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Acoustical and Anatomical Determination of Sound Production and Transmission in West Indian (*Trichechus manatus*) and Amazonian (*T.* inunguis) Manatees

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ABSTRACT

West Indian (*Trichechus manatus*) and Amazonian (*T. inunguis*) manatees are vocal mammals, with most sounds produced for communication between mothers and calves. While their hearing and vocalizations have been well studied, the actual mechanism of sound production is unknown. Acoustical recordings and anatomical examination were used to determine the source of sound generation. Recordings were performed on live captive manatees from Puerto Rico, Cuba and Colombia (*T. manatus*) and from Peru (*T. inunguis*) to determine focal points of sound production. The manatees were recorded using two directional hydrophones placed on the throat and nasal region and an Edirol-R44 digital recorder. The average sound intensity level was analyzed to evaluate the sound source with a *T* test: paired two sample for means. Anatomical examinations were conducted on six *T. manatus* carcasses from Florida and Puerto Rico. During necropsies, the larynx, trachea, and nasal areas were dissected, with particular focus on identifying musculature and soft tissues capable of vibrating or constricting the airway. From the recordings we found that the acoustical intensity was significant ($P < 0.0001$) for both the individuals and the pooled manatees in the ventral throat region compared to the nasal region. From the dissection we found two raised areas of tissue in the lateral walls of the manatee's laryngeal lumen that are consistent with mammalian vocal folds. They oppose each other and may be able to regulate airflow between them when they are adducted or abducted by muscular control of arytenoid cartilages. Acoustic and anatomical evidence taken together suggest vocal folds as the mechanism for sound production in manatees. Anat Rec, 297:1896–1907, 2014. © 2014 Wiley Periodicals, Inc.

Key words: Sirenia; sound production; vocal folds; larynx; communication

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TABLE 1. Manatees recorded live during veterinary examinations (recordings), and specimens dissected for anatomical study (dissections)

Recordings

Species	Name	Sex	Age	Origin of specimen
T. manatus	Aramaná	M	C	Puerto Rico
T. manatus	Guamá	M	C	Cuba
T. manatus	David	M	A	Colombia
T. inunguis	Yuri	M	J	Peru
T. inunguis	Sur America	F	A	Peru

Dissections

Species	Specimen ID	Sex	Age	Length	Origin of specimen
T. manatus	LPZ102921	F	A	228	Florida
T. manatus	MSW1159	F	A	269	Florida
T. manatus	MSE1152	M	C	121	Florida
T. manatus	MEC1190	M	C	144	Florida
T. manatus	SWFTm1115b	M	C	138	Florida
T. manatus	NEPT941	F	A	309	Puerto Rico

M, male; F, female C, calf; J, juvenile; A, adult; Length, Total linear length in centimeters from tip of the snout to the end of the tail.

The mammalian order Sirenia is composed of four extant species in tropical and subtropical aquatic ecosystems: the West Indian manatee (*Trichechus manatus*), the Amazonian manatee (*T. inunguis*), the West African manatee (*T. senegalensis*), and the dugong (*Dugong dugon*). These are considered the only herbivorous marine mammals, and all are vulnerable due to pressure from negative human interactions (Gerstein, 2002).

Sirenians, as other marine mammals, use sound to communicate. Some marine mammals (i.e., toothed whales) use echolocation for navigation and locating prey, while others (e.g., humpback whales, *Megaptera novaeangliae*) produce structured song units during the breeding season (Au et al., 2006). Sirenians produce sound year round, and these are used to maintain social interactions (Hartman, 1979). Manatee vocalizations, categorized as chirps, squeaks, and squeals, are characteristically short tonal complexes that contain several harmonics (Nowacek et al., 2003). The fundamental frequencies range from 2.5 to 5.9 kHz but can extend up to 15 kHz and have a duration between 0.25 and 0.5 s (Nowacek et al., 2003; Phillips et al., 2004). Vocalization rates are higher when manatees socialize (Bengtson and Fitzgerald, 1985), especially between parent and offspring. One vocal function is to maintain acoustical close proximity between mother and calf (Hartman, 1979), particularly as mother/calf pairs frequently separate momentarily. During parental foraging, mothers "park" their calves in protected areas so they may briefly go and graze on sea grass beds. Evidence of individually distinct vocal signals and individual vocal recognition has been documented in Amazonian and West Indian manatees (Sousa-Lima et al., 2002; Sousa-Lima et al., 2008). Individual vocal differences have also been noted in dugongs (Anderson and Barclay, 1995) and between a Florida manatee mother and calf that had been physically separated (Reynolds, 1981).

Human activities (hunting, habitat encroachment, bycatch, habitat destruction, and environmental contamination) have greatly affected sirenian populations. An increase of marine activities and water noise pollution (e.g., motorized boats) has resulted in sirenians being exposed to the effects of noise masking. Noise masking occurs when water noise pollution prevents aquatic animals from perceiving natural sounds in their environment (Marine Mammal Commission, 2007). For example, motor boat or ship engine sounds may disrupt manatee communication because noise amplitudes are so high that they overwhelm their relatively quiet vocalizations, or because noise frequencies overlap, and thus mask, their vocalization frequencies (Miksis-Olds, 2006). Exposure to elevated ambient noise altered the communication behavior of dugongs, resulting in a change of their usual call frequencies of 3–6 kHz to an elevated 6–9 kHz (Ando-Mizobata et al., 2011). Moreover, cases of manatee collisions with watercraft may be due to a number of factors, including inability to hear the approach of individual boats due to combined noise masking by the total number of boats in the region. In an effort to reduce mortality rates due to watercraft collisions, studies of sirenian vocalizations have centered on characterizing their vocalizations and audition. These research data supported the development of a high-frequency alarm placed on the bow of boats to alert manatees and help prevent collisions (Gerstein, 2002). Current efforts are directed at devising an effective system to alert mariners of the presence of manatees based on passive detection of vocalizations (Nowacek et al., 2003).

While these studies have provided ample information on the sound characteristics of their vocalizations and the ranges and capabilities of their hearing, there is no information to date on the mechanism of sirenian sound production. This is due mainly to the limited knowledge of the anatomy of the sirenian upper respiratory tract. Since the description of the larynx of two Antillean manatees by Murie (1872), there have been no detailed anatomical studies of the mechanisms that can generate sound for this particular species. The objective of this study was to define the source and mechanism of sound generation and of transduction (transfer to water), and to characterize the sound in the West Indian and

Amazonian manatees. This entailed two different research approaches: acoustical and anatomical techniques.

MATERIALS AND METHODS

Acoustical Techniques

Five live captive manatees were recorded for acoustic signals during routine medical examinations (Table 1). Protocols used in live animals were previously approved by the Inter American University's Institutional Animal Care and Use Committee (IACUC). Acoustic sampling took place in Puerto Rico and Colombia on three male West Indian manatees (two calves, one from Puerto Rico and one from Cuba, and one adult Colombian manatee, all of the Antillean manatee subspecies [*T. manatus manatus*]), and in Peru on one adult female and one juvenile male Amazonian manatee. One SS03-10 *Sea Phone* directional hydrophone (sensitivity of −169 dB re 1 μPa and a frequency range response of 20 Hz to 50 kHz) with 30.5-cm suction cup was placed on the throat region (Fig. 1A) and another was placed on the nasal region caudal (Fig. 1B) to the nostrils of the manatee to determine the focal point of sound production. The nasal region was selected because some marine mammals produce sound with structures located next to the nares/blowhole (e.g., odontocetes). The throat region was selected because the laryngeal vocal folds in the manatee's closest relative—the elephant (Shoshani, 1998)—are located in that area, and this is also the location of laryngeal sound production in other marine mammals (e.g., mysticetes, pinnipeds). The sounds from each of the two hydrophones were recorded with a digital recorder (Edirol R-44 frequency response 20 Hz to 40 kHz +0/−3 dB) and analyzed with the program *Raven Pro 1.4* to obtain the corresponding spectrograms. The duration and frequency were measured from the most intense harmonic that was visible along the length of the signal with a good signal to noise ratio. The fundamental frequency was the lowest band in a harmonic series. The average sound intensity difference between the throat and nasal areas was analyzed to localize the sound source. The data were tested for statistically significant differences using a *T* test: paired two sample for means ($\alpha = 0.05$). The sample size for each statistical test was 20 vocalizations.

Anatomical Techniques

We examined larynges both *in situ* and excised from the heads of six West Indian manatee carcasses: five Florida manatees (*T. manatus latirostris*) and one Antillean manatee from Puerto Rico (Table 1). Specimens of both sexes and different ages were included. All manatee carcasses were recovered in fresh condition (stranding network designation of code 1). The heads were severed and stored frozen for future examination. The dissections were carried out under the authority of permits from Puerto Rico's Department of Natural and Environmental Resources and the US Fish and Wildlife Service.

Dissections followed the protocol of Bonde et al. (1983). One head was midsagittally sectioned on a bandsaw (butcher's style) while frozen, and all remaining heads were thawed for detailed dissections of the surrounding extrinsic laryngeal musculature. Larynges were removed from the heads and further dissected to reveal intrinsic anatomy. The cricoid cartilage was incised in the dorsal midline and each portion was reflected laterally to view the laryngeal lumen in order to examine for the presence or absence of vocal folds. All larynges were photographed, and then preserved by immersion in 10% formalin. Internal laryngeal anatomy was examined to ascertain whether there were structures that are homologous to the vocal folds of terrestrial mammals. Possible tissues were evaluated for their (1) orientation, (2) cartilaginous attachments, (3) potential cartilage movements and mechanism of control, (4) presence/absence of ligaments, (5) innervations of associated musculature and mucosa, and (6) relationships to adjacent structures, as in Reidenberg and Laitman (2007).

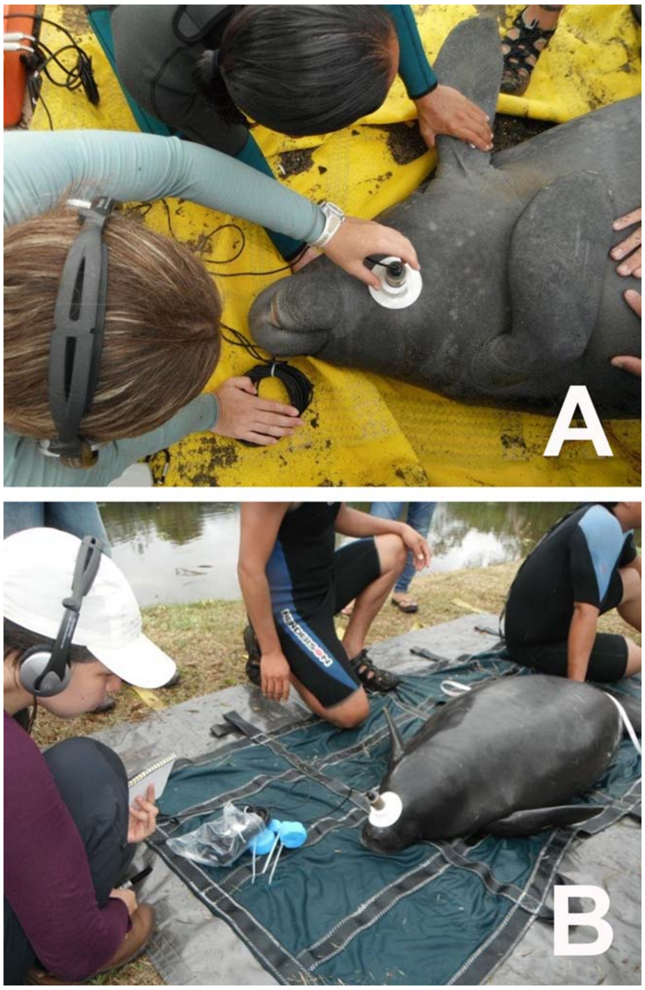

Fig. 1. Recordings of manatees to determine the point of sound transmission. (**A**) Throat region recording of a live *Trichechus manatus* in Puerto Rico. (**B**) Nasal region recording of a live *T. inunguis* in Peru.

RESULTS

Acoustics

Eighty-three separate recording sessions on a total of five manatees were conducted, yielding 526 vocalizations

TABLE 2. Mean values for measured parameters of sounds recorded from manatees during the study

	Trichechus manatus			*Trichechus inunguis*
Variable	Cuba	Puerto Rico	Colombia	Peru
n	121	69	35	103
Mean duration (ms)	231.6 ± 68.5 (40.6–394.7)	251.5 ± 124.8 (64–502.1)	258.8 ± 94 (72.6–421)	224.5 ± 140.3 (70.1–516.7)
Mean fundamental frequency (Hz)	2988 ± 445 (1895–3878)	2390 ± 581[a] (1206–3617.6)	3325 ± 458 (2600–4134.4)	2907 ± 659 (1808–3925)
Mean peak frequency (Hz)	9395 ± 3013 (1034–20155)	5652 ± 3133 (517–11886)	7189 ± 3454 (612–11370)	8706 ± 3133 (918–12248)
Minimum frequency (Hz)	575.8	519.1	450	704.6
Maximum frequency (Hz)	22050	18547.2	20345	17433.2

[a] The subsample size (n) is 64. Dataset range is shown in parenthesis.

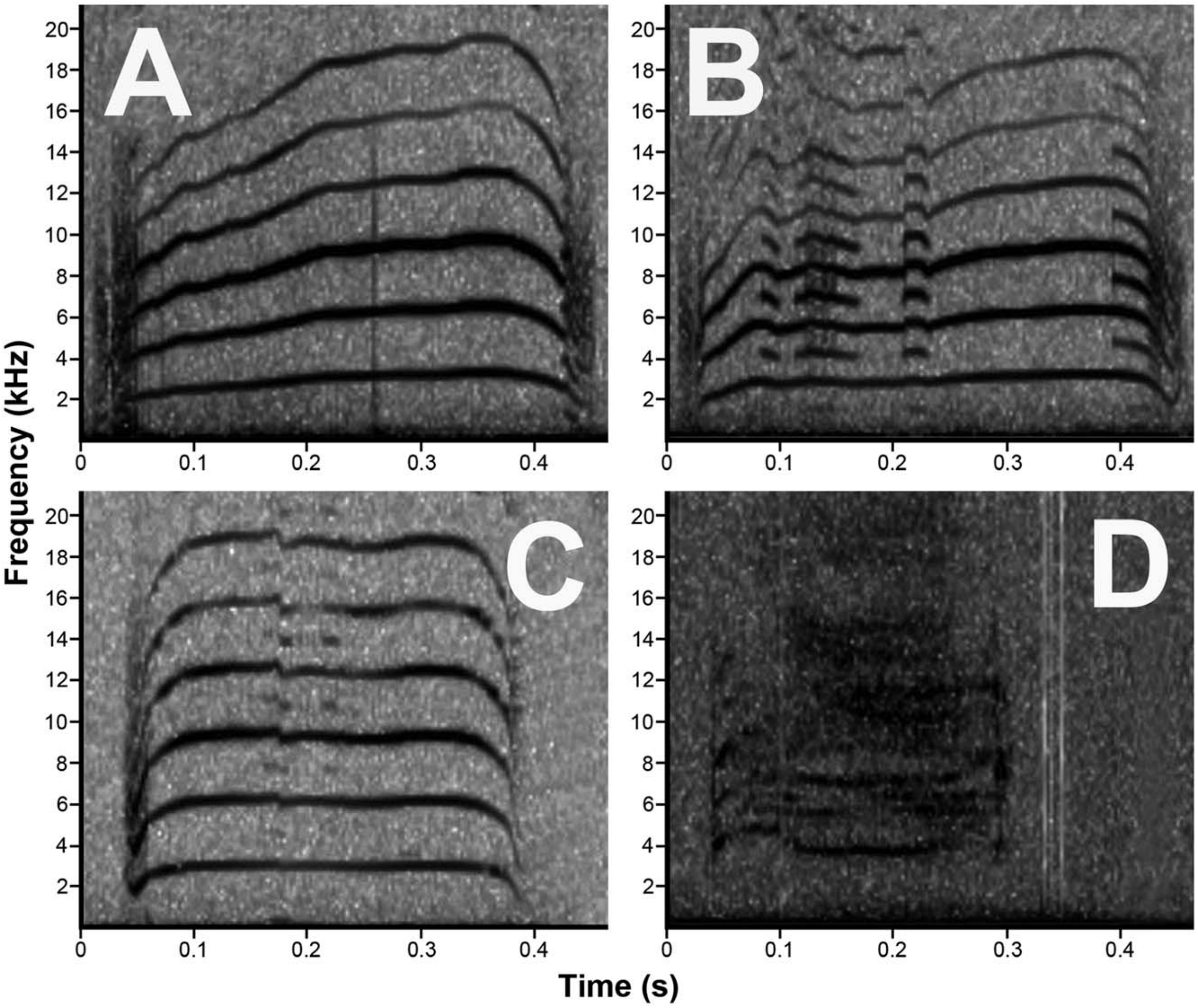

Fig. 2. Spectrograms of vocalizations of *Trichechus manatus* and *T. inunguis*. (**A**) tonal harmonic vocalization (ascending to descending), (**B**) tonal harmonic structure with an upward modulation at the beginning and subharmonics, (**C**) harmonic structure with a nonlinear element (subharmonincs), and (**D**) tonal harmonic vocalization with a noisy quality.

produced by manatees in Puerto Rico, Colombia, and Peru. See Table 2 for a summary of the mean values for measured parameters (duration, fundamental frequency, peak frequency, minimum and maximum frequency) of sounds recorded from the manatees in this study.

TABLE 3. Statistical comparison and intensity data with standard deviation collected from manatees

	Nasal region			Throat region				
	Mean intensity (dB)	Lowest intensity (dB)	Highest intensity (dB)	Mean intensity (dB)	Lowest intensity (dB)	Highest intensity (dB)	*P* value	n
T. manatus								
Aramaná	52.5 ± 4.3	40.2	59.2	65.3 ± 4.9	56	73	<0.0001	20
Guamá	46.1 ± 5.5	32.4	55.2	68.4 ± 6.2	56.7	80.7	<0.0001	20
David	52.6 ± 4.3	40.2	59.8	65.8 ± 5.02	58.2	73	<0.0001	20
T. inunguis								
Yuri	49.4 ± 4.5	39.9	56.8	63 ± 5.9	54.4	74	<0.0001	20
Sur America	51 ± 5.4	38.1	59.2	65.1 ± 6.6	55	76.5	<0.0001	20
All individuals	50.3 ± 5.3	32.4	59.8	65.9 ± 5.9	54.4	80.7	<0.0001	100

Data on this table does not represent absolute intensity values but can be used for relative comparisons within this study.

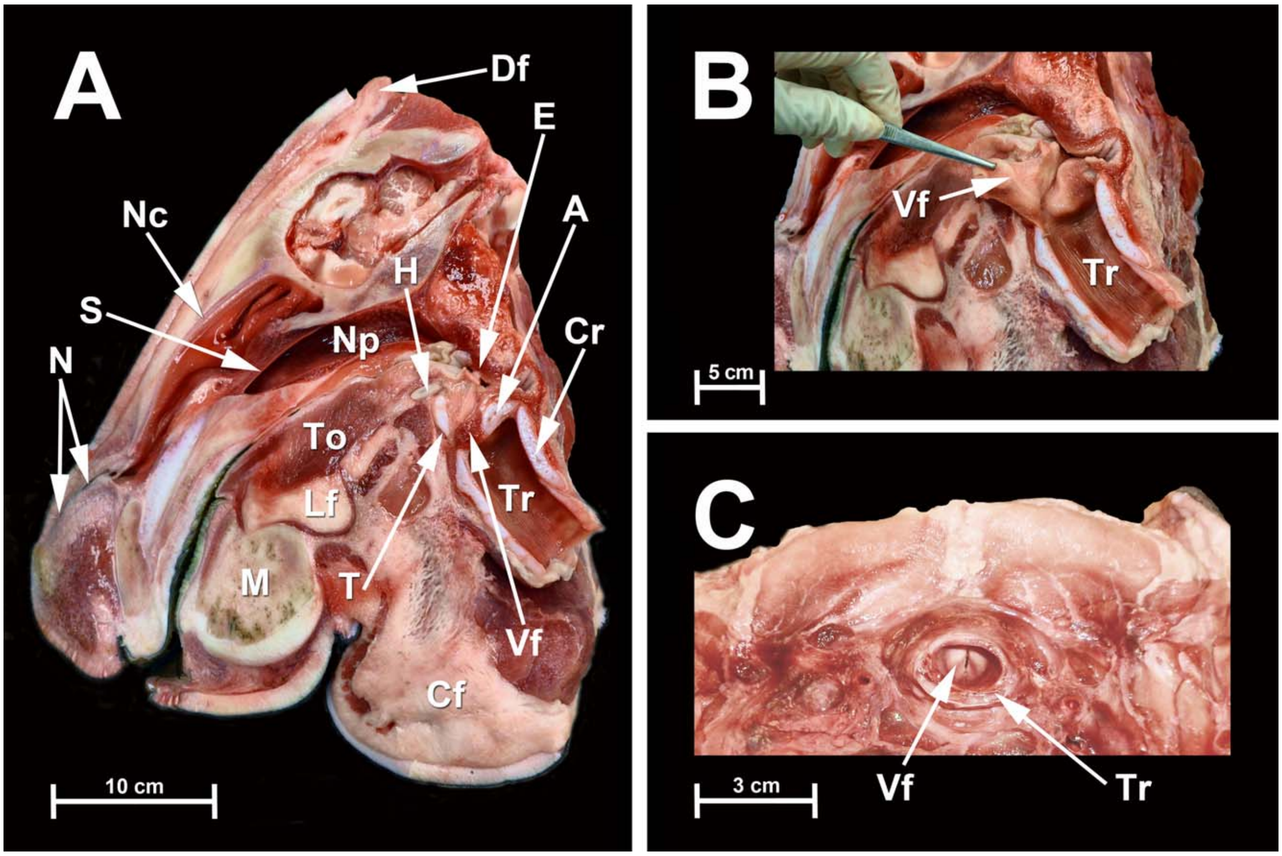

Fig. 3. (**A**) Adult female manatee head (*Trichechus manatus lastrirostris*) cut along the midsagittal plane, right side. Note: cervical region was unnaturally flexed after decapitation when it was stored in freezer prior to bandsaw cutting. The cervical vertebrae are missing, but would normally be positioned roughly parallel to the brain stem, aligned with the foramen magnum. The trachea (Tr) would normally be positioned rotated ~90° dorsal from the position in this figure, running approximately parallel to the vertebral column. In the natural position, the large region of cervical fat (Cf) would lie ventral to the larynx. A, arytenoid cartilage; Nc, nasal cartilage; Cr, cricoid cartilage; Df, dorsal fat; E, epiglottis; H, hyoid bone; LF, lingual fat; M, mandible; N, naris; Np, nasal passage; S, septum; T, thyroid cartilage; To, tongue; Tr, trachea; Vf, vocal fold. (**B**) Adult female manatee head cut along the midsagittal plane (right side) depicting the vocal folds in the larynx. Retraction with the forceps shows the left vocal fold laterally. This reveals the entirety of the right vocal fold traversing across the laryngeal lumen. (**C**) Caudal view of a whole manatee male calf head that was decapitated from the carcass. It shows a close up of the cut trachea (Tr), and the lumen that leads to the larynx. The vocal folds (Vf) can be seen as two opposing masses of tissue that obstruct the lumen. They are approximated in the midline.

West Indian manatee vocalization repertoire from Puerto Rico and Cuba consists mostly of harmonic structures with modulations at the beginning (ascending or descending), middle and end (ascending or descending). In addition, several of the Cuban vocalizations had nonlinear elements (i.e., subharmonics) (Fig. 2A–C).

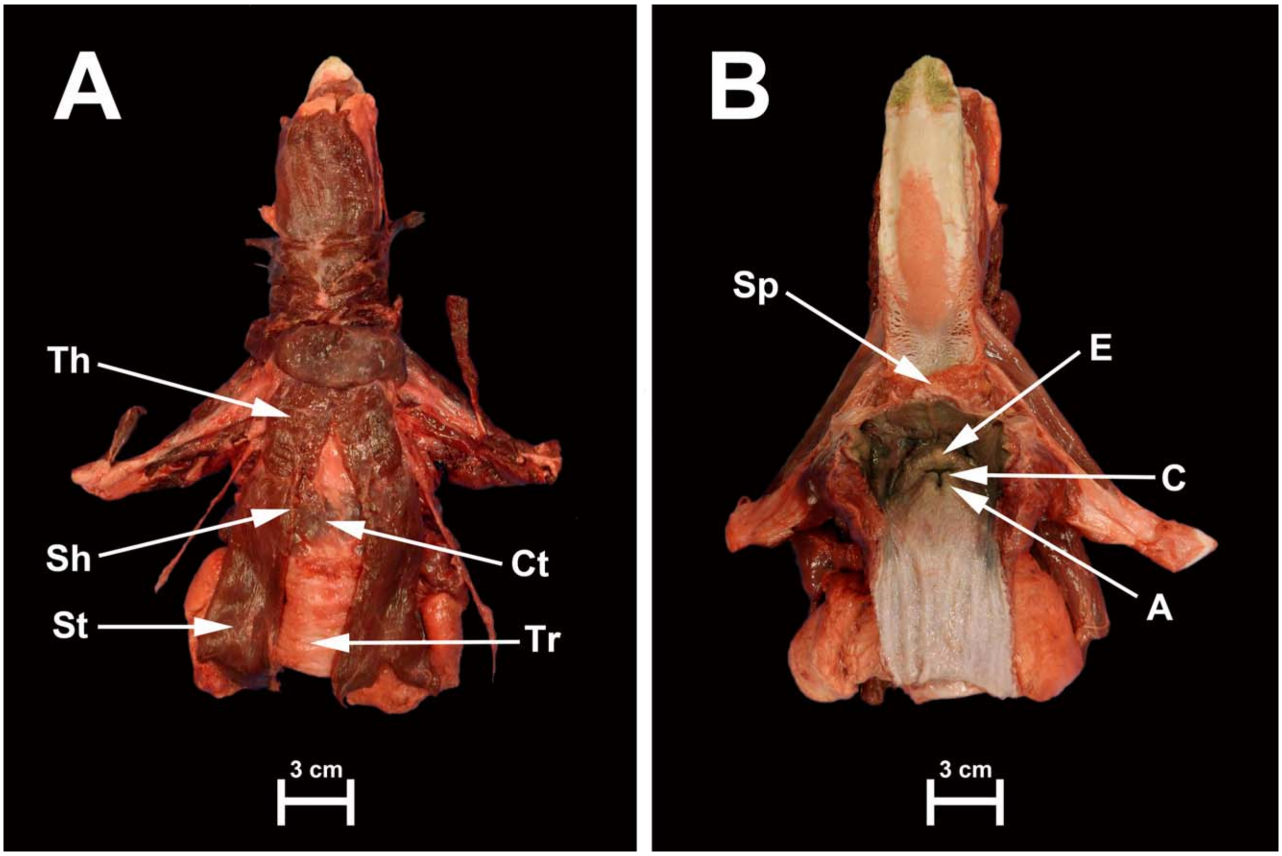

Fig. 4. (**A**) Ventral view of an adult manatee larynx. Notice the paired cricothyroid muscles (Ct) located cranial to the trachea (Tr) and caudal to the midline of the thyroid cartilage. Three paired extrinsic laryngeal muscles are visible: thyrohyoid (Th), sternothyroid (St), and sternohyoid (Sh). All three muslces are fused together, giving the appearance of one large muscular strap, with the sternohyoid spanning medially over the connective tissue seam between the thyrohyoid and sternothyroid muscles. (**B**) Dorsal view of an adult manatee larynx. Note the short epiglottis (E) barely contacting the soft palate (Sp), and narrow "T"-shaped laryngeal aditus opening between it and the closely opposed arytenoid (A) and corniculate (C) cartilages.

Vocalizations of the West Indian manatee from Colombia and Amazonian manatees from Peru consist mostly of harmonic complexes with a noisy quality (Fig. 2D). Both throat and nasal recordings were made on all individuals. The intensity levels of the recordings were higher in the throat region than in the nasal region for each recording of each animal. The difference between the sound intensity levels in the throat and nasal regions was statistically significant for each individual ($P < 0.0001$) (Table 3). In addition, there was a significant difference in the pooled samples for all five individuals ($P < 0.0001$) (Table 3).

Anatomy

Nasal region. The manatee nasal cavity is large and divided by a nasal septum into two nasal chambers that contain conchae along the lateral walls. Each chamber is approximately tubular in shape, and spans the entire length of the rostrum. The lumen of the airway is oriented approximately parallel to the long axis of the vertebral column. The dorsal aspect of each nasal chamber is covered by cartilage and bone (Fig. 3A), but the external nasal passageways are surrounded by soft tissues that were observed to swell and retract in living manatees during vocalizations. A pair of narrow and long air passageways led anteriorly from each chamber through those soft tissues, and each terminates at a small, round nostril located at the tip of the rostrum (Fig. 3A). The passageways are surrounded by nasal plug connective tissue and musculature. No structures capable of vibrating were noted in the nasal cavity.

Throat region. Two fat pads are found in the throat region (Fig. 3A): one is in near the tongue (lingual fat pad) and the other is located in the ventral neck (cervical fat pad). The lingual fat pad is the smaller of the two fat pads, and is colored off-white. It is located at the base of the rostral aspect of the tongue, immediately caudal to the genu of the mandible and dorsal to the geniohyoid muscle. The cervical fat pad is located ventrally in the throat region, but dorsal to the external circumferential fat layer associated with the overlying skin. The cervical fat pad is yellowish in color compared to the white blubber layer, and is much larger than the lingual fat pad.

The manatee larynx resembles the terrestrial mammal larynx in overall structure. It is composed of the unpaired thyroid, cricoid, and epiglottic cartilages, and the paired arytenoid and corniculate cartilages. No

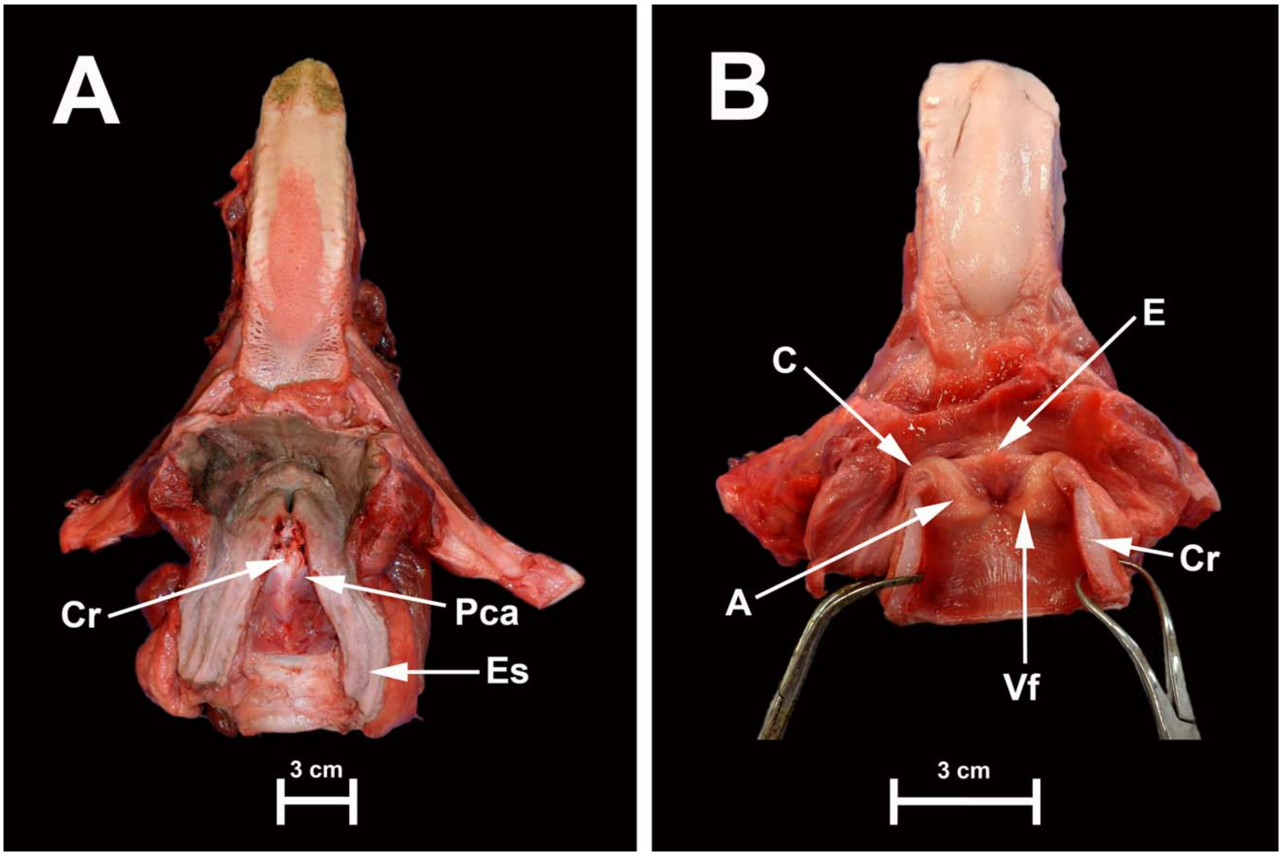

Fig. 5. (**A**) Dorsal view of an adult manatee larynx, with the esophagus (Es) cut in the dorsal midline. The cricoid cartilage (Cr) is revealed below the esophagus. Note the dorsal ridge, which provides additional surface area on either side for the attachments of the posterior cricoarytenoid muscles (Pca). (**B**) Dorsal view into the lumen of the larynx of a manatee calf. The larynx has been cut along the dorsal midline of the cricoid cartilage (Cr), and the left and right halves are reflected laterally. Note the dorsal position of the corniculate cartilage (**C**) relative to the arytenoid cartilage (A). The arytenoid has an L-shape, with the vertical component meeting the corniculate cartilage, and the horizontal component supporting the vocal fold (Vf) directed medially and ventrally towards the thyroid cartilage at the base of the epiglottis (E).

cuneiform cartilages were noted, but they may have been embedded in the undissected aryepiglottic folds. The thyroid cartilage forms the ventral aspect of the larynx and extends laterally and dorsally on both sides. The midline of the thyroid cartilage (thyroid prominence) can be seen between the paired cricothyroid muscles (Fig. 4A). These attach ventrally just lateral to the midline, and extend to the rostral margins of the cricoid cartilage. The paired thyrohyoid muscles attach cranially to the region of the basihyal portion of the hyoid bone. On each side, the thyrohyoid muscle terminates caudally into a connective tissue band located on the thyroid cartilage that separates it from the cranial edge of the corresponding sternothyroid muscle, giving the appearance of the two muscles merging into one broad ribbon of muscle. The paired sternothyroid muscles are the largest of the extrinsic muscles, and attach the laryx to the sternum. The paired sternohyoid muscles are very thin and are positioned closer to the midline than the larger thyrohyoid and sternothyroid muscles. The lateral edges of the sternohyoid are continuous with both the thyrohyoid and sternothyroid muscles, except where they pass ventrally to overlie the connective tissue band separating the latter two muscles. The sternal attachments of the sternothyroid and sternohyoid muscles were severed during decapitation.

The dorsal aspect of the larynx reveals a short epiglottis that approximates the soft palate (Fig. 4B). The epiglottis is unpaired, and is flexible near the pointed tip. The “T”-shaped opening into the laryngeal lumen (laryngeal aditus) is relatively small, and is surrounded by the epiglottis rostrally in the midline and the paired corniculate and arytenoid cartilages caudo-laterally. The arytenoid cartilages are attached to the cranial edge of the unpaired cricoid’s dorsal aspect. The arytenoid is pyramidal in shape with a wider base at the articulation with the cricoid cartilage. Each arytenoid projects ventrally into the lumen of the larynx, supporting a thick fold of tissue. The lateral aspect of the arytenoid cartilage is raised into a muscular process. The posterior cricoarytenoid muscle inserts at this site and fans out dorsally and laterally to attach at its origin on the external dorsal aspect of the cricoid cartilage. The cricoid cartilage has a midline ridge along the dorsal aspect. This provides additional surface area for the attachment of the posterior cricoarytenoid muscles (Fig. 5A).

Direct manipulation of the arytenoid produces a movement which results in abduction and adduction of the

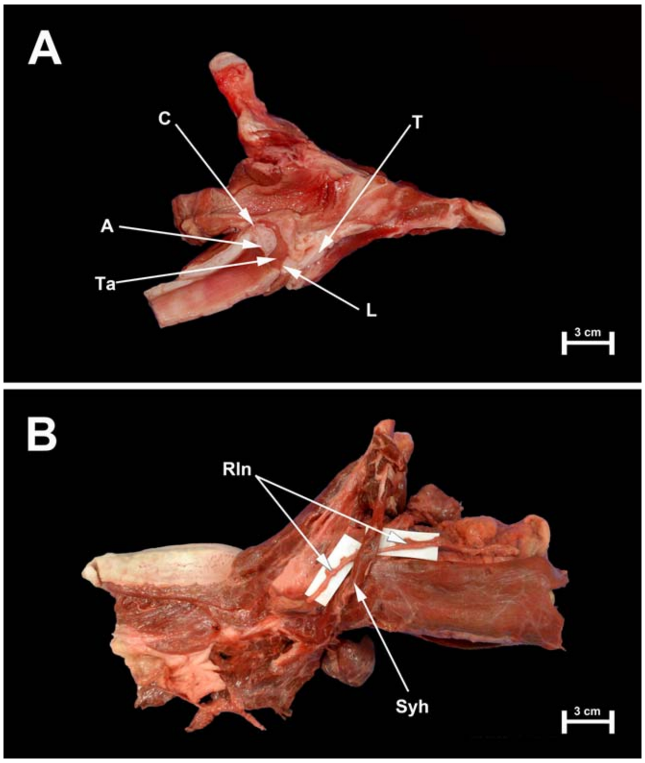

Fig. 6. Medial aspect of the left side of an adult manatee tongue and larynx cut along the midsagittal plane, including the left hyoid apparatus. Detailed dissection of the vocal fold reveals the supporting vocal process of the arytenoid cartilage (**A**) and the ventral extension of a ligament (L) toward the thyroid cartilage (T). The thyroarytenoid muscle (Ta) can be seen immediately above the ligament and arytenoid cartilage. The small corniculate cartilage (C) can be seen immediately dorsal to the arytenoid cartilage. (**B**) Left lateral view of the same adult manatee larynx, tongue and hyoid specimen as Figure A. The recurrent laryngeal nerve (Rln) can be seen on the two white squares. It courses cranially along the side of the trachea to innervate the caudal portion of the larynx. The stylohyoid muscle (Syh) can be seen overlying the recurrent laryngeal nerve.

tissues supported by the vocal process. The abduction movement increases the width of the rima glottidis, whereas adduction narrows the rima. The muscles usually considered for narrowing the laryngeal rima (e.g., lateral cricoarytenoid and interarytenoid) may be present, but were not confirmed in our dissections. The posterior cricoarytenoid muscle was manipulated in the direction of the muscle's fibers: (1) pulling the muscle caudally toward the dorsal origin on the cricoid cartilage causes abduction of the arytenoid cartilage.

The corniculate cartilages are situated dorsally on the larynx, and curve caudally (Fig. 5B). They are fused ventrally to the cranio-dorsal aspect of the arytenoid cartilages. The arytenoid cartilages appear L-shaped when viewed in the midsagittal plane. The vertical component (as oriented in Fig. 5B) meets the corniculate cartilage dorsally, and the horizontal component is directed medially and ventrally toward the thyroid cartilage.

The manatee has two raised areas of tissue in the lateral walls of the laryngeal lumen that are homologous to vocal folds based upon both position and structure. These folds oppose each other and may be able to regulate airflow between them when they are adducted or abducted by muscular control of the arytenoid cartilages. A midsagittal view of the larynx reveals these two fleshy tissue bulges are oriented perpendicular to airflow (Fig. 3B). These tissues can be adducted (by manipulation of the arytenoid cartilage) into opposition at the midline to accomplish occlusion of the airway. A caudal view (Fig. 3C) shows how these tissue masses can function as a valve and obstruct the laryngeal lumen when opposed. A detailed dissection of the vocal fold (Fig. 6A) reveals the supporting vocal process of the arytenoid cartilage. On gross exaination, the vocal processof the arytenoid cartilage is connected to a thin ligament that extends ventrally towards the thyroid cartilage. Immediately cranial to these structures is the thyroarytenoid muscle.

The innervation of the manatee larynx is similar to that of other terrestrial mammals (Fig. 6B). The superior laryngeal nerves branch from the vagus nerves and pass caudal to the hyoid bone to innervate the larynx at its cranial aspect. The internal branch of the superior laryngeal nerve was noted to pass into the wall of the laryngeal vestibule cranial to the vocal folds. The external branch was not dissected, but is presumed to innervate the cricothyroid muscle, as this is the pattern in other mammals. The recurrent laryngeal nerves were not traced from their origins off the vagus nerves, due to severing of the head in the region of the cervical-thoracic junction. However, they were noted to course cranially along the sides of the trachea to innervate the caudal aspect of the larynx. The recurrent laryngeal nerve was dissected along its course into the posterior cricoarytenoid muscle, but was not dissected further into any other muscles. It is presumed to innervate the remaining intrinsic laryngeal muscles, including thyroarytenoid.

DISCUSSION

Acoustic Analysis

The manatees responded to stimuli by generating various forms of vocal behaviors that, depending on the emotional state, were different in amplitude, structure, and duration. Our recordings were within the frequency range reported in previous literature for both species of manatees (Schevill and Watkins, 1965; Evans and Herald, 1970; Sonoda and Takemura, 1973; Steel, 1982; Sousa-Lima et al., 2002, 2008; Nowacek et al., 2003; O'Shea and Poché, 2006). Amazonian and West Indian manatees' higher harmonics (2nd and 3rd) usually contained more energy than the fundamental frequency in our study. O'Shea and Poché (2006) suggested that the inclusion of multiple harmonics with greatest intensities at harmonics higher than the fundamental will help circumvent the canceling out of lower frequencies in shallow waters (the Lloyd Mirror Effect is insubstantial at 0.2 kHz in waters about 1.5 m deep [Gerstein, 2002]), and these higher harmonics coincide more closely with the range of auditory best frequencies reported by Gerstein et al. (1999). Recorded clicks for Antillean manatees were found mainly in the 2–7 kHz frequency range (Sonoda and Takemura, 1973) and reached peak frequencies up to 14 kHz (Sousa-Lima et al., 2008). Nowacek et al. (2003), however, did not report clicks in wild West Indian manatees. Our recordings of West Indian manatees also do not show clicks in their vocal repertoire.

The subspecies of the West Indian manatee, the Antillean manatee (*T. m. manatus*) and the Florida manatee (*T. m. latirostris*), are distinguishable by mostly quantitative cranial morphologic characters (Domning and Hayek, 1986) and are genetically distinct groups (Hunter et al., 2012). The Antillean manatee is found in the Greater Antilles, including Cuba, Hispaniola, Puerto Rico and Jamaica (Lefebvre et al., 2001). The visible sound, from the Cuban calf manatee, extended from 575.8 to 22050 Hz. The energy of 22 kHz is the highest frequency published for sirenians compared to other literature (Schevill and Watkins, 1965; Alicea-Pou, 2001; O'Shea and Poché, 2006; Sousa-Lima et al., 2008). Higher-frequency sounds are easier for manatees to localize because they produce larger interaural intensity differences (Mann et al., 2005), which is important for mother and calf communication when traveling or when they are separated.

The sound intensity level is the measure of sound power over a particular area (Au and Hastings, 2008). As a sound wave carries its energy through a medium (e.g., soft tissues), the intensity of the sound wave decreases with increasing distance from the source. This decay is referred to as transmission loss (Au and Hastings, 2008). Analysis of the recordings from the manatees examined revealed significant differences between the sound intensity levels of the nasal versus the throat region (Table 3), with higher intensities measured in the throat region. This indicates that the sound received by the nasal region microphone had traveled a larger distance than the sound received by the throat microphone, and thus the sound source is closer to the throat region than to the nasal region.

Anatomical Analysis

Vocal fold. The first description of manatee vocal folds in literature was by Murie (1872), wherein he states "…Stannius and Rapp have failed to notice the existence of a small recess or pseudo-sacculus laryngis at the anterior extremity of the vocal cord, as in the Dugong…the vocal cords are the reverse of prominent, and deficient in inferior excavation." However, after Murie (1872), previous studies of the sirenian larynx have yielded conflicting observations on the existence of vocal folds (vocal "cords"), the source of vocalizations in terrestrial mammals. Nair and Lal Mohan (1975) observed during dugong vocalization that the nostrils were closed and wrinkles appeared on the skin of the frontal area. Such wrinkles were not observed in the larger female dugong, which did not make any sound, and they commented that there seemed to be some coordination between the movements of the wrinkles and the sound production (Nair and Lal Mohan, 1975). Domning (1977) observed that the vocalis and ventricularis muscles are absent in both dugongs and, based on literature, in manatees, although he later found possible equivalents of these muscles in *T. inunguis* (Domning, 1978). Harrison and King (1980) reported that sirenian vocal folds are absent and are replaced by fleshy, prominent cushions. Gambaryan and Sukhanov (1986), give a detailed description of the laryngeal and other muscles of *T. manatus*, with interpretations that differ from those in Domning (1977). Dong et al. (1992) also reported that dugongs have no vocal "cords" in the larynx, but described false vocal "cords" that have lost their function as such at the bottom of the laryngeal vestibule. Reidenberg and Laitman (1995), however, describe the thick opposing tissues in the laryngeal lumen as likely homologs of mammalian vocal folds. Although postulated, these laryngeal tissues were never proven to be homologous to the vocal folds of terrestrial mammals, and they further postulated that not all sounds appear to be generated at the larynx. According to Anderson and Barclay (1995), low-pitched whistles of dugongs are more likely an abnormality in the respiratory system rather than a means of communication, given their production during breathing. Behavioral observations indicate that chirp-squeaks and other sounds of the dugong originate in the frontal region of the head rather than in the larynx (Anderson and Barclay, 1995), suggesting a mechanism similar to that of odontocetes (Cranford et al., 1996). However, not all cetaceans (whales, including dolphins and porpoises) produce sounds from the nasal region, as evidenced by the discovery of vocal fold homologs in the larynges of mysticetes (Reidenberg and Laitman, 2007). Other marine mammals (e.g., pinnipeds) also use the larynx for sound production (Reidenberg and Laitman, 2010).

The only structure found to be suitable for generating sounds in our specimens were the tissues in the laryngeal lumen. These tissues met all the criteria for being called vocal fold homologs (Reidenberg and Laitman, 2007): they are oriented perpendicular to airflow, they are attached to the arytenoid cartilage, they can be abducted and adducted, they are controlled by the lateral and dorsal intrinsic muscles including the posterior cricoarytenoid, they contain a ligament that attaches to the thyroid cartilage, they are innervated on the cranial surfaces by the superior laryngeal nerve (internal branch) and appear to be innervated on their caudal surfaces by the recurrent laryngeal nerve, and they can completely occlude the airway.

Manatee vocal folds are the homologous structures to the true vocal folds of other mammals; however, they do not have a sharp edge as in many land mammals. This blunt edge may affect the quality of their fundamental frequencies. Sound production occurs as opposition of the manatee vocal folds restricts airflow through a narrow slit, resulting in fold vibrations that produce the fundamental frequency. Laryngeal vibrations can pass through the overlying fatty tissues of the throat (lingual and cervical), that in turn may transfer sound to water with very little energy loss. Although dissections on Amazonian manatees were not conducted, the same mechanism for sound production as in the West Indian manatee is thought to occur. Researchers found the vocalizations to be very similar in most respects except that they differ in duration and fundamental frequency (ranged from 2.5 to 5 kHz for *T. manatus* and 2.6–5.9 kHz for *T. inunguis*) (Evans and Herald, 1970; Sonoda and Takemura, 1973; Nowacek et al., 2003).

Sound Transmission. The velocity of sound transmission through solids (e.g., bone), gels (e.g., fat, skin, muscle, cartilage), and fluids (e.g., seawater, freshwater) is linearly related to the densities of the media (Mast, 2000). Soft tissue density varies around 10% from that of seawater and velocity varies around 15% (Aroyan, 1996). Tissues containing more structural elements (e.g., collagen) have higher densities and sound velocities than water (Goold and Clarke, 2000), while those with greater fat content retain lower densities and sound velocities (Mast, 2000). Energy is reflected at density interfaces, resulting in a transmission loss as sound travels between the various media. Tissues with a density close to water will allow a more efficient energy transfer from inside the body to the surrounding water. Chapla et al., (2007) found that the soft tissues of the manatee head have a density similar to that of seawater, suggesting that sound waves could propagate easily from one medium to the other.

The composition of the lingual and cervical fatty tissue in manatees has not been studied. The fat could provide an impedance matching mechanism for more efficient sound propagation into water. The odontocete melon, which is also composed of fatty tissues, occupies the forehead region of the skull and acts as an acoustic channel for sounds propagating out of the head (Cranford and Amundin, 2003). Muscles surrounding the melon appear to change its shape, and may enable it to function as a variable acoustic lens (Norris and Harvey, 1972). The lingual fat pad may allow rostro-ventral sound transmission through the floor of the mouth, while the cervical fat pad may allow latero-ventral projection of sounds from the throat region.

Fats are useful for sound transmission to water due to their relatively low density (compared with thicker connective tissues) that is impedance-matched with water. Sound transmission is not limited to outgoing sounds. Bullock et al. (1980) and Ketten et al. (1992) noted that the manatee's zygomatic process is lipid-filled, and suggested that it may conduct received sounds to the ear, much like the acoustic fat found in the acoustic window in the mandibles of cetaceans (Norris, 1968). The zygomatic process was found to have significantly lower density than other bones (Fawcett, 1942; Caldwell and Caldwell, 1985); however, the lipids it contained were composed almost entirely of triacylglycerols (Ames et al., 2002) and not the isovaleric acid typical of cetacean acoustic fat by which sounds are conducted (Varanasi and Malins, 1971). Cranford et al. (2008) found an intriguing finite element model (FEM) result concerning the pathway by which sounds reach the ears of a Cuvier's beaked whale (*Ziphius cavirostris*). The simulations revealed a previously undescribed "gular pathway" for sound reception in the whale. The propagated sound pressure waves enter the head from below and between the lower jaws, pass through an opening created by the absence of the medial bony wall of the posterior mandibles, and continue toward the bony ear complexes through the internal mandibular fat bodies (Cranford et al., 2008).

Although manatees do not have nasal fat, they may still use that region to transfer sounds to the water. The dorsum of the nasal cavity was observed to swell and collapse during vocalizations of the live manatees in the study, both the Antillean and Amazonian. These movements may act as a drum-head, transferring pulses as pressure waves into the water. Alternatively, the movements may indicate flow of air through the larynx. A flexible wall in the nasal cavity could expand to allow airflow for a longer period before the respiratory tract becomes pressurized. Once the outflow reservoir (nasal cavity) is fully expanded and pressurized, airflow will cease and so will sound production. Reversal of this flow, however, may allow the air to be recycled for another vocalization without losing any air out of the nostrils. This could allow manatees to remain submerged longer while continuously vocalizing between breaths. An expandable/collapsible nasal cavity has additional advantages: it can serve as a variable resonating chamber and act to amplify or mute certain frequencies termed formant frequencies (or formants). Air spaces (e.g., nasal cavity) within soft tissues are efficient reflectors of acoustic energy (Aroyan, 1996). The manatee's closest relative, the elephant, also produces variable formants. The elephant's expandable trunk and large nasal cavity likely are involved in modifying these sounds (Soltis, 2010). Stoeger et al. (2012) found that African elephants may be switching vocal paths (nasally and orally emitted rumbles) to actively vary vocal tract length (with considerable variation in formants) according to context.

The definitive path of sound transference to water has not yet been established for manatees; however, the presence of both a flexible nasal cavity and multiple fat pads may indicate an ability to vary the nature of the laryngeally emitted sounds, much like their elephant cousins. Manatees may be transferring sounds through several different transmission pathways: floor of the mouth (lingual fat pad), throat (cervical fat pad), and nose (flexible drum-head of the nasal cavity).

Sound travels a greater distance than light under water. Light only travels a few hundred meters in the ocean before it is absorbed or scattered. Given that sound travels much farther underwater than in the air, for marine mammals the use of sound in an aquatic environment is indispensable compared to vision. It is therefore no surprise that marine mammals have evolved different mechanisms for sound transmission and reception. Bullock et al. (1980), Ketten et al. (1992), and Ames et al. (2002) suggest that the position, porosity and oil-filled nature of the zygomatic process of the squamosal bone (ZPSB) of the Florida manatee may have a similar sound conduction function to that of the intramandibular fat body (IMFB) of the bottlenose dolphin and other odontocetes. The ability to use lipids to permit or enhance directional hearing underwater would be extremely useful for manatees to communicate or avoid oncoming boats (Ames et al., 2002). Even though the lipid composition in the manatee ZPSB differs in some ways from the lipid composition in odontocete IMFB and melon (Ames et al., 2002), the presence of the porous bone of the ZPSB may, in conjunction with the lipids of that bone, provide a channel for sound conduction as Bullock et al., (1980) suggested. Future comparisons between disparate species may indicate that convergent evolution mechanisms are present.

CONCLUSION

Taken together, the acoustical and anatomical findings support the vocal folds as the manatee's prime sound production mechanism. The vocal folds are composed of twin projections of mucous membrane, which can be moved to regulate the amount of air flowing between them. This airflow could cause the vocal folds to vibrate, thereby producing the vibrations that constitute the fundamental frequencies of their vocalizations. These vibrations are likely transmitted through the lingual or cervical fat pads and then transferred to water as communication sounds. The nasal region may also serve as a reservoir for receiving and recycling air for continued vocalizations, and may also transfer some vibrations through the dorsal nasal skin to water.

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