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Phenotype constrains the vocal tract in the most dimorphic mammal, the southern elephant seal (*Mirounga leonina*)

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Abstract

The study of mammal acoustic communication was revolutionized by the application of the source–filter theory, originally developed for human speech. The theory states that the vocal tract is constrained by body anatomy and, therefore, creates a structural link between phenotype and acoustic formants, providing a basis for honest signaling. The phenotype–formant link was validated in many species, but the phenotype–vocal tract link was rarely assessed. We used two dimensional (2D) videogrammetry to estimate the vocal tract length of wild southern elephant seal (*Mirounga leonina* (Linnaeus, 1758)) males during their normal vocalization behavior. We showed that (*i*) the vocal tract can be measured noninvasively in a large wild mammal; (*ii*) the vocal tract depends on the structural phenotype (age, body length, and skull size); (*iii*) the nasal tract is more related to the structural phenotype than the buccal tract; and (*iv*) the dependence on size, and body length in particular, is stronger than the dependence on age. Altogether, the phenotypic constraint on the vocal tract provides the anatomical basis for honest signaling in elephant seals.

Key words: vocal tract, age, body length, skull size, vocalizations, source–filter theory, honest signaling, marine mammals, southern elephant seal, *Mirounga leonina*

Résumé

L'application de la théorie source–filtre, proposée à l'origine pour la parole humaine, a révolutionné l'étude de la communication acoustique chez les mammifères. Cette théorie postule que le tractus vocal est contraint par l'anatomie du corps et établit donc un lien structural entre le phénotype et les formants acoustiques, fournissant ainsi une base pour la signalisation honnête. Si le lien phénotype–formants a été validé chez de nombreuses espèces, le lien phénotype–tractus vocal a rarement été évalué. Nous utilisons la vidéogrammétrie bidimensionnelle (2D) pour estimer la longueur du tractus vocal d'éléphants de mer austraux (*Mirounga leonina* (Linnaeus, 1758)) mâles à l'état sauvage durant leur comportement de vocalisation normal. Nous démontrons que (*i*) le tractus vocal peut être mesuré de manière non invasive chez un grand mammifère à l'état sauvage, (*ii*) le tractus vocal dépend du phénotype structural (âge, longueur du corps et taille du crâne), (*iii*) le tractus nasal est plus étroitement relié au phénotype structural que le tractus buccal, (*iv*) la relation à la taille et la longueur du corps en particulier est plus forte que la relation à l'âge. Dans l'ensemble, la contrainte imposée par le phénotype sur le tractus vocal fournit le fondement anatomique de la signalisation honnête chez les éléphants de mer. [Traduit par la Rédaction]

Mots-clés : tractus vocal, âge, longueur du corps, taille du crâne, vocalisations, théorie source–filtre, signalisation honnête, mammifères marins, éléphant de mer austral, *Mirounga leonina*

Introduction

Acoustic signals may convey honest information about the emitter's phenotypic traits, such as body size or age, and can be used for assessment by the receiver (Taylor and Reby 2010). Signals are defined as "honest" if they provide accurate information to the receivers, either about the signaler itself (i.e., advertisement calls) or about the environment (i.e., alarm calls; Fitch and Hauser 2003). The structural basis of acoustic honest signaling had been uncertain for a long while (Maynard Smith and Harper 2003; Seyfarth and Cheney 2017), until the source–filter theory, originally developed for human speech (Fant 1960), was applied to animal communication. The source–filter theory provides a robust framework to study the anatomical constraints that may generate honesty in acoustic communication (Taylor and Reby 2010; Taylor et al. 2016), and suggests which traits may be more promising to study the relationships between vocalizations and phenotype (Fitch 2000*a*; Fitch and Hauser 2003).

In the source–filter theory, the vocal tract is modeled as a uniform tube closed at one end, the glottis, and open at the other end, the mouth (Fitch 1997). The sound production system includes two main components: the vocal folds (the source) and the vocal tract (the filter). The frequencies of the sound produced by the vocal folds are filtered by the vocal tract; for example, some frequencies are attenuated and others are amplified. The amplified frequencies, the formants, depend on the size of the vocal tract, and a longer tract will produce lower formants (Fant 1960). Therefore, if the vocal tract is anatomically constrained by the rest of the phenotype, the formants can be an honest signal (Hauser et al. 2002; Taylor et al. 2016).

The expected relationship between phenotype and formants has been validated in many mammal species (Briefer and McElligott 2011; Garcia et al. 2016, 2017; Bowling et al. 2017; Garcia and Ravignani 2020), although the strength of the relationship was variable, and in some cases acoustic communication was found to be honest but imprecise (Sanvito et al. 2007*a*). In contrast, excluding a few exceptions (McElligott et al. 2006; Frey et al. 2011, 2020; Fitch et al. 2016), most demonstrations of the structural link between phenotype and vocal tracts have come from studies that applied radiography or other imaging methods to sedated or dead subjects (Plotsky et al. 2013; Garcia et al. 2017; Volodin et al. 2017). Although those studies have provided accurate measurements of the vocal tract parts, being performed on sedated or dead subjects, they have only provided a static snapshot of the tract that may not be highly representative of what happens in naturally vocalizing animals. Many mammal species have a mobile larynx (Fitch and Reby 2001), which can change position during vocalization (McElligott et al. 2006; Frey et al. 2008, 2011), and are able to modify the size and shape of their vocal tract (Reby et al. 2005; Charlton and Reby 2016; Garcia and Ravignani 2020). Furthermore, in most mammals, including humans, the nasal cavities can contribute to the filtering of vocalizations when the velum is lowered and air passes through the nostrils (Fitch 1994). Therefore, multiple resonators may reinforce or reduce the effects of each other (Fry 1979). This is particularly relevant for species that present flexible elongations of the tract that can be inflated or deflated. Those species can be effectively studied only when the subject is actually using those flexible structures to vocalize. Altogether, the best approach to study the vocal tract and its relationship with the rest of the phenotype is to work on wild, naturally vocalizing subjects.

Elephant seals (genus *Mirounga* Gray, 1827) are model species to study the honesty of acoustic signals, because of the very intense competition among adult males for access to females (Galimberti et al. 2002; Lloyd et al. 2020) and the importance of vocalizations in the resolution of agonistic contests (Sanvito et al. 2007*a*; Casey 2020; Casey et al. 2020). The role of acoustic communication in elephant seal behavior was clear from the earliest studies (Laws 1956; Bartholomew and Collias 1962), but detailed information on the acoustic structure of vocalizations has been obtained only recently (Sanvito and Galimberti 2000*a*, 2000*b*; Mathevon et al. 2017; Casey et al. 2018; Southall et al. 2019; Casey 2020). Although the fine structure of the elephant seal larynx has been described (Schneider 1964; Dong et al. 1993; Reidenberg and Laitman 2018), the vocal tract anatomy is poorly known (Murphy 1914), but elephant seals definitely have a mobile larynx, which is lowered during vocalization (Sanvito et al. 2007*a*). Furthermore, male elephant seals have a prominent proboscis that changes in size and shape with age (Sanvito et al. 2007*b*; Galimberti et al. 2019) and greatly elongates the vocal tract (Sanvito et al. 2007*a*). The proboscis of elephant seals is a soft structure, which is expanded during vocalization, presents large differences between individuals, and changes within each individual depending on breeding status and phase of the vocalization (Galimberti et al. 2019). Although its role during vocalization is not fully clear, it is likely that the proboscis acts as a resonator during sound emission as air flows through the open nostrils. As Sanvito et al. (2007*a*) have reported, the lowest frequencies of males' vocalizations are incompatible with an exclusive buccal production. The authors have suggested that such low frequencies can be reached by lengthening the sound path and thus through nasal sound emission (Sanvito et al. 2007*a*). Therefore, to produce vocal tract measurements that can be useful to study the signal honesty in elephant seals, the tract needs to be measured in subjects showing their natural vocalization behavior.

The aims of this study were as follows:

(1) To assess the feasibility of noninvasive vocal tract measurement in wild male southern elephant seals (*Mirounga leonina* (Linnaeus, 1758)),
(2) To estimate the repeatability of various vocal tract measures and the correlation between them, and
(3) To investigate the relationship between the vocal tract and the structural phenotype (age, body length, and skull size) that may constrain it.

To conclude, the findings of our study would validate the use of a minimally invasive approach to study the application of the source–filter theory to honest signaling in wild mammals.

Materials and methods

Fieldwork was completed at Sea Lion Island (Falkland Islands; latitude 52°26′S, longitude 59°05′W) during the 2016 breeding season (September–November), when the local breeding elephant seal population comprised 70 males and 629 females (unpublished data).

Tagging, dye marking, and age

As part of an ongoing long-term study of elephant seal life history (Galimberti and Boitani 1999), all Sea Lion Island pups were routinely tagged at birth, and double tagged at weaning, using numbered plastic cattle or sheep tags (various models, but mostly Jumbo Rototag, Dalton Supplies Ltd) placed in the interdigital membrane of the rear flippers. Any lost tag was routinely replaced by a new tag as soon as possible. Upon haul out, seals were routinely marked by writing an identification code on their sides and back using black hair dye activated by peroxide. Dye marks were temporary, and were lost with the molt. Dye marking, and replacement of lost tags on adults, was performed by an operator approaching the animal from behind while it was resting. The tagging at birth permitted us to exactly know the age in years of the males of the study, while the joint use of tags and dye marks permitted a safe recognition of individual males. Further details about the marking protocol can be found elsewhere (Galimberti and Boitani 1999).

Measurement of body length

We estimated body length using a previously validated two dimensional (2D) photogrammetric method (Galimberti et al. 2007, 2019). This method required the animal to lie straight on a flat surface. If the subject was not in the right position, an operator slowly walked around the subject to move it to the correct position. Once the seal was in an appropriate position, another operator approached the subject from behind, holding a telescopic surveying pole (4 m long, 1 cm graduation) above the subject's body and coplanar to the subject's midline (Supplementary Fig. S1). The first operator checked the alignment of the animal and the pole, using the midline of the body as a reference, and then took photos from the side of the seal using a digital camera (Olympus E-M10 Mark II, 14–40 mm lens, 16.1 MP), at a distance of 5–15 m from the subject, with the camera 50–100 cm above the ground. The camera was aligned to the center of the body and was kept parallel to its midline, to avoid perspective distortion of the photo. Several photos were taken for each session, with a slight variation in the camera's angle and distance from the subject, checking the alignment and eventually adjusting the animal's position. Afterward, measurements from the same series of photos were averaged, and only measurements from series in which the animal's position significantly changed were considered independent estimates of size. Pictures were shot as RAW (4608 pixels × 3456 pixels) and then converted to high-resolution JPG (3200 pixels × 2400 pixels) to be measured in ObjectJ software (version 1.04; Vischer 2017), using the pole as a reference scale (Supplementary Fig. S1). The photogrammetric length, measured as a straight line from the beginning of the proboscis to the beginning of the tail (see L in Supplementary Fig. S1), is about 91% of the standard body length (or straight-line nose-to-tail length; Committee on Marine Mammals 1967). Further details on the body length measurement procedure, including repeatability estimates, are presented elsewhere (Galimberti et al. 2007; Sanvito et al. 2007*a*).

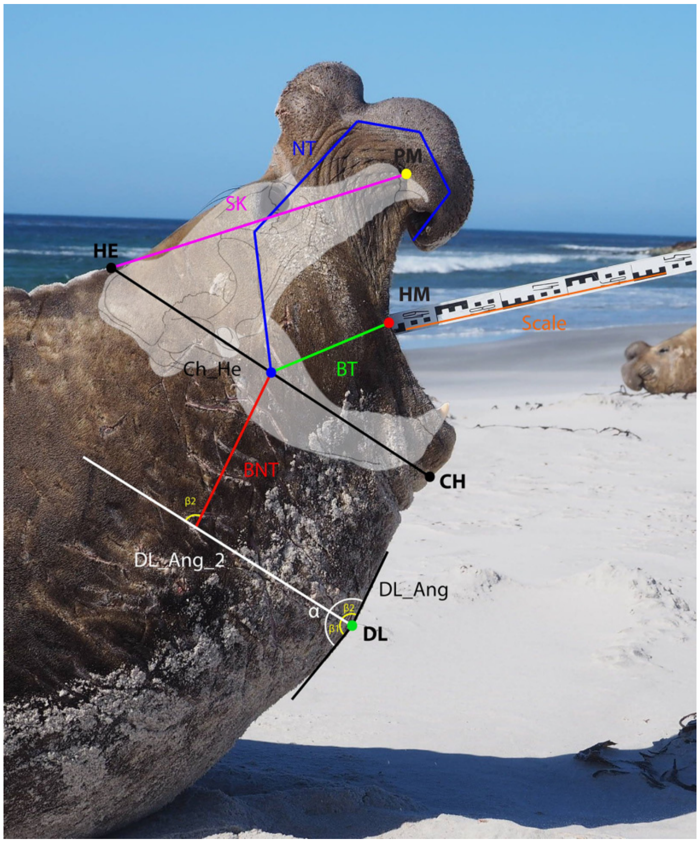
Measurement of the vocal tract and skull size

We measured the vocal tract and skull size in images obtained with a 2D videogrammetric technique similar to the photogrammetric one described above, but based on extraction of frames from videos. An operator approached the subject from the front at close distance, and the subject usually reacted with the normal behavioral sequence used during agonistic contests, starting with the vocalization. A similar stimulation was used in previous studies of proboscis morphology (Sanvito et al. 2007*b*; Galimberti et al. 2019) and acoustics of vocalizations (Sanvito et al. 2007*a*, 2008). While the animal was vocalizing, the operator placed the tip of the surveying pole (1.5 m) at the entrance of the subject's mouth, keeping the pole parallel to the ground and in line with the male's sagittal plane, using the two lower canine teeth as a reference. When the pole was aligned and the male was vocalizing with the proboscis inflated, a second operator, placed at a distance of 5–15 m, took a high-resolution video (4K, 29.97 frames/s) of the head and chest area from the side using a digital camera (see above). The camera was kept parallel to the sagittal plane of the subject and at least 40 cm of the surveying pole was included in the camera frame (Supplementary Video S1). Acoustic features and behavioral responses of natural and stimulated vocalizations were previously shown to be equivalent (Sanvito and Galimberti 2000*a*, 2000*b*). Moreover, there were no signs of habituation, and males performed their normal vocalizations even when regularly stimulated (one stimulation per week for the length of the breeding season, up to 14 weeks).

We examined the videos using Premiere software (version CS6, Adobe) to identify the frames in which the proboscis was fully expanded and the larynx best visible and maximally lowered down. The choice of full proboscis inflation and maximum larynx lowering was motivated by our interest in the maximum vocal tract length that should correspond to the lowest frequency formants that a male may produce.

For each male, we selected three independent videos, and from each video we extracted three high-resolution frames (1920 pixels × 1080 pixels), possibly from three different vocalizations, to obtain nine independent frames for each male. If the videos did not comprise three different suitable vocalizations, three frames were extracted from the available vocalizations, using different "bouts" (a bout is a single air emission that constitutes the basic acoustic unit of elephant seal aggressive vocalizations; Sanvito and Galimberti 2000*a*). Six breeding males had no suitable videos, but they had photos from photographic sequences of vocalizations obtained from a study of proboscis morphology carried out at the same time, and involving the same breeding males (Galimberti et al. 2019). For those males, we chose photos at maximum proboscis inflation and maximum larynx lowering, and we processed them in the same way as we processed video frames. We also checked the alignment of the subject to the camera, and we selected the video frames where the camera was coplanar with the sagittal plane of the animal. If the alignment was not correct and (or) the larynx was high and (or) not clearly visible, the video frames were discarded. We converted the video frames to JPEG format (1920 pixels × 1080 pixels) and measured them together with the photos using the surveying pole as the scale in ObjectJ (see above). The final sample was not balanced, comprising 166 video frames and photos of 32 males (mean ± SD = 5.2 ± 3.3 per male, range = 2–15 frames per individual). The dataset included a representative sample of breeding males ranging from 6 to 11 years of age (mean ± SD = 8.7 ± 1.4 years) and from 327 to 457 cm of body length (mean ± SD = 405.4 ± 29.8 cm). Our sample was composed of 2 males of 6 years of age, 5 males of 7 years of age, 6 males of 8 years of age, 10 males of 9 years of age, 6 males of 10 years of age, and 3 males of 11 years of

Fig. 1. Schematic drawing of the measures of the vocal tract on a southern elephant seal (*Mirounga leonina*) male. BNT (red line): length of the common part of the buccal and nasal tract; BT (green line): length of the buccal-only tract; NT (blue line): length of the nasal-only tract (the sum of BNT and BT gives the length of the total buccal tract (BT_TOT), while the sum of BNT and NT gives the length of the total nasal tract (NT_TOT)); SK (pink line): size of the skull. The landmarks and lines shown in the figure but not described above were used during the intermediate steps of the vocal tract and skull measurement, and are described in Table A3 of Appendix A.



age. Descriptive statistics of body length and vocal tract for each age are reported in Tables A1 and A2 of Appendix A, respectively. In particular, we analyzed 5 frames for 6-year-old males, 32 frames for 7-year-old males, 32 frames for 8-year-old males, 59 frames for 9-year-old males, 25 frames for 10-year-old males, and 13 frames for 11-year-old males.

Using ObjectJ, in each image we placed landmarks corresponding to either external or internal anatomical features, from which, using a strict protocol of geometric relations, we traced lines that permitted us to estimate the vocal/nasal tract and skull size. A schematic drawing of all the landmarks and lines is presented in Fig. 1, and a detailed description is reported in Table A3 of Appendix A.

Although the limitations and assumptions of the methodology are further considered in the "Discussion" section, it is necessary to introduce some of them here. While some of the previously mentioned landmarks were placed on externally visible structures (e.g., chin, mouth middle point, and the point where proboscis and muzzle meet), others were placed in correspondence to internal bony parts or organs (e.g., head end and larynx). Therefore, while the position of the former could be identified with great precision, the placement of the latter was based on the morphology that these structures are likely to assume when looked at from the outside. Nevertheless, some mobile organs, such as the larynx during vocalization, are easily detectable from the outside (see McElligott et al. 2006; Frey et al. 2011 for previous studies using this approach). In southern elephant seals, the larynx is clearly visible when observing the animal from the side, as a bump on the throat moving rhythmically during the vocalization (Supplementary Video S1). In our case, the most difficult landmark to identify was the one that defines the end of the head, because elephant seal males are very fat (Fig. 1, point HE). This landmark corresponds to the upper part of the supraoccipital skull bone (see Table A3 of Appendix A for description; Rommel et al. 2009). In general, the shape of the animal's head suggested a likely placement at the level of the second fold that formed when the male lifted the head to vocalize. To help locate this landmark, a skull outline (properly scaled using external features such as teeth and eyes) was superimposed on the image (see Fig. 1).

Using the landmarks mentioned above, we measured (Fig. 1) (*i*) the common part of the vocal tract (BNT) from the point of maximum descent of the larynx to the point of bifurcation between the nasal and buccal tracts; (*ii*) the buccal tract (BT) from the point of bifurcation of the vocal and nasal tracts to the opening of the mouth; (*iii*) the nasal tract (NT) from the bifurcation point of the nasal and vocal tracts to the nostrils/end of proboscis, passing by the nasal bones; (*iv*) the whole buccal tract length (BT_TOT), summing the buccal tract to the common part; and (*v*) the whole nasal tract (NT_TOT), summing the nasal tract to the common part. To decide how to measure skull sizes for our study, we consulted literature on the skull anatomy of carnivores (Radinsky 1984), marine mammals (Rommel et al. 2009), and southern elephant seals (Tarnawski et al. 2014), and previous work on the role of the skull size in acoustic signaling (Fitch 2000*b*). Taking those references into consideration, we decided to measure the skull size (SK) as the distance between the anterior tip of the premaxilla (positioned where the proboscis is inserted on the snout, PM) and the upper part of the supraoccipital skull bone (HE).

To estimate measurement error, we performed a blind test. An operator randomly selected a subset of 20 images from the full dataset, and a second operator performed the whole measurement procedure on those images, repeating it three times on each image. The images of each trial were in random order, and the trials were carried out at one day's distance to avoid biases. We calculated the repeatability of blind test measures using variance components obtained from a random factor linear model, in which the image replicate was the random factor (Lessells and Boag 1987), and its confidence limits using bootstrap (5000 samples, bias corrected accelerated). We calculated the blind test measurement error as the mean absolute deviation from the mean. The repeatability of measures was high, ranging from 0.89 to 0.99, with a mean of 0.95. The measurement error was low, ranging from

Table 1. Descriptive statistics and repeatability of measures of the vocal tract and skull.

Variable	Mean	SD	CV	Minimum	Maximum	*R*	LCL(*R*)	UCL(*R*)
BNT	24.44	4.13	0.17	15.81	33.37	0.81	0.73	0.84
BT	23.73	2.63	0.11	19.04	29.17	0.79	0.72	0.81
NT	79.62	8.22	0.10	61.89	91.63	0.86	0.80	0.88
BT_TOT	48.17	4.95	0.10	34.86	57.43	0.86	0.78	0.89
NT_TOT	104.05	10.16	0.10	78.89	122.15	0.91	0.86	0.92
SK	52.93	4.52	0.09	46.26	62.30	0.80	0.69	0.83

Note: Variable abbreviations—BNT, buccal–nasal common tract; BT, buccal-only tract; NT, nasal-only tract; BT_TOT, total buccal tract; NT_TOT, total nasal tract; SK, skull size. All the measures are given in centimetres. Statistical abbreviations—SD, standard deviation; CV, coefficient of variation; *R*, repeatability; LCL(*R*) and UCL(*R*), lower and upper 95% confidence limits of repeatability, respectively. Descriptive statistics were calculated on $N = 32$ males; repeatability was calculated on $N = 166$ measures. For schematics of the measures see Fig. 1.

0.4 to 0.8 cm (percentage error = 0.6%–2.14% of total variance); repeatabilities and measurement errors are presented in Table A4 of Appendix A.

Data analysis

We calculated the repeatability (and its confidence limits) of the vocal tract measures in the study sample as above for the blind test, but using the male identity as the random factor. For each measure, we averaged the values of each male, and we used those averages in the following analyses. We calculated the correlation between the vocal tract parts using Pearson's coefficient. To visually assess the relationships between components of the vocal tract and structural phenotype, we fitted a local polynomial smoother with confidence bands to each scattergram (Simons and Wagner 2007). We found no indication of nonlinear relationships and, therefore, we carried out all the following analyses using linear regression models including age, body length, and skull size as independent variables. Standard errors and confidence limits of regression coefficients were calculated using the bootstrap (5000 resamplings, bias corrected accelerated; Manly et al. 2007). We checked the multicollinearity of independent variables by calculating tolerances, which were all above 0.55 (age = 0.65, body length = 0.55, and skull size = 0.78). As a measure of effect size of the whole models, we calculated the adjusted coefficient of determination (Grissom and Kim 2005). For each independent variable, we calculated three measures of effect size: (1) standardized regression coefficient (β; Schielzeth 2010); (2) eta squared (η^2; Levin and Hullett 2002), which is the ratio of the partial sum of squares associated with the independent variable and the total sum of squares; and (3) omega squared (ω^2; Grissom and Kim 2005), which is an unbiased version of eta squared. Data analysis was carried out using Stata software (version 16 MP; StataCorp 2007) and R software (version 3.6.0; R Core Team 2018).

Ethical statement

Fieldwork was carried out (*i*) under research license R10/2013 granted by the Environmental Planning Department of the Falkland Islands Government and (*ii*) in accordance with the local legislation on protection of wildlife species (Conservation of Wildlife and Nature Ordinance, 1999) and marine mammals (Marine Mammals Ordinance, 1992). Sea Lion Island is a National Nature Reserve and, therefore, any field research carried out on the island is subject to auditing and approval by the Environmental Committee of the Falkland Islands Government. The experimental stimulation of males was audited and approved in 2002 and 2003 by the Animal Care Committee of the Memorial University of Newfoundland.

Tagging of pups at birth and of adults to replace lost tags is minimally invasive, is carried out by surprise, does not require any handling or restraint of subjects, lasts just a few seconds, and has no evident long-term effect (Galimberti and Boitani 1999). Dye marking of adults is noninvasive, is carried out on resting subjects without waking them up, uses cosmetic products approved for humans, and is short term (dye marks being lost during the annual molt; Galimberti and Boitani 1999). Photogrammetric measure of size is noninvasive and is carried out on resting subjects that should be unaware of the operator approaching them from behind to place the surveying pole. Videogrammetric measures of vocal tract require male stimulation but (*i*) the reaction to the approaching operator is equivalent to the normal reaction to another approaching male; (*ii*) all stimulations were carried out by an operator with great experience of elephant seal behavior (30+ seasons of fieldwork on both species of elephant seals), and the stimulation was interrupted if the subject showed any unusual behavior; (*iii*) Sea Lion Island elephant seals show an unusually low reaction to human beings (compared with other places; personal observation), possibly because of the many tourists who visit the island; (*iv*) the latency of reaction to approaching humans is very short, and seals resume their previous behavior, usually resting, as soon as the person moves away; and (*v*) all males included in the study completed their normal breeding cycle and no male left the island or changed breeding area after being stimulated for videogrammetry.

Results

Descriptive statistics of the components of the vocal tract are presented in Table 1. The total male vocal tract, summing the nasal tract, the buccal tract, and the common tract, was, on average, 127.8 cm long. The common tract was 24.4 cm (19.1% of the total), the nasal-only tract was 79.6 cm (62.3%), and the buccal-only tract was 23.7 cm (18.6%). The average skull size was 52.9 cm. The repeatability of the measures was high ($R \geq 0.79$; Table 1).

Table 2. Pearson's correlation between the parts of the vocal tract.

	BNT	BT	NT	BT_TOT
BT	0.02			
NT	0.27	0.58		
BT_TOT	**0.85**	0.55	0.54	
NT_TOT	0.63	0.48	**0.92**	**0.78**

Note: Variable abbreviations—BNT, buccal–nasal common tract; BT, buccal-only tract; NT, nasal-only tract; BT_TOT, total buccal tract; NT_TOT, total nasal tract. $N = 32$ males. For schematics of the measures see Fig. 1. The strongest correlations are highlighted in boldface type.

Table 3. Relationships between the vocal tract and the phenotypic traits.

Tract	Phenotype	R^2_a	b	SE(b)	LCL(b)	UCL(b)	β	η^2	ω^2
BNT	Age	0.06	–0.13	0.56	–1.24	0.97	–0.05	0.00	–0.03
	Body length		0.06	0.03	0.01	0.12	0.45	0.11	0.08
	Skull size		–0.10	0.19	–0.47	0.27	–0.11	0.01	–0.02
BT	Age	0.81	0.21	0.19	–0.16	0.57	0.11	0.01	0.01
	Body length		0.00	0.01	–0.01	0.02	0.01	0.00	–0.03
	Skull size		0.51	0.04	0.42	0.59	0.87	0.59	0.75
NT	Age	0.63	2.02	0.97	0.12	3.91	0.34	0.07	0.14
	Body length		0.14	0.05	0.05	0.23	0.51	0.14	0.26
	Skull size		0.20	0.27	–0.33	0.73	0.11	0.01	–0.01
BT_TOT	Age	0.37	0.07	0.58	–1.07	1.21	0.02	0.00	–0.03
	Body length		0.06	0.03	0.01	0.12	0.38	0.08	0.08
	Skull size		0.41	0.19	0.03	0.79	0.37	0.11	0.12
NT_TOT	Age	0.60	1.88	1.11	–0.30	4.06	0.26	0.04	0.07
	Body length		0.20	0.06	0.09	0.32	0.60	0.19	0.30
	Skull size		0.10	0.31	–0.51	0.71	0.05	0.00	–0.03

Note: Variable abbreviations—BNT, buccal–nasal common tract; BT, buccal-only tract; NT, nasal-only tract; BT_TOT, total buccal tract; NT_TOT, total nasal tract. Statistical abbreviations—R^2_a, adjusted coefficient of determination; b, regression coefficient; SE(b), standard error of b; LCL(b) and UCL(b), 95% lower and upper confidence limits of the regression coefficient, respectively; β, standardized regression coefficient; η^2, eta-squared measure of effect size; ω^2, omega-squared measure of effect size. $N = 32$ males. For schematics of the measures see Fig. 1.

Correlations among the components of the vocal tract were variable (Table 2). We found a strong correlation between the common part of the vocal tract and the total buccal tract (Pearson's $r = 0.85$, SE = 0.10, 95% CI = 0.71–0.92), between the nasal-only tract and the total nasal tract (Pearson's $r = 0.92$, SE = 0.07, 95% CI = 0.84–0.96), and between the total buccal tract and the total nasal tract (Pearson's $r = 0.78$, SE = 0.15, 95% CI = 0.59–0.89).

The results of the linear regression models of the components of the vocal tract versus the structural phenotype (age, body length, and skull size) are presented in Table 3 (see Fig. 2 for scattergrams of some representative relationships). We found four general patterns: (1) the linear models explained a large proportion of the variance of the vocal tract components (adjusted $R^2 = 0.37$–0.81), the only exception being the common part of the tract, which was not related to phenotype; (2) the models explained a greater percentage of variance for the total nasal tract than for the total buccal tract; (3) the effect of size (body and (or) skull) was greater than the effect of age in all cases; and (4) in most cases the effect of body length was greater than the effect of skull size, with the notable exception of the buccal tract, for which skull size showed the greatest of all effect sizes obtained in the study.

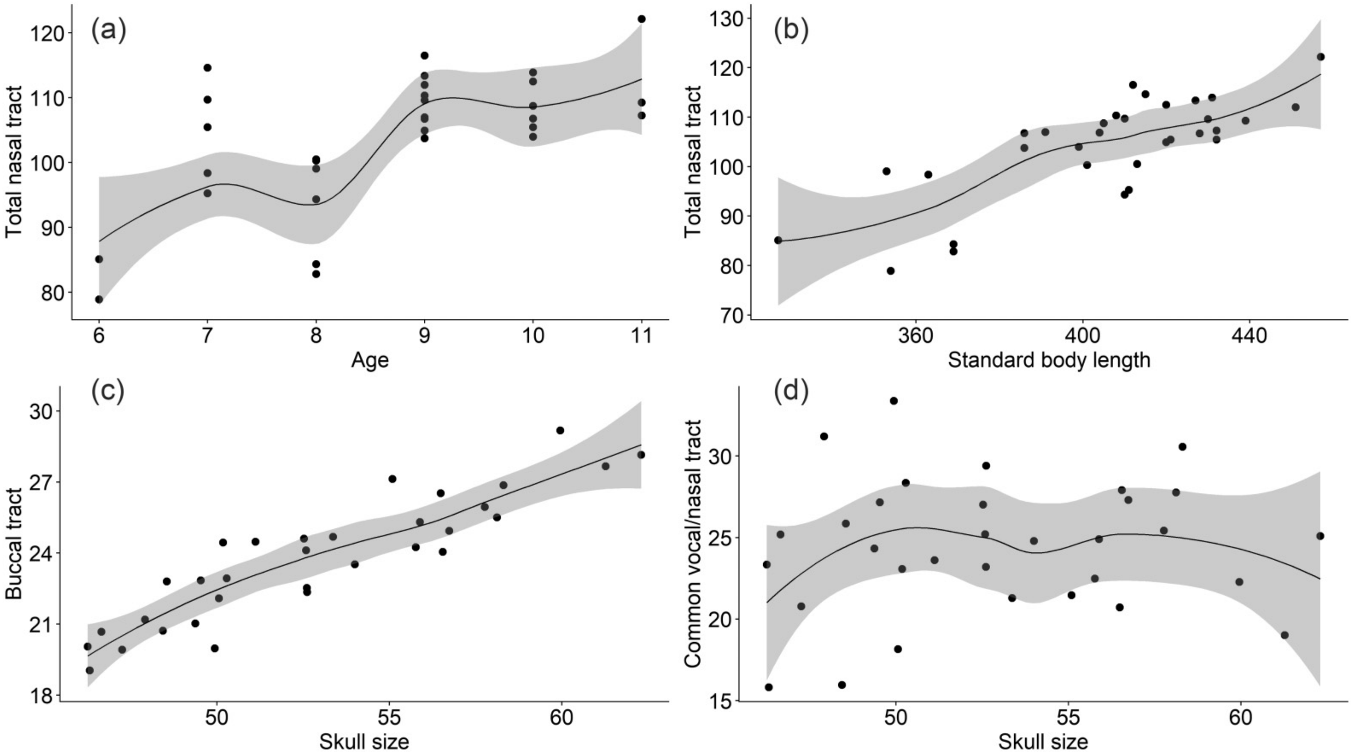
Discussion

Field videogrammetry of the vocal tract

Most studies about the relationship between vocal tract and size were carried out on sedated or dead subjects using some kind of laboratory imaging (Plotsky et al. 2013; Dunn et al. 2015; Shearer et al. 2015, 2016; Fitch et al. 2016; Reby et al. 2018). This direct measurement of the vocal tract, although potentially very accurate (Garcia et al. 2017), is applicable only to captive or laboratory subjects of rather small size, and can capture only the static state of the tract of fully relaxed subjects. The dynamic variation in the vocal tract length and shape during actual vocalization is more relevant for the study of vocal communication than the static snapshot mentioned above. In fact, there are robust demonstrations that different animal species can significantly move their larynx during sound emission (Fitch and Reby 2001; Charlton and Reby 2016; Volodin et al. 2017), and alter the size and shape of their vocal tract (Gamba et al. 2011; Ravignani et al. 2016). These findings suggest that the tract should be measured in wild subjects, during their actual vocalizations.

In the study of wild animals, there is an increasing interest for the development of noninvasive methods (Zemanova 2020) that permit us to obtain data without affecting the

Fig. 2. Scatterplots of the relationships between phenotypic traits and vocal tract parts: (*a*) total nasal tract versus age, (*b*) total nasal tract versus body length, (*c*) buccal tract versus skull size, and (*d*) common buccal/nasal tract versus skull size. Age is given in years; body length, skull size, and all tract components are in centimetres. The line is a local polynomial smoother; the gray area is the 95% confidence band.



welfare of the subjects (Pauli et al. 2010). Close-range photogrammetry is being increasingly used to obtain noninvasive estimates of body size (Weisgerber et al. 2015), mass (Alvarado et al. 2020), condition (Kotik 2020), sexual dimorphism (Breuer et al. 2007), and shape of morphological traits (Galimberti et al. 2019). The use of photogrammetry to obtain estimates of the length of the vocal tract, or parts of it, has been very limited and, until our study, has been applied to small samples (McElligott et al. 2006; Sanvito et al. 2007*a*; Frey et al. 2011). Here, we used photogrammetry to target the maximal length of the vocal tract, which should, in turn, correspond to the minimum frequency formants that a male can produce. While this configuration can also be considered a static snapshot, it is very different from the measurement of the vocal tract in dead or sedated animals, in which the measurement not only is static but also, and more importantly, has no clear relationships with what would be measured in a live, naturally vocalizing, subject. Our study of elephant seal vocal tract showed that 2D videogrammetry of vocalizing wild subjects can be effectively used to estimate the size of the vocal tract with good repeatability, although a rigorous measurement protocol is required. In the case of elephant seals, landmarks placed on video frames taken while the animal is vocalizing will likely have bigger placement errors than landmarks collected on cleaned skulls and larynxes or sedated/immobilized subjects. To partially overcome this problem, we developed a strict protocol to obtain our tract measures, to make them as reliable and repeatable as possible. Once some main anatomical features were identified on the images, the final measurements were obtained applying simple geometrical relationships. The measurement error for the analyzed features was low (maximum = 2.14%).

It should be emphasized that although in univariate regression measurement error in the independent variable would bias the regression coefficient toward zero, in multivariate regression the impact of measurement error on regression coefficients is difficult to forecast, and depends on the correlations among the regressors (Gillespie 1983; Liu 1988). In our models, age was the regressor measured with the smallest error (age known for all males), while of the two size regressors, skull size was the one with the greatest error. A reasonable assumption is that measurement error was not correlated among our regressors, because they were estimated using different methodologies. Therefore, the most likely effect of measurement error in our models was an attenuation of the regression coefficients, smaller for age, greater for body length, and greatest for skull size. Altogether, our estimates of the strength of the observed relationships were probably conservative, and probably more for skull size than for body length and age.

In the specific case of elephant seals, there is the additional effect of the proboscis that plays a crucial role in elongating the phonatory tract and, therefore, affecting the vocalizations (Sanvito et al. 2007*a*). Measurement of the proboscis is possible only when it is fully expanded during displays and vocalizations. Although direct measures on dead or sedated subjects, using radiography or magnetic resonance imaging, may help clarify the internal anatomy of the vocal tract, there are enormous logistical and ethical problems of applying such an approach to vocalizing elephant seals, and big mammals in general. Altogether, 2D videogrammetry seems a promising approach to estimate vocal tract length in field studies of elephant seals and wild mammals at large.

Phenotypic constraints on the vocal tract

Our results confirmed the general expectation of a positive relationship between the vocal tract and the structural phenotype (Fitch 1997), and our regression models explained a large percentage of the variation in the vocal tract, in particular if compared with explained variances usually found in field studies of vocal tract and honest signaling.

The relationships between the phenotype and the different parts of the vocal tract were variable, depending on the phenotypic trait and part considered. Analysis of the relationship between the vocal tract and the structural phenotype is often complicated by the fact that age and body length are strongly correlated with each other due to the growth process. However, in southern elephant seals there is a very large size variation within each male age (McLaren 1993), which should be mirrored by an equally large variation in vocal tract length. In fact, our sample included a 7-year-old male that was a very large individual for its age, and had an unusually long vocal tract. In all cases, the relationship between age and vocal tract (whole and parts) was weaker than the relationship with size (either body or skull) in most cases. The nasal tract of male elephant seals is elongated by the proboscis, a greatly enlarged nasal vestibulum that is a typical exaggerated sexually selected trait (Darwin 1871; Sanvito et al. 2007*b*), is kept expanded during the breeding season (Hindell 2018), and presents a large variation between ages (Sanvito et al. 2007*b*; Galimberti et al. 2019). The role of mammal vocal tract extensions is currently debated (Clifford and Witmer 2004; Charlton and Reby 2016), but in southern elephant seals, male vocalizations often present a peak of power at frequencies (the “minor formant”) that are so low that it is not compatible with the emission through the buccal tract, and is likely due to the nasal tract elongated by the proboscis (Sanvito et al. 2007*a*). Dominant males can produce extremely loud vocalizations that can be heard over a 500–1000 m range (Southall et al. 2003). During these vocalizations, the proboscis is fully expanded and may act as a resonator to increase the sound pressure level (Sanvito and Galimberti 2003) and lower the frequency of the sounds (Sanvito et al. 2007*a*, 2007*b*). In southern elephant seal males, the relationship between age and frequency formants is not linear, and presents a change point at age 7–8 depending on the formant considered (Sanvito et al. 2008). After the change point, the regression slope decreases in magnitude but remains negative, in particular for the minor formant mentioned above. Therefore, we expected the nasal part of the vocal tract to be related to age, and to potentially provide the anatomical basis for honest signaling of age in southern elephant seals. In fact, the only vocal tract parts for which the effect of age was not negligible, although smaller than the effect of size, were the nasal tract and the total nasal tract.

The vocal tract was positively related to size, and with the exception of the buccal tract, the relationship was stronger for body length than for skull size. Compared with previous studies, we found two notable differences: (1) the effect size of the relationships was somehow lower that that obtained in studies based on imaging methods and sedated subjects and (2) the relationships with the common part of the tract were weak, and the regression model explained only 6% of the variance. The difference can be in part due to the lower accuracy of our field measures compared with measures obtained by imaging on sedated subjects and the difficulty of correctly locating the larynx, but our blind trial showed low measurement errors, so other factors should be operating. The weak relationship between the common part of the tract and phenotype is particularly interesting because the lower end point of the common tract is the larynx (Fig. 1, lower end of BNT tract). In many mammals, the larynx can be moved during vocalization, descending and producing an elongation of the vocal tract (Fitch and Reby 2001; McElligott et al. 2006; Frey et al. 2008, 2011). However, it is usually assumed that the anatomical constraints at the neck–thorax junction will strongly limit the laryngeal descent, constraining the elongation of the vocal tract and permitting the production of honest signals (Reby and McComb 2003; Frey et al. 2011). In southern elephant seals, the common part of the vocal tract has the least phenotypic constraint of all tract parts and, therefore, males of this species seem to be particularly able to change the position of the larynx. This may reduce the anatomical constraints between size, vocal tract, and frequency formants, reducing the accuracy of the phenotypic information transmitted by the vocalizations. This is in accordance with the results of our previous study in which we showed that southern elephant seal communication is honest but imprecise (Sanvito et al. 2007*a*). The effect of the flexibility of the common tract on the whole tract length is expected to be greater for the total buccal tract than for the total nasal tract, because the buccal tract is smaller than the nasal tract and, therefore, the common tract is a greater proportion of the total buccal tract. In fact, the total nasal tract showed a greater phenotypic constraint than the total buccal tract (variance explained = 60% vs. 37%).

Some parts of the vocal tract show a particularly strong anatomical constraint because they are limited mostly by hard skeletal structures. In particular, the length of the buccal part of the tract is largely determined by the skull size and the length of the palate (Fitch 1997). In fact, the regression model of the buccal tract showed the greatest percentage of variance explained (81%), and the relationship with phenotype was almost completely due to skull size.

Honest signaling in southern elephant seals

In a previous study (Sanvito et al. 2007*a*), we showed that in southern elephant seals the formants decrease with age and body length and are related to male competitive and reproductive success. With the current study, we integrated these previous results by demonstrating that the relationship between phenotype and formants is determined by the anatomical constraints acting on the vocal tract. The biggest elephant seals, which also have a bigger skull, have longer vocal tracts and, therefore, emit vocalizations with lower formant frequencies. At the same time, we showed that the positive relationships between the structural phenotype and vocal tract are less strong than expected (Fitch and Suthers 2016), and that there is a considerable individual variation, even within each age. This result is in agreement with a previous study on southern elephant seal vocalizations, in which it was shown that the relationship between formants and the structural phenotype is variable, and the formants are an honest, but imprecise, proxy of structural phenotype (Sanvito et al. 2007*a*). Southern elephant seal males make extensive use of vocalizations during agonistic interactions and vocalizations are the single behavior that more frequently resolves those interactions (Sanvito et al. 2008). On the other hand, vocalizations settle contests only when phenotypic differences between males are large, while when males have similar resource holding potential, they resort to direct fights (Galimberti et al. 2007).

Our study confirmed that there is an anatomical basis for the relationship between structural phenotype and formants that was previously demonstrated (Sanvito et al. 2007*a*), and that the inaccuracy in the communication of information on the structural phenotype through the vocal signals is probably due to the weaker relationship between size and the vocal tract, which, in turn, could be due to the unusually large mobility of the larynx.

In conclusion, the methodology developed for this study is an effective tool to study the vocal tract of large wild mammals in field conditions, as long as (*i*) a well-defined choice of what to measure is carried out at the very beginning of the study (e.g., as in our case, measurement of the maximum vocal tract length), (*ii*) a representative sample of subjects is obtained (e.g, as in our study, a good sample of the breeding males), and (*iii*) a strict and replicable measurement protocol is applied to the video frames. The biggest problem in applying our methodology to subjects less collaborative than southern elephant seal males would be the placing of a scale in the video frames, but there are various possible approaches that may permit us to operate from a distance, including the use of parallel lasers (Bergeron 2007) or high-accuracy three dimensional range-finding devices (Sanvito et al. 2019).

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Supplementary material

Supplementary Fig. S1 and Video S1 are available with the article at https://doi.org/10.1139/cjz-2021-0188.

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Appendix A

Table A1. Descriptive statistics of body length measurements by age.

Age	Mean	SD	CV	Minimum	Maximum	*n*
6	340.5	19.09	0.06	327	354	2
7	406.2	25.72	0.06	363	432	5
8	385.83	25.29	0.07	353	413	6
9	415.7	19.58	0.05	386	451	10
10	410.33	16.63	0.04	386	431	6
11	442.67	12.90	0.03	432	457	3

Note: Age is given in years. All the measures are given in centimetres. Statistical abbreviations—SD, standard deviation; CV, coefficient of variation; *n*, sample size.

Table A2. Descriptive statistics of vocal tract parts by age.

Tract	Age	Mean	SD	CV	Minimum	Maximum
BNT	6	20.30	4.02	0.20	15.12	23.71
	7	25.95	2.82	0.11	20.21	31.41
	8	22.55	4.25	0.19	13.46	27.34
	9	25.42	4.39	0.17	15.50	34.18
	10	24.72	2.66	0.11	20.93	29.16
	11	27.27	5.47	0.20	16.31	32.84
BT	6	21.80	1.23	0.06	19.77	22.83
	7	24.96	2.79	0.11	21.48	30.13
	8	20.73	2.30	0.11	17.68	30.21
	9	24.63	2.63	0.11	19.27	29.04
	10	25.01	2.10	0.08	22.58	29.84
	11	25.56	2.30	0.09	19.71	28.05
NT	6	62.31	0.99	0.02	60.89	63.68
	7	78.23	5.93	0.08	65.12	89.83
	8	69.75	4.83	0.07	62.11	83.50
	9	83.78	3.25	0.04	73.90	89.77
	10	83.45	5.08	0.06	74.07	96.48
	11	89.62	5.67	0.06	77.23	95.91
BT_TOT	6	42.10	4.98	0.12	36.55	46.37
	7	50.91	3.04	0.06	44.20	56.73
	8	43.28	5.53	0.13	31.16	51.88
	9	50.05	2.71	0.05	41.65	54.29
	10	49.73	2.72	0.05	45.48	54.83
	11	52.82	7.40	0.14	40.07	59.06
NT_TOT	6	82.61	3.53	0.04	78.80	86.24
	7	104.18	6.50	0.06	91.27	114.79
	8	92.30	7.27	0.08	78.66	105.17
	9	109.20	4.84	0.04	99.74	119.83
	10	108.17	5.30	0.05	95.36	117.73
	11	116.89	7.37	0.06	103.25	123.86
SK	6	50.94	2.66	0.05	46.83	53.81
	7	55.78	5.69	0.10	47.92	64.83
	8	47.91	4.16	0.09	42.58	66.47
	9	54.22	4.73	0.09	46.53	64.36
	10	53.86	3.85	0.07	47.66	61.91
	11	56.38	3.78	0.07	48.47	60.14

Note: Variable abbreviations—BNT, buccal–nasal common tract; BT, buccal-only tract; NT, nasal-only tract; BT_TOT, total buccal tract; NT_TOT, total nasal tract; SK, skull. Age is given in years. Sample sizes are the same as in Table A1. Statistical abbreviations—SD, standard deviation; CV, coefficient of variation. For schematics of the measures see Fig. 1.

Table A3. Measurement of the vocal tract using ObjectImage software.

	Description
Landmark	
DL	Descended larynx. It was the lowest visible point reached by the larynx during vocalization as it is constrained by the sternum (Reby and McComb 2003)
CH	Chin. The point that divided the chin from the rest of the neck, i.e., the lower anterior tip of the jaw
HM	Half-mouth. The point that divided the mouth contour in half; it was the joining point of the lower and upper lips
PM	Proboscis–muzzle. The point where the proboscis was inserted into the muzzle, used as an indicator of the front tip of the premaxilla
HE	Head end. The point of attachment of the head with the rest of the body, i.e., the upper part of the supraoccipital bone
Lines	
Scale	Line drawn on the surveying pole, measuring 40 cm. It was traced as close to the animal's mouth as possible to limit perspective distortions and was used to scale all the measurements
DL_Ang	Descended larynx angle. The two line segments forming an angle (β), the vertex of which was at point DL and whose sides were tangent to the curve produced by the larynx protuberance
DL_Ang_2	Descended larynx angle bisector. The line originating in landmark DL and bisecting the angle α formed by line DL_Ang in two equal angles ($\beta_1 = \beta_2$)
Ch_He	Chin–head. The line that joined the landmarks CH and HE
SK	Skull size. The line that joined landmarks PM and HE
BNT	Buccal–nasal common tract. The line joining the midpoint of line Ch-He with line DL_Ang_2. This line was drawn in such a way that the angle formed with the portion of line DL_Ang_2 facing the back of the animal was equal to the angles β_1 and β_2. It was the length of the vocal tract from the lowest point reached by the larynx during vocalization to the bifurcation of the buccal and nasal tracts
BT	Buccal-only tract. The line joining the middle of the mouth (landmark HM) with the point that divided line Ch-He into two equal parts. It was the portion of the vocal tract that goes from the bifurcation of the buccal and nasal tracts to where the air is exhaled through the mouth during vocalization
NT	Nasal-only tract. Broken line, consisting of five segments, from the middle point of line Ch-He (where also line BT converges) to the nostrils' opening. The first segment started from the midpoint of line Ch-He and ended just before the eye. Second segments went from there toward the intersection of the two proboscis bumps, stopping approximately at the center of the proboscis width. The other three segments were traced following the proboscis, roughly in the middle of its width; the last segment ended at the nostrils' opening, in their middle. It was the portion of the vocal tract from the point where the air path bifurcates in two ways (nasal and buccal) to the point where the air comes out of the proboscis
BT_TOT	Total buccal tract. Line BNT + line BT. It was the total buccal tract starting from the maximum point of descent of the larynx to the exit point of the sound through the mouth (in its center point HM)
NT_TOT	Total nasal tract. Line BNT + line NT. It was the total nasal tract, starting from the maximum point of descent of the larynx to the exit point of the sound through the nostrils

Note: List of landmarks and lines placed on the images to measure vocal tract parts of male southern elephant seals (*M. leonina*; see also Fig. 1).

Table A4. Repeatability and measurement error of vocal tract and skull measures of the blind test.

Variable	*R*	SE(*R*)	LCL(*R*)	UCL(*R*)	ME (cm)	ME (%)
BNT	0.97	0.01	0.92	0.97	0.46	2.14
BT	0.91	0.02	0.83	0.94	0.44	1.85
NT	0.98	0.01	0.96	0.99	0.56	0.72
BT_TOT	0.99	0.01	0.97	0.99	0.41	0.89
NT_TOT	0.99	0.01	0.98	0.99	0.62	0.61
SK	0.89	0.03	0.81	0.92	0.78	1.5

Note: Variable abbreviations—BNT, buccal–nasal common tract; BT, buccal-only tract; NT, nasal-only tract; BT_TOT, total buccal tract; NT_TOT, total nasal tract; SK, skull size. Statistic abbreviations—*R*, repeatability; SE(*R*), standard error of repeatability; LCL(*R*) and UCL(*R*), lower and upper 95% confidence limits of repeatability, respectively; ME, mean measurement error (see the "Materials and methods" section). $n = 20$ images; measures replicated three times per image. For a schematic drawing of the measures see Fig. 1.