animals. Intact reproductive tracts, from the external uro-genital slit through to the ovaries, were obtained. Specimens used for gross morphological measurements were analyzed while fresh or were frozen as soon as

possible and transferred to facilities located at Texas A&M University at Galveston. Separate specimens were used for the histological analysis that were collected exclusively from Texas and were formalin-fixed while fresh. One specimen was used for both gross morphological and histological analysis (Table 1). For this specimen, gross morphological measurements were collected while the tissue was fresh and prior to formalin-fixation. Information about each specimen was provided by the stranding network that collected the animal, including the date, location, and degree of decomposition at the time of stranding. In addition, total body length, state of sexual maturity (based on the presence of *corpora lutea* and/or *corpora albicantia* on either ovary, or body length), and reproductive state (i.e., pregnant, lactating, or resting) data were provided. All reproductive tracts were collected under a National Marine Fisheries Service (NMFS) Southeast Regional Office salvage permit letter to one of the authors (D.N.O.)

Gross Morphological Measurements

Up to fifteen measurements were recorded for fresh or frozen-thawed specimens. Anatomical landmarks and measurements are shown in Figure 1. All measurements were collected with the excised reproductive tracts oriented in dorsal recumbency (ventrum-up). Each ovary was assessed for the absence or presence of *corpora lutea* and/or *corpora albicantia* (Ivashin, 1984). The specimens were bisected by a ventral incision along the longitudinal midline of the reproductive tract. The incision was made from the external clitoris through to the internal bifurcation of the uterine horns (Fig. 1). The uterine horns were opened longitudinally and their appearance was characterized as either longitudinally banded (Fig. 2) or non-banded in texture (Fig. 3). Measurements were consistently collected with the specimens in a relaxed state rather than stretched out.

Measurements of the upper reproductive tract (Table 2) included the:

- Left and Right Uterine Horn Lengths: from the cranial external bifurcation of the uterine horns to the distal *fimbria*; the oviduct was not measured separately from the uterine horn, because the point of delineation was unclear in some specimens
- Uterine Horn Septal Length: cranial-caudal length of the common median wall along the external bifurcation to the internal bifurcation of the uterine horns
- Uterine Length: cranial-caudal length from the internal bifurcation of the uterine horns to the cranial tip of the endocervix
- Uterine Width: in transverse plane; measured midway between the internal bifurcation of the uterine horns and the cranial tip of the endocervix. The cylindrical tube was bisected and spread open
- Total Reproductive Tract Length: cranial-caudal length from the external bifurcation of the uterine horns to the cranial limit of the vulva (delineated by the distal position of the *glans clitoridis*). We did not add uterine horn lengths to the total reproductive tract lengths, because of discrepancies between left and right horn lengths

Measurements of the lower reproductive tract (Table 2) included the:

- Cervical Length: cranial-caudal length from the cranial tip of the endocervix to the caudal tip of the ectocervix (*portio vaginalis*)
- Ectocervical Length: cranial-caudal length of the ectocervix from its base (where it met the dorsal vaginal wall) outward to its distal tip that projected into the vaginal lumen; measurement was on the dorsal side of the ectocervix

- Total Vaginal Length: cranial-caudal length from the base of the ectocervix (where it met the vaginal wall) to the cranial limit of the vulva
- Cranial Vaginal Length: cranial-caudal length from the base of the ectocervix (where it met the vaginal wall) to the cranial tip of the primary vaginal fold (the fold with the greatest projection into the vaginal lumen)
- Caudal Vaginal Length: cranial-caudal length from the base of the primary vaginal fold (where it met the vaginal wall) to the cranial limit of the vulva; measurement was on the dorsal side of the tissue
- Vaginal Fold Width: in the transverse plane; the width of the vaginal fold at its cranial end where it met the vaginal wall. The cylindrical tube was bisected and spread open
- Vaginal Fold Length: cranial-caudal length of the primary vaginal fold from its base (where it met the dorsal vaginal wall) outward to its distal tip that projected into the vaginal lumen; measurement was on the dorsal side of the vaginal fold
- Vaginal Fold Width-to-Vaginal Width Ratio: in transverse plane; ratio of vaginal fold width divided by total vaginal width. Vaginal fold widths and total vaginal widths were measured along the cranial line where the two structures met
- Number of Vaginal Folds: counted as those exceeding 5 mm in vaginal fold length

Small transverse ridges (< 5 mm long from their base to distal tip) were counted and their distances from other vaginal landmarks were recorded. Transverse ridges were not included in subsequent analyses, because they would not likely impede the penis, were not deep crypts to trap or store sperm, and some were so fine the distinction between a ridge and smooth vaginal tissue was unclear in some specimens. With the exception of the left and right uterine

horn lengths, ectocervical lengths, and vaginal fold lengths, all data were collected with digital calipers. The left and right uterine horn lengths were measured with dental floss as a curvilinear line. The ectocervical and vaginal fold lengths were collected with a clear plastic ruler positioned at the base (the lateral attachment at the fornix) of the structure (where it met the vaginal wall), to its distal tip. Additionally, measurements were taken down the longitudinal midline of the vagina except for the cervical length, ectocervical length, and vaginal fold length. These three measurements could be offset from the midline and were measured to the distal tips of the anatomical landmarks.

We established baseline reproductive tract measurements for female common bottlenose dolphins across sexual maturity states, reproductive states, and geographic areas (Table 1). The specimens were categorized as sexually mature or immature based on visual assessment of their ovaries. When ovaries were not available for examination, published region-specific asymptotic body lengths for maturity were used instead (Mead and Potter, 1990; Fernandez and Hohn, 1998; Mattson et al., 2006; Mallette et al., in press). Only mature dolphins were used in the reproductive state and geographic area analyses, while immature and mature dolphins were used in the sexual maturity state analysis. Mature animals were subcategorized as pregnant, lactating, or resting (non-pregnant and non-lactating) based on data provided by the stranding networks (i.e., fetus present or milk in mammary gland). To account for potential confounding effects associated with reproductive state, we conducted analyses for sexual maturity classes and geographic areas using: 1) only sexually mature resting dolphins, and 2) all sexually mature animals. Reproductive tract measurements were standardized by total body lengths for comparisons across specimens. When reproductive tracts were incomplete, some measurements

could not be collected (e.g., measurements of the total reproductive tract length, total vaginal length, and caudal vaginal length were not included in the analysis if the vulva was missing). Accordingly, there are inconsistent numbers of measurements for each specimen (Table 2). Due to our small and inconsistent sample sizes, and the large number of outliers (based on the 1.5x interquartile range rule), statistical analyses were not performed. The data scaled by total body lengths are presented as Tukey's box and whisker plots.

Histology

Specimens collected from Galveston, Texas, were preserved in ten percent physiologically-buffered formaldehyde for histological processing. Two samples (~ 6.5 cm²) of cranial vaginal fold tissue were collected from each specimen. Two similarly-sized samples of (non-fold) vaginal tissue were collected along the same longitudinal plane, caudal to the region of vaginal folds. The tissues were fixed, dehydrated, cleared, and infiltrated with paraffin wax under vacuum. Tissues were then embedded in paraffin blocks, and cut longitudinally at 7-10 μm on a Leica 2235 RM rotary microtome. Samples were collected from the muscularis layer of the tissue, since other regions of the vaginal wall have not been reported to contain muscle (Coleman, 2001). The resulting ten sections per sample were mounted on gelatin-coated slides and stained with a modified Masson's trichrome stain (Masson, 1929) following Marshall et al. (2006) to visualize the muscle tissue. Muscle fascicles were stained pink, connective tissues were stained green-blue, and cell nuclei were stained black (Luna, 1968). A total of forty slides (ten per sample) were prepared for each specimen.

To examine the microstructure of the vaginal tissue, we selected five slides at random from each tissue sample. Micrographs were collected using a Diagnostic Instrument Spot Pursuit camera fitted to a Nikon Eclipse E400 light microscope and SPOT Advanced Image software. One micrograph was collected per slide at 20x magnification. We imaged portions of the slides in which no other structures were present (fat cells, blood vessels, etc.), muscle bands (fascicles) were parallel and in the same plane, and muscle bands occupied a minimum width of 2 units on the reticle at 20x magnification. No adjustments besides the additions of scale bars, contrast, and brightness were made to the micrographs. The microstructure of the vaginal tissues was assessed in Image J (v.1.44p) by three researchers unaware of the tissue location (cranial vaginal fold or caudal vaginal wall tissue).

The presence or absence of smooth and skeletal muscle bands were evaluated visually, using reference images in Geneser (1985) and Hammersen (1985) as guides. Differences in the density of muscle banding patterns were compared between cranial vaginal fold and caudal vaginal tissue. A diagonal line was placed across each image and the diagonal length was measured. Then the number of muscle bands that were crossed by the diagonal line and the sum of their collective thicknesses along the diagonal line were tallied. The density of muscle banding was derived by dividing the sum of muscle thicknesses by the length of the diagonal line. The average percent density of muscle banding was calculated per sample when the measurements of the three independent researchers were within 5 units (%) of each other. If the intermediate measurement was within 5 units of the upper and lower measurements, but the span of the three measurements was >5 units, the average of the two measurements with the smallest difference was used. Samples that resulted in >5 units discrepancies between the researchers

were considered ambiguous and excluded from statistical analysis. A mixed model repeated measure Analysis of Variance test (with animal identity as the random variable) was used to determine if there was a difference in the density of muscle banding between vaginal fold and vaginal tissue, sexually mature or immature specimens, and the interaction between the tissue type and sexual maturity (Stata, ver. 14, Stata Corp, College Station, TX). The predicted standardized residuals of the final model were evaluated for normality using quantile normal plots. Data with non-normal distributions (based on Shapiro-Wilk tests) were transformed to meet assumptions of normality (based on the best transformation method; Ladder test, Stata®) and reanalyzed. Pairwise comparisons of estimated marginal means were conducted with a Šidák adjustment to control for type 1 errors ($P < 0.05$).

Results

Gross Morphological Measurements

A suite of 15 reproductive tract measurements was obtained for 18 specimens representing different sexual maturity states, reproductive states, and geographic areas (Tables 1, 2). The general characteristics of the reproductive tracts are described. Color pigmentations throughout the reproductive tract varied with specimens and related to decomposition (Figs. 1-4). The ovaries were oval-shaped (Figs. 1-2) and every sexually mature specimen had more *corpora lutea* and/or *corpora albicantia* in the left than right ovary. In sexually immature specimens, the left and right uterine horns were small in diameter and marked internally with fine longitudinal bands that gave the tissue a striped appearance (Fig. 2). In sexually mature specimens, the left and right uterine horns were greater in diameter and not banded internally, because the tissue was

comparatively distended (Fig. 3). In pregnant females, both uterine horns were greatly distended. The distal tips of the ectocervix and endocervix were uneven and serrated in shape (Figs. 1, 3). The cervix contained fine internal longitudinal textured bands (Figs. 1, 3). Longitudinal bands were particularly prevalent on the endocervix and ectocervix (Fig. 1). Thick and sticky cervical mucus was congealed within the cervical cavity of many specimens, although the mucus sometimes extended into the vaginal lumen in a more diluted consistency. The ectocervix created a deep fornix in the cranial vagina that had the greatest length on the dorsal wall.

The vagina was bisected in the transverse plane by a single vaginal fold, which was large, deep, and protruded from the entire width of the vagina caudally into the vaginal lumen (Figs. 1, 3-4). One lactating sexually mature female from Florida and the one immature female from Virginia had a secondary vaginal fold that was shorter in length. In the mature specimen, the secondary vaginal fold was in the caudal vagina (caudal to the primary vaginal fold) and was substantially less developed than the primary vaginal fold (6 mm vs 29 mm in vaginal fold length, respectively). In the immature specimen, the secondary vaginal fold was in the cranial vagina and was moderately less developed than the primary vaginal fold (6 mm vs 10 mm in vaginal fold length, respectively). Only the primary vaginal fold data were included in the analysis. Across all specimens, the vaginal folds had the greatest lengths on the dorsal wall of the vagina (Fig. 4). The folds had fine textured bands running longitudinally on their interior surface (oriented towards the vaginal lumen; Figs. 1, 3-4). The distal tips of the folds ranged from thin and serrated to thick and non-serrated (Figs. 1, 3-4). The caudal vagina had up to two shallow transverse ridges (<5 mm in length). If these ridges were present, they were located in the cranial end of the caudal vagina, were often branching, did not extend around the entire circumference

of the vaginal lumen, and were most prominent on the dorsal vaginal wall (Figs. 3-4). No vaginal band (hymen) was observed in any specimen. The clitoris was keeled, dense, and projected into the genital slit. The glans (distal tip) of the clitoris pointed directly to the cranial limit of the vulva, located caudo-dorsally and demarcated by a darker tissue color than the interior of the vagina.

The raw reproductive tract measurements are presented in Table 2. The data scaled by body length are presented in Figures 5 and 6. We present data on all mature females for the sexual maturity state and geographic area analysis, regardless of reproductive state; this yielded a larger sample size and no differences were found between all mature specimens and resting-only specimens for any of the morphological measurements.

Sexual Maturity State: The left and right uterine horns were proportionally longer in sexually mature than immature dolphins, and much of the skew was driven by pregnant females (Fig. 5a, Table 2). The vaginal fold was marginally more developed (longer and therefore greater surface area) in sexually mature animals (Fig. 6a, Table 2).

Reproductive State: The upper reproductive tracts of pregnant females were greater in all measurements, except for the total reproductive tract length, compared to lactating or resting females (Fig. 5b, Table 2). There were no differences between lactating and resting females in any of the six measurements for the upper reproductive tract. One pregnant female had longer vaginal lengths (total, cranial, and caudal) than lactating or resting females (Fig. 6b, Table 2). The other two pregnant females were incomplete specimens and their vaginal lengths could not

be measured. Vaginal folds were wider in pregnant females than lactating females (Fig. 6b, Table 2). There was an outlier in the vaginal fold width data for resting females, and it is unclear if pregnant females also had wider vaginal folds than resting females.

Geographic area: Adult specimens from the four geographic areas had overlapping ranges of body lengths (Table 2). Samples from the Texas population of dolphins had marginally longer left and right uterine horn lengths than samples from the Florida population (Fig. 5c, Table 2). The samples from the North Carolina population had slightly longer cervical lengths, vaginal fold widths, and vaginal fold lengths than those from Virginia (Fig. 6c, Table 2). All vaginal length measurements decreased gradually from Texas to Virginia (Fig. 6c, Table 2).

Histology

We assessed the microanatomy of five specimens, all of which had only one vaginal fold (Table 1). The microstructure of the cranial vaginal fold and caudal vaginal wall tissues consisted of smooth muscle bands (fascicles), mixed with dense irregular connective tissue, blood vessels, and adipose tissue (Fig. 7). Skeletal muscle was not found in any of the 100 micrographs analyzed. The data from 90 micrographs were included in the mixed model analysis. The variance partition coefficient indicated that 45% of the model variance was explained by inter-animal differences. There was no significant difference in estimated marginal means for the density of muscle banding between vaginal fold ($n = 42$, $\bar{x} = 49.29 \pm 5.3\%$) and vaginal wall tissues ($n = 48$, $\bar{x} = 44.56 \pm 5.3$; $z = -1.84$, $P = 0.07$; Fig. 7) or between sexually mature ($n = 57$, $\bar{x} = 45.47 \pm 6.6$) and immature specimen tissues ($n = 33$, $\bar{x} = 49.01 \pm 8.1$; $z = 0.34$, $P = 0.74$). However, there was a significant interaction between tissue type and maturity

state ($P = 0.002$), with the significant differences ($z = -3.51$, $P = 0.003$) limited to the vaginal walls ($\bar{x} = 41.92 \pm 8.3$) and folds ($\bar{x} = 57.1 \pm 8.5$) of sexually immature specimens.

Discussion

The goal of our study was to describe the anatomy of female common bottlenose dolphin reproductive tracts and to lay the foundation for future investigation of potential functions of vaginal folds. We found little variability in the morphological measurements of reproductive tracts between sexually mature and immature specimens and across reproductive states. Female common bottlenose dolphins generally had only one large vaginal fold. We did not find skeletal muscle or differences in the densities of smooth muscle bands between the cranial vaginal folds and caudal vaginal walls or between states of sexual maturity.

Female Anatomy: Landmarks

Our observations of more *corpora lutea* and/or *corpora albicantia* on the left than right ovary and of longer left than right uterine horns are consistent with what is known for common bottlenose dolphins (Robeck et al., 2005) and some other odontocetes (toothed whales) after their first pregnancy (Ohsumi, 1964; Sljper, 1966). *Corpora lutea* and *corpora albicantia* accumulate on ovaries following ovulations and pregnancies (Mackintosh and Wheeler, 1929; Ohsumi, 1964; Sljper, 1966; Harrison, 1969; Harrison and Ridgway, 1971; Plön and Bernard, 2007; Rommel et al., 2007). A fetus had been removed from the left uterine horn of all three pregnant specimens that we dissected. Our data support observed laterality in ovulation and pregnancy in common bottlenose dolphins, and are congruent with previous reports of odontocetes' tendency

towards left uterine horn ovulation, implantation, and development (Slijper, 1966; Robeck et al., 2005). Both the left and right uterine horn lengths that we report for common bottlenose dolphins were substantially longer than those reported by Robeck et al. (1994). This disparity may result from our inclusion of the oviduct in the left and right uterine horn length measurements. Our findings suggest that the presence or absence of fine longitudinal bands in the uterine horns may be useful in categorizing sexual maturity states if the ovaries are not present. The cetacean uterus is bicornate and is completely separated from the lower reproductive tract by the cervix (Slijper, 1966). The uteri in the current study were shorter in length (scaled by body length), yet similar in appearance to the bicornate uteri of dogs, pigs, and elephants that develop the fetus within the uterus and not the uterine horn (Schroeder, 1990). Our measurements of uterine length are congruent with those reported by Robeck et al. (1994) for common bottlenose dolphins.

The cervix was almost the same length as the uterus in both this and Robeck et al.'s (1994) study of common bottlenose dolphins. The uterus length relative to the cervix length is highly variable across mammals (König and Liebich, 2007). The presence of longitudinal bands in the cervix could provide a passageway for spermatozoa to reach the uterus by travelling between the bands and avoiding the dense mucus barrier produced by females in the central cervical canal (bovines, Mullins and Saacke, 1989; goats and cattle, Mattner, 1968). The presence of similar longitudinal bands on the interior surface (lumen-oriented) of the vaginal folds (Pycraft, 1932; Harrison, 1949) supports the hypothesis that vaginal folds function in sperm movement. Our observed patterns of the greatest ectocervical and vaginal fold lengths on the dorsal wall of the vagina have been reported in other cetaceans (bowhead whales, *Balaena mysticetus*; Tarpley and Hillman, 1999), although the functional importance is unclear.

The majority of our measurements focused on the lower reproductive tract, and specifically the vaginal folding. Although the common bottlenose dolphins in Robeck et al.'s (1994) study had longer body lengths than those in our study, the total vaginal lengths of our specimens were 2 cm longer on average. In contrast to other cetaceans, common bottlenose dolphins have one large vaginal fold (Green, 1977). However, one sexually mature and one sexually immature specimen in our study possessed a second, smaller vaginal fold. The second vaginal fold varied in its degree of development, position relative to the primary fold (cranial or caudal), and shape of its distal tip. There was individual variation in vaginal morphology, although most characteristics were shared among the specimens we observed. In short-beaked common dolphins (*Delphinus delphis*) and other unspecified cetacean species, vaginal structures decreased in length and width from the cranial to the caudal direction (Hunter, 1787; Pycraft, 1932). However, the opposite pattern was found in bowhead whales (Tarpley and Hillman, 1999).

Variation across sexual maturity state, reproductive state, and geographic area

We found a small range of variation among specimens in the general reproductive tract morphology of female common bottlenose dolphins. Our finding expands the pool of suitable specimens for future research; we suggest that specimens, regardless of their sexual maturity state, reproductive state, or geographic location, can be used interchangeably in comparative morphological studies of the cetacean vagina.

Maturation-related developmental shifts occur in the morphology of some tissues associated with reproduction in mammals (e.g., genital swelling in primates, Nunn, 1999). However, the only variation observed in this study between sexually mature and immature specimens in the lower reproductive tract was the extent of vaginal fold development, and the difference was minimal. In the upper reproductive tract, sexually mature specimens were observed to have relatively longer left and right uterine horn lengths than immature specimens, as expected, just as humans display maturity-related increases in uterine size (Salardi et al., 1985; Herter et al., 2002). Based on our observations of minimal variation in reproductive tract morphometrics, sexually immature specimens look like small mature specimens.

As expected, pregnant females had more distended upper reproductive tracts than lactating and resting sexually mature dolphins to accommodate the developing fetus, amniotic cavity, and placenta (Wislocki and Enders, 1941). Changes in uterine dimensions reflect vascular alterations of the mucosa and are related to reproductive state in other cetaceans as well (fin whales, *Balaenoptera physalus*, Mackintosh and Wheeler, 1929). Lower reproductive tract variations were minimal across reproductive states. We cannot comment on relative vaginal length variations since we were only able to obtain measurements for one of the three pregnant females. The few variations in the lower reproductive tract morphometrics of pregnant and non-pregnant females could reflect individual variation or adaptations related to conception, pregnancy, or estrus, as seen in primates (Nunn, 1999).

Specimens from Texas, Florida, North Carolina, and Virginia represent different populations of common bottlenose dolphins (Rosel et al., 2011). There were few clear patterns of

variation in upper or lower reproductive tract morphology across the population samples we assessed. Albeit tentative, the differences we observed could potentially indicate real geographic variation across populations of common bottlenose dolphins (e.g. decreases in vaginal lengths from Texas to Virginia). As *T. truncatus* has a near-global distribution, investigations of reproductive morphology at a broader geographical scale and with a larger sample size are warranted and could yield interesting comparisons.

Histology

No skeletal muscle was present in the cranial or caudal vaginal wall of common bottlenose dolphins, congruent with other mammals (domestic cats, *Felis catus*, Rosengren and Sjöuber, 1967; domestic rabbits, *Oryctolagus cuniculus*, Rodríguez-Antolín et al., 2009; rats, *Rattus norvegicus*, Berger et al., 2005). No changes were found in the density of muscle banding between cranial vaginal fold and caudal vaginal wall tissues in common bottlenose dolphins, in contrast to Harrison's (1949) results for long-finned pilot whales. He qualitatively described muscles in the cranial vaginal fold tissue as large and sparse and muscles in the caudal vaginal wall tissue as small and abundant. Harrison (1949) did not differentiate between the microstructure of fetal and adult vaginal fold and wall tissues, potentially indicating no maturity-specific variation. However, we found that the variance observed in percent muscle banding between tissue types was significantly explained by differences within sexually immature specimens. We emphasize the importance of assessing ontogeny to understand functionality.

Form and Function

A number of non-mutually exclusive hypotheses – sexual selection, natural selection, and phylogenetic similarity and constraint, among others – might explain the presence of cetacean vaginal folds (see above; Clarke et al., 1994). It has been hypothesized that vaginal folds might function in selective sperm movement (Clarke et al., 1994). We found no evidence that the common bottlenose dolphin vaginal wall muscle is under somatic control. However, in other taxonomic groups where females eject sperm, muscle contractions also appear to be under autonomic control (dunnocks, *Prunella modulari*; Davies, 1983; damselflies, *Calopteryx haemorrhoidalis asturica*; Cordoba-Aguilar, 1999). The stimulus itself may be more important than the muscle type in inducing spontaneous or reflexive contractions (coital reflex, Carro-Juarez and Rodríguez-Manzo, 2000; acoustic reflex, Thompson et al., 1980). Further analyses of the muscle architecture of dolphin vaginal walls, particularly focusing on the innervation, histochemistry, biophysical properties, and force production, will advance the understanding of vaginal fold functionality (bottlenose dolphin muscles, Pabst et al., 1993; Etnier et al., 2004). On the one hand, vaginal folds could divert sperm away from the cervix and upper reproductive tract; vaginal folds project caudally into the lumen of the vagina and form deep dead-end crypts. Semen trapped caudal to the vaginal folds could come into contact with lethal seawater. On the other hand, the longitudinal textured bands on the interior of the vaginal folds could provide a passageway for spermatozoa to reach the upper reproductive tract, and/or the vaginal folds could hold or reduce the loss of semen. For example, Green (1977) reported more fluid that looked like semen cranial than caudal to the vaginal fold in a dissected female common bottlenose dolphin. Future studies that tag and track the movements of semen through the female reproductive tract *in vivo* will be able to test if vaginal folds create storage crypts (fruit flies, *Drosophila melanogaster*, Manier et al., 2010) or facilitate semen uptake or expulsion.

Much of a female's reproductive success relates to her reproductive anatomy, yet research on female genitalia has been largely under-represented in the scientific literature (Ah-King et al., 2014). In cetaceans, female mating tactics have been generally overlooked, often because of logistical challenges. Our exploration of the reproductive tract morphology of the female common bottlenose dolphin establishes the framework necessary to conduct intra- and interspecific comparative studies; we can now begin to test alternative hypotheses for the evolution of these unusual vaginal folds. We demonstrate that sexual maturity state, reproductive state, and geographic area do not appear to influence vaginal morphology within *T. truncatus* from the southeastern USA. While mating tactics have been inferred from female reproductive tract morphology in eutherian mammals (Gomendio and Roldan, 2003), few other studies have recognized the potential for vaginal morphology to advance our understanding of mammalian mating systems.

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Figure Legends:

Fig. 1: Dissected reproductive tract of a female common bottlenose dolphin with labels of morphological landmarks (A) and measurements (B). The frozen-thawed specimen is oriented in dorsal recumbency. The specimen was splayed open, but the tissue was not stretched for any measurements. The right, but not the left ovary is included in this specimen. The arrows delineate the start and end points of the individual measurements. Curvilinear lengths are distinguished with an asterisk (*) symbol. Dashed lines denote measurements on the underside of

tissue not visible in the figures. Width measurements in transverse plane correspond with the circumference of the lumens straightened out.

Fig. 2: Uterine horns and uterus of a sexually immature female common bottlenose dolphin. The frozen-thawed specimen is oriented in dorsal recumbency. The uterine horns and uterus were opened longitudinally. The specimen was splayed open, but the tissue was not stretched. The right, but not the left ovary was included in this specimen. The internal tissue of the uterine horns had a banded texture pattern along its longitudinal axis that gave it a striped appearance. The uterine horn and uterus tissue were not distended.

Fig. 3: Dissected reproductive tract of a sexually mature female common bottlenose dolphin. The frozen-thawed specimen is oriented in dorsal recumbency. The uterine horns and entire reproductive tract were opened longitudinally. The specimen was splayed open, but the tissue was not stretched. The uterine horns were wide in diameter proximal to the uterus and tapered off in length closer to the ovary. The internal tissue of the uterine horns did not have a banded texture pattern along its longitudinal axis. The bottom of the ruler demarcates the cranial limit of the cervix. The cervix had a banded texture pattern along its longitudinal axis. The cranial and caudal limits of the cervix were serrated and uneven. The vaginal fold was located immediately caudal to the cervix in the transverse plane and spanned the entire width of the vaginal canal. The vaginal fold had a longitudinal banded pattern on its internal surface and an uneven tip. Two yellow pins demarcate a shallow transverse ridge in the lateral plane caudal to the vaginal fold. This shallow ridge was non-linear, did not span the entire width of the vagina, and was deepest on the dorsal body wall.

Fig. 4: Dissected reproductive tract of a female common bottlenose dolphin in the transverse plane. The frozen-thawed specimen was oriented in dorsal recumbency. In this figure, the vaginal lumen was intact (we did not make a longitudinal incision). The vaginal fold encircled the entire interior circumference of the vagina and its distal tip projected caudally towards the vulva. The vaginal fold had the greatest length and created a recess on the dorsal side of the vaginal wall. The distal tips of the vaginal fold were serrated and uneven. A banded texture pattern was present along the longitudinal axis of the vaginal fold on the interior of the lumen. The ectocervix was visible through the lumen of the vaginal fold. A shallow transverse ridge was positioned caudal to the vaginal fold and did not extend along the complete circumference of the vaginal lumen.

Fig. 5: Tukey's box and whisker plots of the upper reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red 'X' symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

Fig. 6: Tukey's box and whisker plots of the lower reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red

‘X’ symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

Fig. 7: Micrographs of dolphin tissue at 20x magnification. The slides were stained with a modified Masson’s trichrome stain to distinguish the muscle fascicles (pink) from connective tissue (green-blue), and from cell nuclei (black). The left micrographs (A and C) show vaginal fold tissue, while the right micrographs (B and D) show vaginal tissue collected from the caudal vagina. The top two micrographs (A and B) show low densities of muscle banding from the same adult dolphin, while the bottom two micrographs (C and D) show high densities of muscle banding from a second adult dolphin.

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Table 1: Count of the number of specimens used in the study. Common bottlenose dolphin specimens are divided by geographic area and state of sexual maturity. Within the sexually mature state, specimens are further divided into reproductive states. Within the geographic area columns, specimens from Texas are divided into those used for gross morphological (top row) or histological (bottom row) analysis. Different specimens were used for gross morphological and histological techniques with the exception of one specimen denoted with an * symbol.

State of Sexual Maturity	Reproductive State	Geographic Area			
		Texas	Florida	North Carolina	Virginia
Mature	Pregnant	2	0	1	0
		1*			
	Lactating	1	2	0	0
		1			
	Resting	3	3	2	1
		1			
Immature		0	0	2	1
		2			

Table 2. Measurements of reproductive tract variation in common bottlenose dolphin. Mean (\pm SD) of gross morphological measurements collected from the reproductive tracts of up to eighteen female bottlenose dolphins. The sample size for the measurement is italicized below the mean. All length, width, and depth measurements are in mm. Values are raw data and are not scaled by body length.

Measurement	Sexual Maturity State		Reproductive State (Sexually Mature Only)			Geographic Area (Sexually Mature Only)			
	Mature	Immature	Pregnant	Lactating	Resting	Texas	Florida	North Carolina	Virginia
Total Body Length	2512.87 (\pm 182.18) <i>15</i>	1763.33 (\pm 587.31) <i>3</i>	2590.00 (\pm 314.80) <i>3</i>	2500.00 (\pm 219.31) <i>3</i>	2491.44 (\pm 135.92) <i>9</i>	2436.67 (\pm 170.37) <i>6</i>	2484.00 (\pm 160.87) <i>5</i>	2636.67 (\pm 197.57) <i>3</i>	2473.00 (\pm 0) <i>1</i>
Left Uterine Horn Length	510.57 (\pm 218.42) <i>7</i>	119.75 (\pm 33.59) <i>2</i>	792.50 (\pm 228.40) <i>2</i>	N/A <i>0</i>	397.80 (\pm 53.70) <i>5</i>	710.00 (\pm 3457) <i>2</i>	382.00 (\pm 50.91) <i>2</i>	463.33 (\pm 150.48) <i>3</i>	N/A <i>0</i>
Right Uterine Horn Length	375.88 (\pm 144.85) <i>8</i>	94.00 (\pm 38.18) <i>2</i>	595.00 (\pm 24.04) <i>2</i>	273.00 (\pm 79.20) <i>2</i>	317.75 (\pm 55.72) <i>4</i>	452.50 (\pm 177.48) <i>2</i>	265.67 (\pm 57.42) <i>3</i>	435.00 (\pm 158.29) <i>3</i>	N/A <i>0</i>
Uterine Horn Septal Length	73.84 (\pm 52.14) <i>13</i>	27.05 (\pm 10.00) <i>3</i>	236.12 (\pm 0) <i>1</i>	54.66 (\pm 16.23) <i>3</i>	62.20 (\pm 20.74) <i>9</i>	94.60 (\pm 79.73) <i>5</i>	54.03 (\pm 24.60) <i>5</i>	79.32 (\pm 18.70) <i>2</i>	58.12 (\pm 0) <i>1</i>
Uterine Width	86.69 (\pm 70.18) <i>14</i>	27.27 (\pm 11.14) <i>3</i>	238.30 (\pm 44.83) <i>2</i>	44.75 (\pm 11.01) <i>3</i>	66.99 (\pm 29.565) <i>9</i>	92.84 (\pm 73.76) <i>5</i>	62.19 (\pm 25.07) <i>5</i>	130.78 (\pm 120.60) <i>3</i>	46.17 (\pm 0) <i>1</i>
Uterine Length	57.74 (\pm 29.41) <i>13</i>	16.58 (\pm 13.59) <i>3</i>	148.87 (\pm 0) <i>1</i>	52.05 (\pm 11.22) <i>3</i>	49.17 (\pm 11.72) <i>9</i>	68.03 (\pm 46.25) <i>5</i>	52.61 (\pm 14.59) <i>5</i>	52.94 (\pm 1.20) <i>2</i>	41.50 (\pm 0) <i>1</i>
Total Reproductive Tract Length	272.67 (\pm 53.54) <i>9</i>	148.69 (\pm 51.62) <i>3</i>	305 (\pm 0) <i>1</i>	237.00 (\pm 7.07) <i>2</i>	279.17 (\pm 61.71) <i>6</i>	288.40 (\pm 56.11) <i>5</i>	250.01 (\pm 61.05) <i>3</i>	/ <i>0</i>	262.00 (\pm 0) <i>1</i>
Cervical Length	48.77 (\pm 11.97) <i>14</i>	31.46 (\pm 10.50) <i>3</i>	59.21 (\pm 15.74) <i>2</i>	50.86 (\pm 13.01) <i>3</i>	45.75 (\pm 10.98) <i>9</i>	48.75 (\pm 12.37) <i>5</i>	43.13 (\pm 1.13) <i>5</i>	59.62 (\pm 10.61) <i>3</i>	44.48 (\pm 0) <i>1</i>
Ectocervical length	32.36 (\pm 7.02) <i>14</i>	13.33 (\pm 6.03) <i>3</i>	38.50 (\pm 6.36) <i>2</i>	24.67 (\pm 4.51) <i>3</i>	33.56 (\pm 6.06) <i>9</i>	36.80 (\pm 5.67) <i>5</i>	28.80 (\pm 8.53) <i>5</i>	31.00 (\pm 5.20) <i>3</i>	32.00 (\pm 0) <i>1</i>
Total Vaginal Length	154.26 (\pm 35.03) <i>9</i>	89.67 (\pm 20.18) <i>3</i>	201.00 (\pm 0) <i>1</i>	119.01 (\pm 12.80) <i>2</i>	158.21 (\pm 31.38) <i>6</i>	171.16 (\pm 33.94) <i>5</i>	132.51 (\pm 31.55) <i>3</i>	/ <i>0</i>	134.98 (\pm 0) <i>1</i>
Cranial Vaginal Length	28.57 (\pm 11.76) <i>12</i>	18.31 (\pm 9.35) <i>3</i>	52.83 (\pm 0) <i>1</i>	29.29 (\pm 9.68) <i>3</i>	25.26 (\pm 9.69) <i>8</i>	36.04 (\pm 11.43) <i>5</i>	28.12 (\pm 8.27) <i>4</i>	20.25 (\pm 4.15) <i>2</i>	9.65 (\pm 0) <i>1</i>
Caudal Vaginal Length	111.23 (\pm 27.07) <i>9</i>	67.97 (\pm 8.36) <i>3</i>	152.00 (\pm 0) <i>1</i>	87.92 (\pm 5.42) <i>2</i>	112.20 (\pm 24.81) <i>6</i>	123.83 (\pm 27.87) <i>5</i>	94.37 (\pm 21.85) <i>3</i>	/ <i>0</i>	98.78 (\pm 0) <i>1</i>
Number of Folds	1.07 (\pm 0.26) <i>15</i>	1.33 (\pm 0.58) <i>3</i>	1.00 (\pm 0) <i>3</i>	1.33 (\pm 0.58) <i>3</i>	1.00 (\pm 0) <i>9</i>	1.00 (\pm 0) <i>6</i>	1.20 (\pm 0.45) <i>5</i>	1.00 (\pm 0) <i>3</i>	1.00 (\pm 0) <i>1</i>
Vaginal Fold Width	91.94 (\pm 30.62) <i>14</i>	39.30 (\pm 11.37) <i>3</i>	130.24 (\pm 32.44) <i>2</i>	69.38 (\pm 16.64) <i>3</i>	90.94 (\pm 27.64) <i>9</i>	86.00 (\pm 29.03) <i>5</i>	90.57 (\pm 31.41) <i>5</i>	113.92 (\pm 34.40) <i>3</i>	62.52 (\pm 0) <i>1</i>
Vaginal Fold Length	27.63 (\pm 5.78) <i>15</i>	9.00 (\pm 2.65) <i>3</i>	28.46 (\pm 6.66) <i>3</i>	28.33 (\pm 5.03) <i>3</i>	27.11 (\pm 6.35) <i>9</i>	24.06 (\pm 4.68) <i>6</i>	28.80 (\pm 5.76) <i>5</i>	33.33 (\pm 4.62) <i>3</i>	26.00 (\pm 0) <i>1</i>
Vaginal Fold Width-to-Vaginal Width Ratio	1.00 (\pm 0) <i>15</i>	1.00 (\pm 0) <i>3</i>	1.00 (\pm 0) <i>3</i>	1.00 (\pm 0) <i>3</i>	1.00 (\pm 0) <i>9</i>	1.00 (\pm 0) <i>6</i>	1.00 (\pm 0) <i>5</i>	1.00 (\pm 0) <i>3</i>	1.00 (\pm 0) <i>1</i>

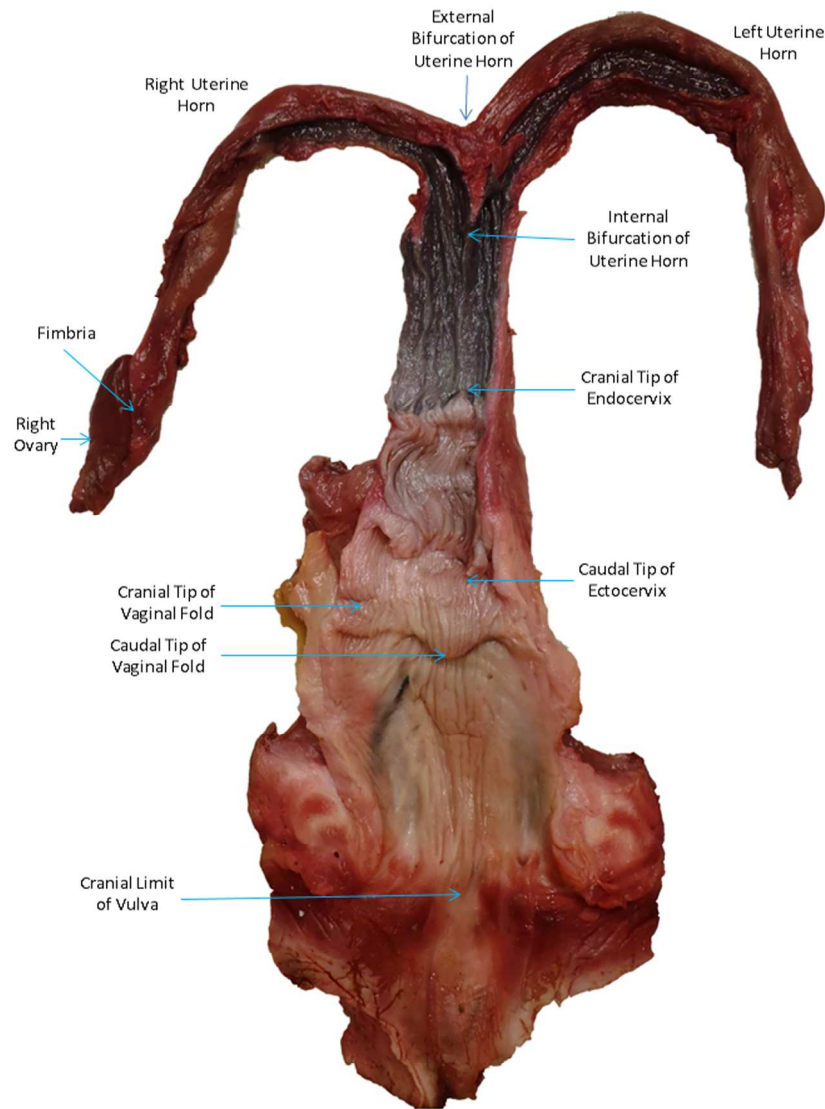


Fig. 1: Dissected reproductive tract of a female common bottlenose dolphin with labels of morphological landmarks (A) and measurements (B). The frozen-thawed specimen is oriented in dorsal recumbency. The specimen was splayed open, but the tissue was not stretched for any measurements. The right, but not the left ovary is included in this specimen. The arrows delineate the start and end points of the individual measurements. Curvilinear lengths are distinguished with an asterisk (*) symbol. Dashed lines denote measurements on the underside of tissue not visible in the figures. Width measurements in transverse plane correspond with the circumference of the lumens straightened out.
254x338mm (300 x 300 DPI)

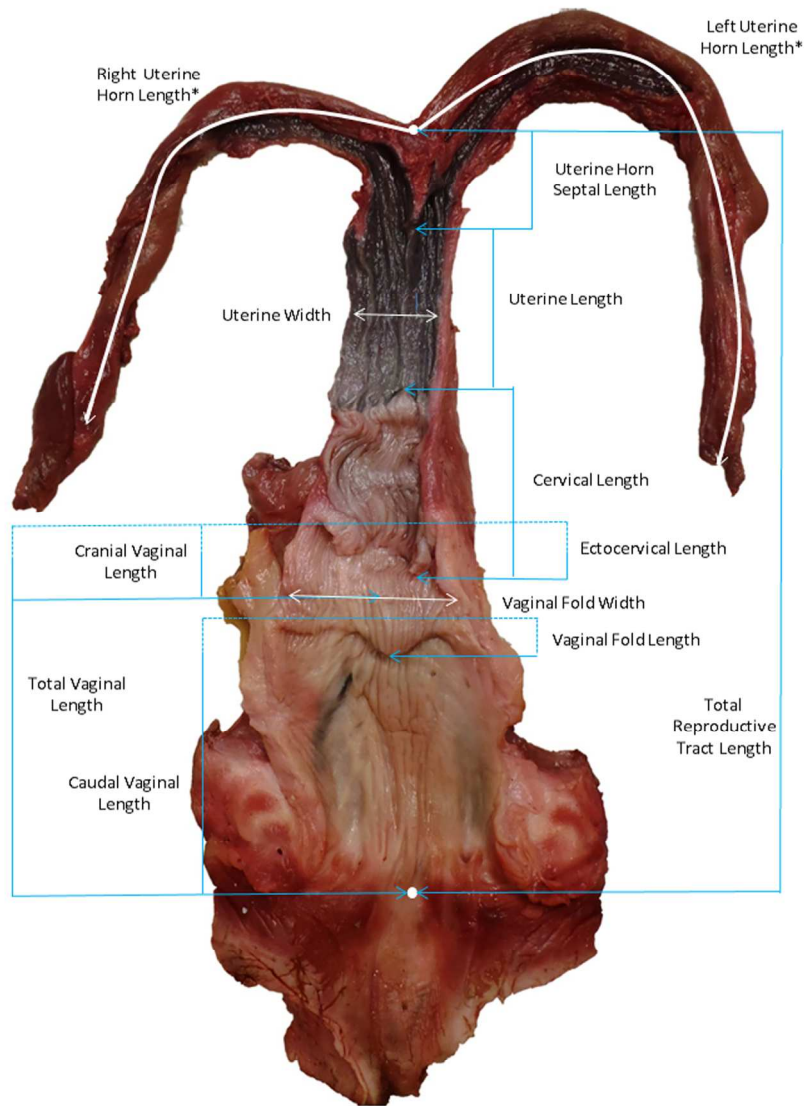


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254x338mm (300 x 300 DPI)

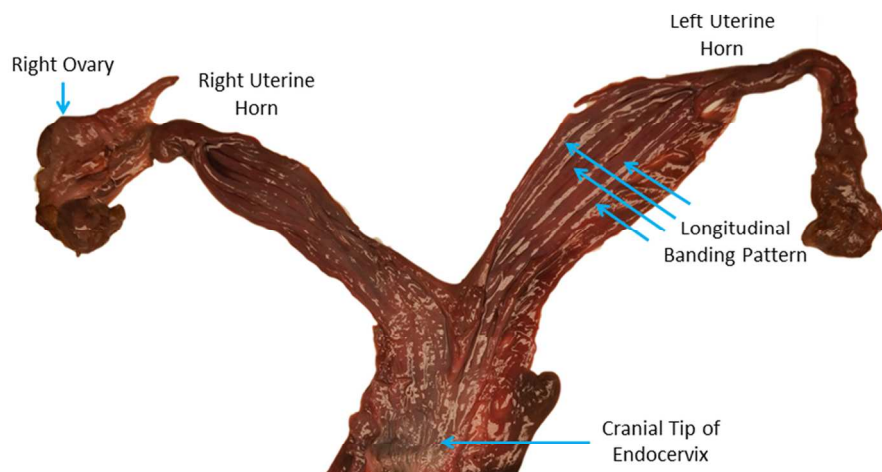


Fig. 2: Uterine horns and uterus of a sexually immature female common bottlenose dolphin. The frozen-thawed specimen is oriented in dorsal recumbency. The uterine horns and uterus were opened longitudinally. The specimen was splayed open, but the tissue was not stretched. The right, but not the left ovary was included in this specimen. The internal tissue of the uterine horns had a banded texture pattern along its longitudinal axis that gave it a striped appearance. The uterine horn and uterus tissue were not distended.

114x85mm (300 x 300 DPI)

ACCE

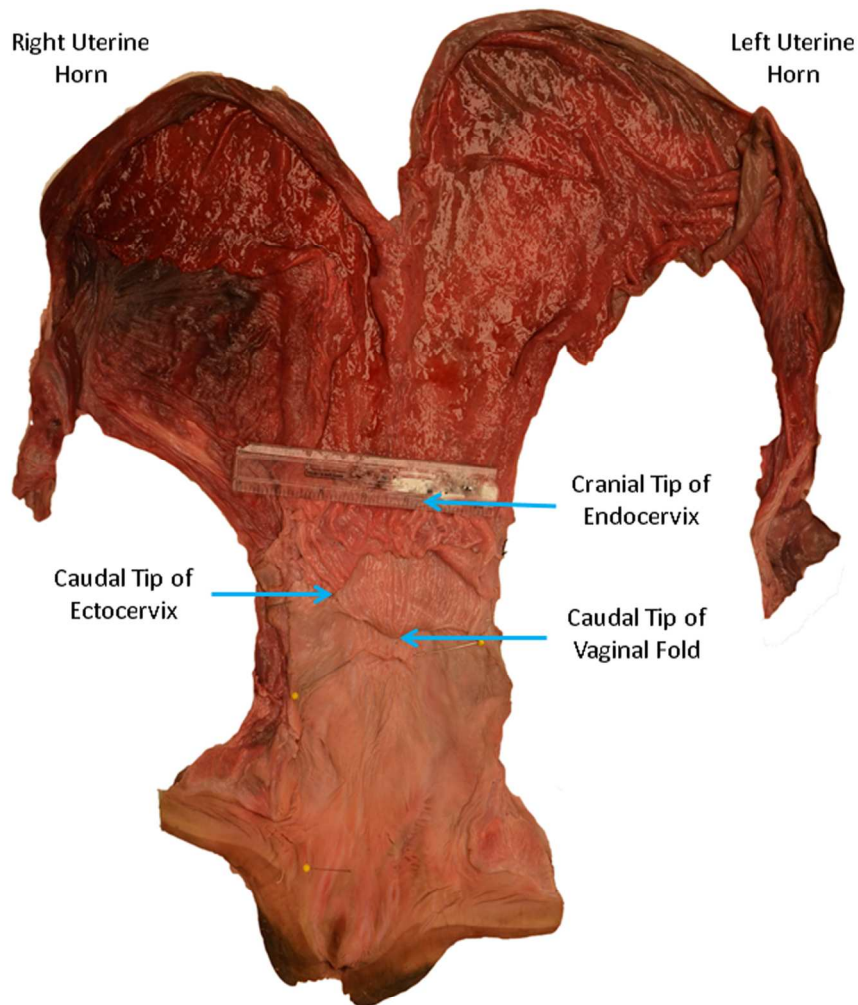


Fig. 3: Dissected reproductive tract of a sexually mature female common bottlenose dolphin. The frozen-thawed specimen is oriented in dorsal recumbency. The uterine horns and entire reproductive tract were opened longitudinally. The specimen was splayed open, but the tissue was not stretched. The uterine horns were wide in diameter proximal to the uterus and tapered off in length closer to the ovary. The internal tissue of the uterine horns did not have a banded texture pattern along its longitudinal axis. The bottom of the ruler demarcates the cranial limit of the cervix. The cervix had a banded texture pattern along its longitudinal axis. The cranial and caudal limits of the cervix were serrated and uneven. The vaginal fold was located immediately caudal to the cervix in the transverse plane and spanned the entire width of the vaginal canal. The vaginal fold had a longitudinal banded pattern on its internal surface and an uneven tip. Two yellow pins demarcate a shallow transverse ridge in the lateral plane caudal to the vaginal fold. This shallow ridge was non-linear, did not span the entire width of the vagina, and was deepest on the dorsal body wall.
224x264mm (300 x 300 DPI)

Accepted Article

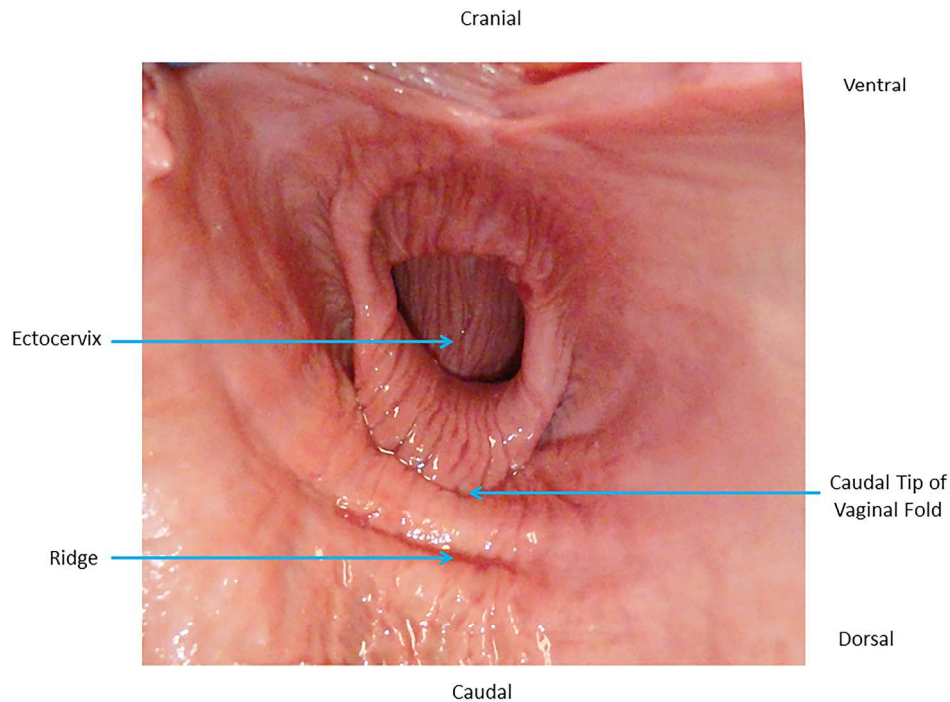


Fig. 4: Dissected reproductive tract of a female common bottlenose dolphin in the transverse plane. The frozen-thawed specimen was oriented in dorsal recumbency. In this figure, the vaginal lumen was intact (we did not make a longitudinal incision). The vaginal fold encircled the entire interior circumference of the vagina and its distal tip projected caudally towards the vulva. The vaginal fold had the greatest length and created a recess on the dorsal side of the vaginal wall. The distal tips of the vaginal fold were serrated and uneven. A banded texture pattern was present along the longitudinal axis of the vaginal fold on the interior of the lumen. The ectocervix was visible through the lumen of the vaginal fold. A shallow transverse ridge was positioned caudal to the vaginal fold and did not extend along the complete circumference of the vaginal lumen.

190x142mm (300 x 300 DPI)

ACC

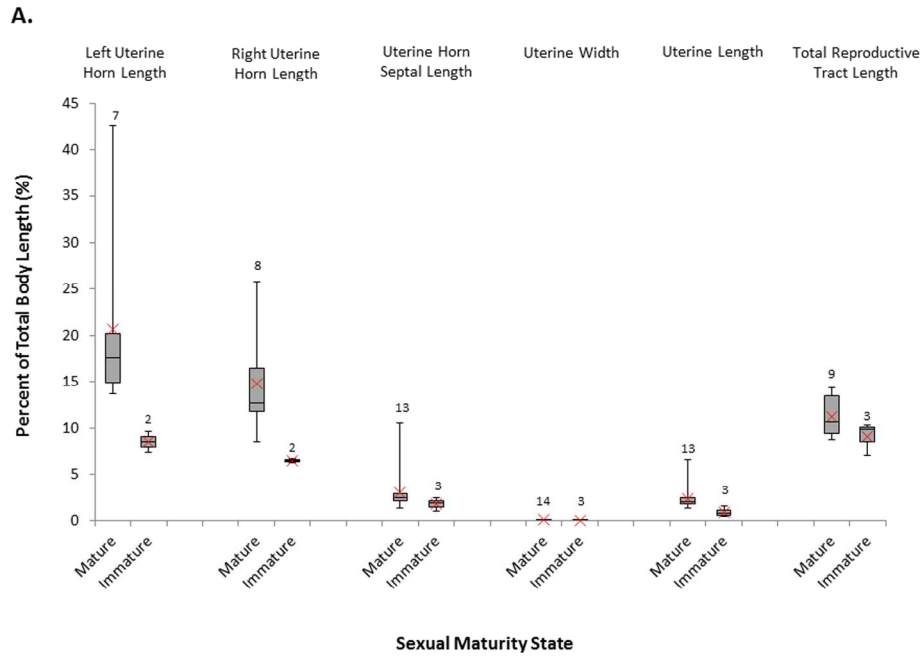


Fig. 5: Tukey's box and whisker plots of the upper reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red 'X' symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

107x75mm (600 x 600 DPI)

Accel

B.

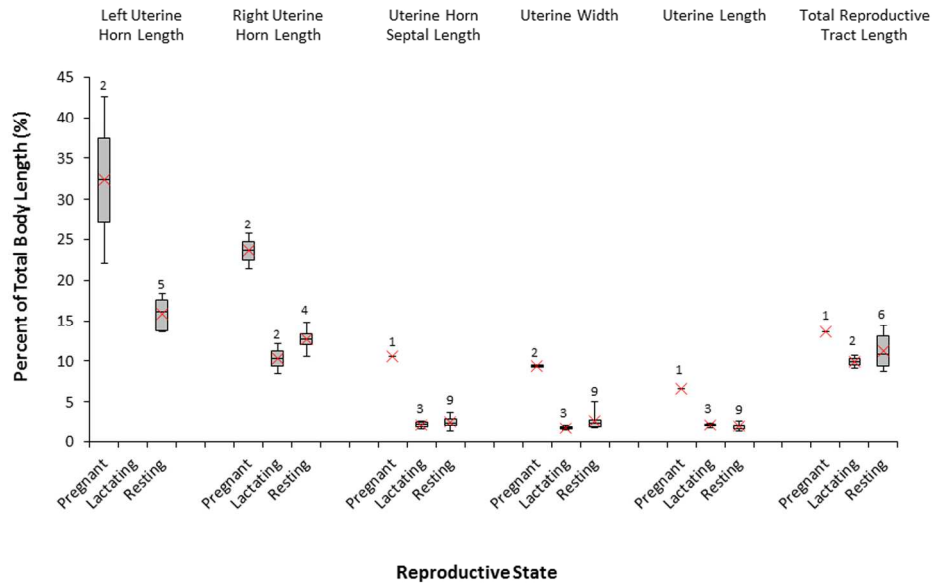


Fig. 5: Tukey's box and whisker plots of the upper reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red 'X' symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

107x75mm (600 x 600 DPI)

Accel

C.

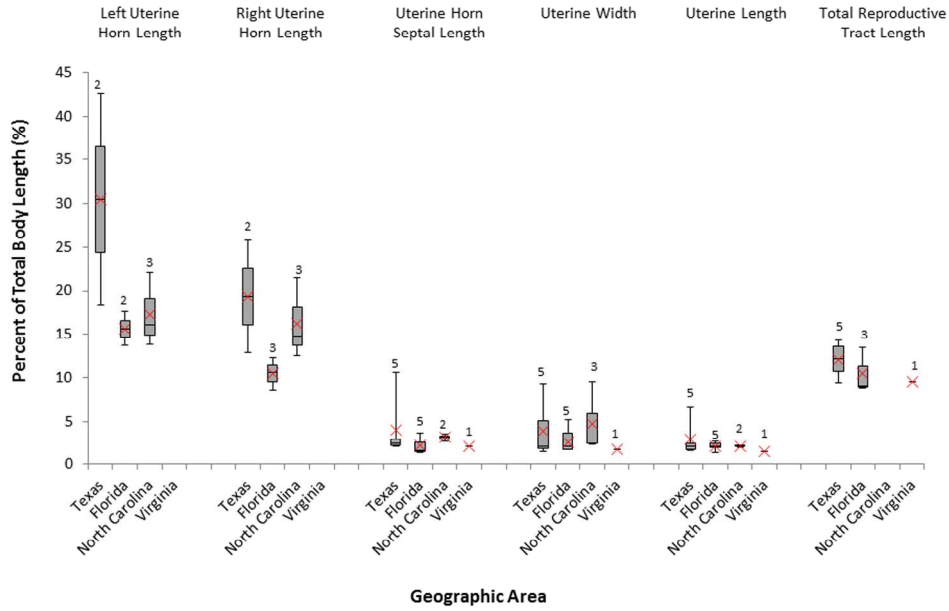


Fig. 5: Tukey's box and whisker plots of the upper reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red 'X' symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

107x76mm (600 x 600 DPI)

Accel

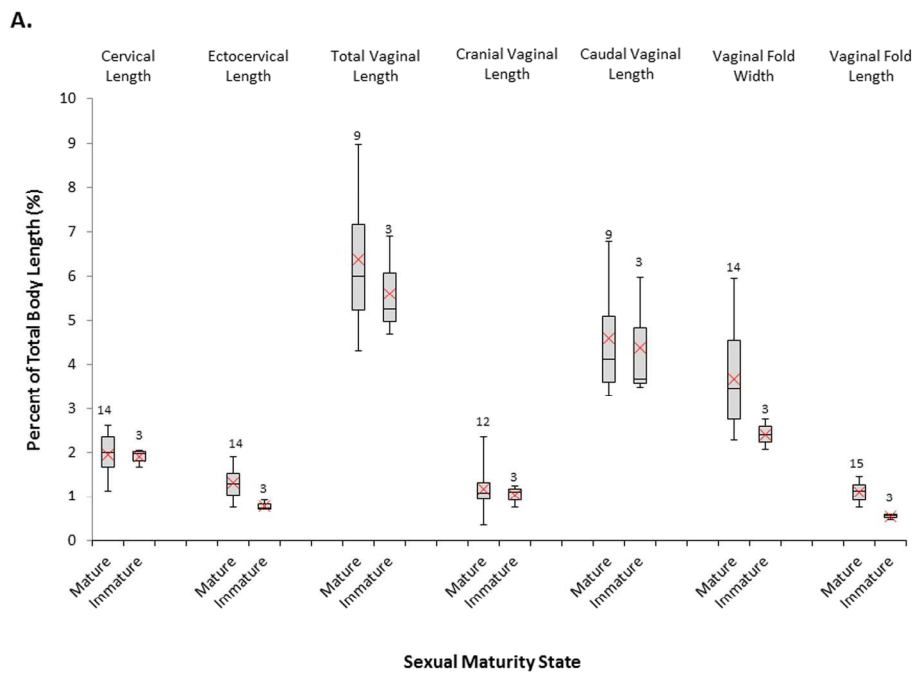


Fig. 6: Tukey's box and whisker plots of the lower reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red 'X' symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

107x76mm (600 x 600 DPI)

Accel

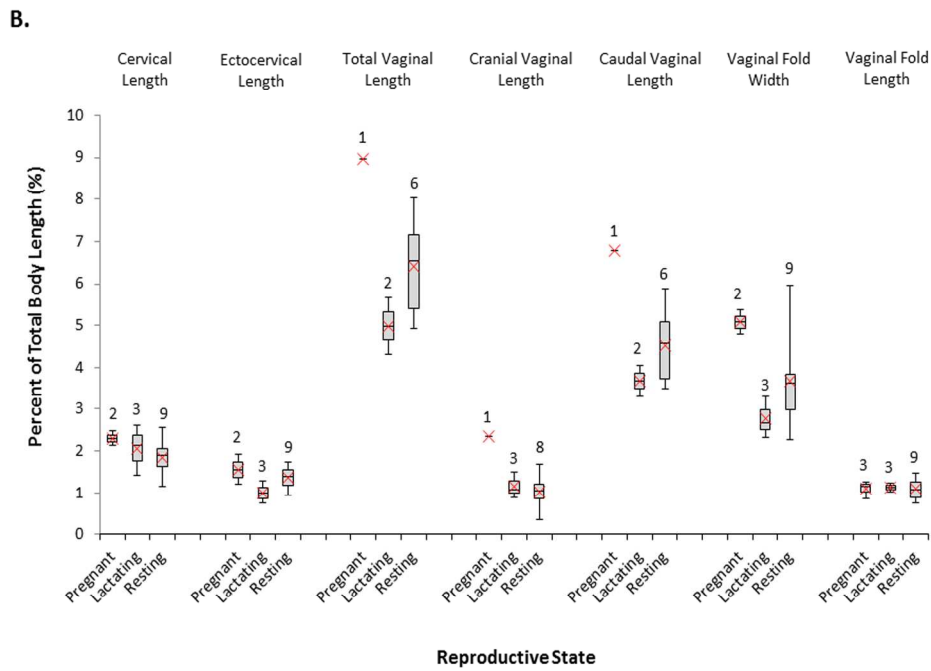


Fig. 6: Tukey's box and whisker plots of the lower reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red 'X' symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

107x76mm (600 x 600 DPI)

Accel

C.

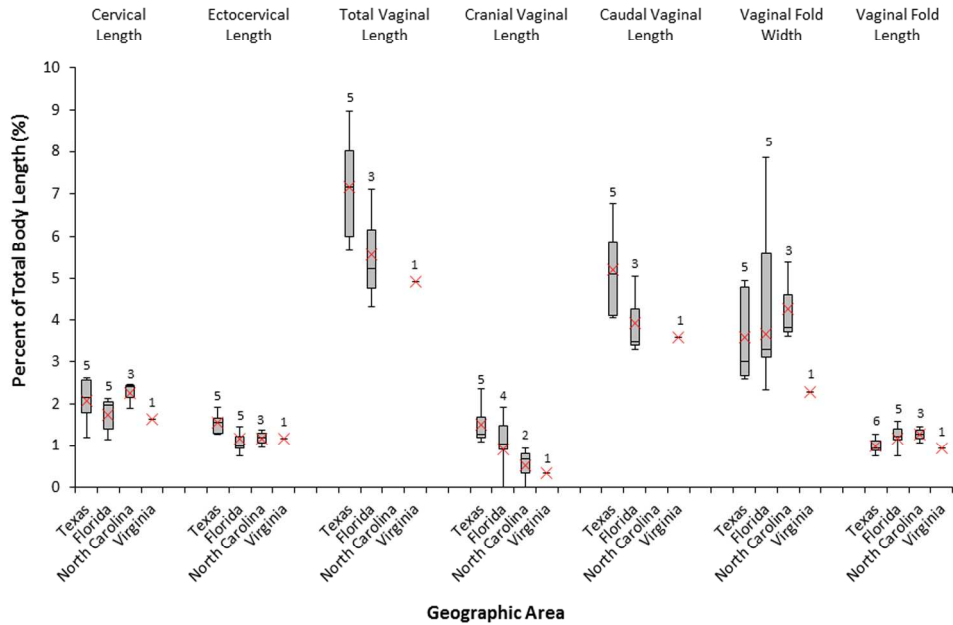


Fig. 6: Tukey's box and whisker plots of the lower reproductive tract measurements of female common bottlenose dolphins divided by: A) sexual maturity state (including all reproductive states), B) reproductive state (adults only), and C) geographic area (adults only). All data are shown as a percentage of the dolphin's total body length. The '-' represents the median. The red 'X' symbol denotes mean percentages. The specimen count for each measurement is above the whiskers.

107x76mm (600 x 600 DPI)

Accel

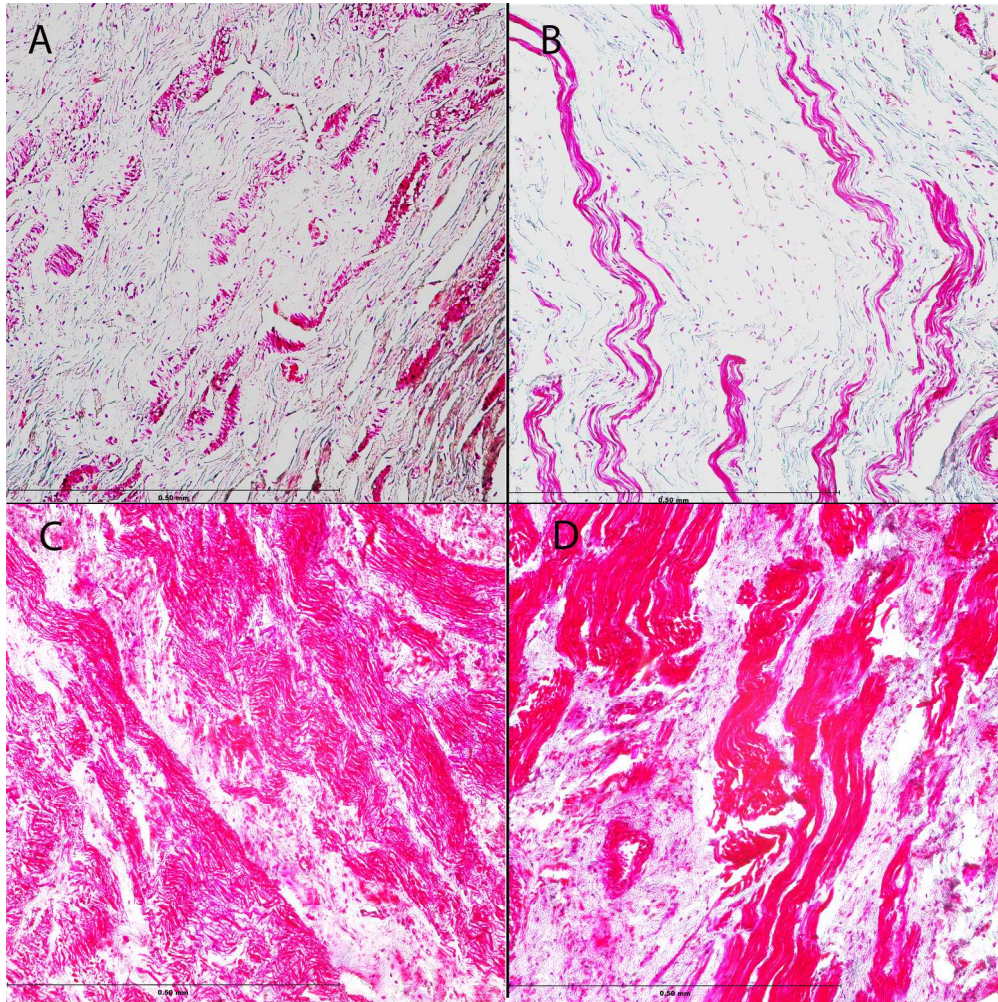


Fig. 7: Micrographs of dolphin tissue at 20x magnification. The slides were stained with a modified Masson's trichrome stain to distinguish the muscle fascicles (pink) from connective tissue (green-blue), and from cell nuclei (black). The left micrographs (A and C) show vaginal fold tissue, while the right micrographs (B and D) show vaginal tissue collected from the caudal vagina. The top two micrographs (A and B) show low densities of muscle banding from the same adult dolphin, while the bottom two micrographs (C and D) show high densities of muscle banding from a second adult dolphin.

203x203mm (300 x 300 DPI)

A