VOCAL TRACT ALLOMETRY IN A MAMMALIAN VOCAL LEARNER

Koen de Reus[1,2,3*#], Daryll Carlson[3,4*], Alice Lowry[3,5], Stephanie Gross[6], Maxime Garcia[7,8], Ana Rubio-Garcia[3], Anna Salazar-Casals[3], Andrea Ravignani[1,3#]

[1]Comparative Bioacoustics Group, Max Planck Institute for Psycholinguistics, Nijmegen, the Netherlands
[2]Artificial Intelligence Lab, Vrije Universiteit Brussel, Brussels, Belgium
[3]Research Department, Sealcentre Pieterburen, Pieterburen, the Netherlands
[4]Department of Earth System Science, Stanford University, Stanford, USA
[5]School of Environmental Sciences, University of Liverpool, Liverpool, United Kingdom
[6]Institute for Terrestrial and Aquatic Wildlife Research (ITAW), University of Veterinary Medicine Hannover, Foundation, Büsum, Germany
[7]Animal Behaviour, Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zürich, Switzerland
[8]Center for the Interdisciplinary Study of Language Evolution, University of Zurich, Zürich, Switzerland

* These authors share joint first authorship.
[#] Corresponding Authors: kdereus95@gmail.com; andrea.ravignani@mpi.nl

Abstract

Acoustic allometry occurs when features of animal vocalisations can be predicted from body size measurements. Despite this being considered the norm, allometry sometimes breaks, resulting in species sounding smaller or larger than expected. A recent hypothesis suggests that allometry-breaking animals cluster into two groups: those with anatomical adaptations to their vocal tracts and those capable of learning new sounds (vocal learners). Here we test this hypothesis by probing vocal tract allometry in a proven mammalian vocal learner, the harbour seal (*Phoca vitulina*). We test whether vocal tract structures and body size scale allometrically in 68 individuals. We find that both body length and body weight accurately predict vocal tract length and one tracheal dimension. Independently, body length predicts vocal fold length while body weight predicts a second tracheal dimension. All vocal tract measures are larger in weaners than in pups and some structures are sexually dimorphic within age classes. We conclude that harbour seals do comply with allometric constraints, lending support to our hypothesis. However, allometry between body size and vocal fold length seems to emerge after puppyhood, suggesting that ontogeny may modulate the anatomy-learning distinction previously hypothesised as clear-cut. Species capable of producing non-allometric signals while their vocal tract scales allometrically, like seals, may then use non-morphological allometry-breaking mechanisms. We suggest that seals, and potentially other vocal learning mammals, may achieve allometry-breaking through developed neural control over their vocal organs.

Key words: pinniped, harbour seal, vocal anatomy, acoustic allometry, trachea, larynx, vocal tract

Introduction

In many species, acoustic signals help mediate social interactions among individuals such as competition for mates and territory, and parent-offspring recognition (Bradbury & Vehrencamp, 1998; Martin, Tucker, & Rogers, 2017). Signals can encode information about the caller's biology which can be readily deciphered by the receiver, including age (Reby & McComb, 2003; Charlton, Zhihe, & Snyder, 2009), sex (Vignal & Kelley, 2007; Charlton et al., 2009), body size (Fitch, 1997; Charlton et al., 2009; Charlton et al., 2011, Garcia, Wondrak, Huber, & Fitch, 2016), hormone levels (Koren & Geffen, 2009), and physical condition (Wyman, Mooring, McCowan, Penedo, & Hart, 2008; Koren & Geffen, 2009).

In particular, body size often shapes mammalian sounds by constraining the geometry of the vocal tract (Fitch, 2000; Reby & McComb, 2003). Acoustic cues relating to the body size of the caller can inform the receiver about the caller's competitive ability and reproductive success (Poole, 1999; Reby & McComb, 2003; Kuester, Paul, & Arnemann, 1995; Pfefferle & Fischer, 2006). For example, in primates and carnivores, there is an inverse relationship between body size and the call frequency parameters, where larger animals produce calls with lower frequencies, i.e., have a 'deeper' voice (Bowling et al., 2017). This relationship between acoustical call features and their accurate reflection of body size is known as 'acoustic allometry' (Taylor & Reby, 2010; Fitch, 1997). Here, signalling is considered *honest* when the acoustic parameters of observed vocalisations accurately reflect an individual's body size (Fitch & Hauser, 2003). Deviations from allometry can generate *dishonest* signals, with animals sounding unexpectedly small or large for their body size (Garcia & Ravignani, 2020). Dishonest signals may be produced when an animal has a non-allometric vocal tract, or enhanced control over their vocal organs which allows them to learn or modify existing vocalisations: an ability known as 'vocal learning'. Recent cross-species work indeed showed that, given a cross-species regression between sounds produced and body size, outlier species seem to cluster either on one side of the line – those with anatomical adaptations – or on the other – the vocal learners. This led to a morphology vs. learning hypothesis (Garcia & Ravignani, 2020; Ravignani & Garcia, 2021): dishonest signals in mammals may arise either from anatomical adaptations or vocal learning capacities. This prediction has the potential to identify new vocal learners or species with unexpected vocal tract morphology. Vocal learners should therefore violate acoustic allometry while possessing an allometric vocal tract. For the first time, we test this prediction, asking whether vocal tract allometry or a deviation thereof occurs in a vocal learning species which is known to violate acoustic allometry. We do this in harbour seals, a pinniped rapidly becoming a model species for studying mammalian vocal learning.

Pinnipeds are a group of semi-aquatic mammals, with many species exhibiting marked vocal flexibility (Bowen, 1991; Ravignani et al., 2016). Harbour seals are among the few pinnipeds showing vocal learning abilities (Bjørge, Desportes, Waring, & Rosing-Asvid, 2010; Ralls, Fiorelli, & Gish, 1985; Borda, Jadoul, Rasilo, Salazar-Casals, & Ravignani, in press). They are particularly vocal during the first few weeks following birth (Perry & Renouf, 1988). Harbour seal pups produce individually distinctive mother attraction calls (Renouf, 1984) which vary with age, sex, and body length (Khan, Markowitz, & McCowan, 2006; Sauvé, Beauplet, Hammill, & Charrier, 2015). After weaning, however, these calls disappear entirely from their vocal repertoire, with most vocalisations ceasing aside from occasional clicks and growls (Renouf, 1984). During adulthood, female harbour seals remain almost entirely vocally inactive (Van Parijs & Kovacs, 2002), but males start vocalising again, producing underwater calls during the mating season (Hanggi & Schusterman, 1994). The large variation in vocal repertoire observed across individuals, sexes, and age classes makes harbour seals ideal candidates to test the morphology vs. learning hypothesis, i.e., whether a vocal learning mammal does indeed escape acoustic allometry via learning instead of via anatomical adaptations.

Most mammalian vocalizations are described via the source-filter theory of vocal production. Within this framework, vocal signals are initially produced by a *source* and are then *filtered* by the vocal tract before being released into the environment (Fant, 1970). In mammals, the source of sound production is the larynx, and the filter is composed of the cavities making up the supralaryngeal vocal tract (Fant, 1970) (see Figure 1A). The vocal folds in the larynx are shelves of tissue lying across the airway that attach ventrally and laterally to the thyroid cartilage and dorsally to the arytenoid cartilage (see Figure 1B). When vocalising, the air expelled from the lungs rushes between the vocal folds, causing vibration of their edges and producing sound (Reidenberg & Laitman, 2010). The sound then continues to propagate along the supralaryngeal vocal tract, being filtered by its geometry, and is finally released as vocalisation. The source-filter framework highlights which vocal tract structures determine specific features present in acoustic signals. The rate of vibration of the vocal folds determines the fundamental frequency (F0) and the cavities of the vocal tract determine formant frequencies (Taylor & Reby, 2010). Measurements of these vocal tract structures can thus be used to estimate the acoustic features of vocalisations.

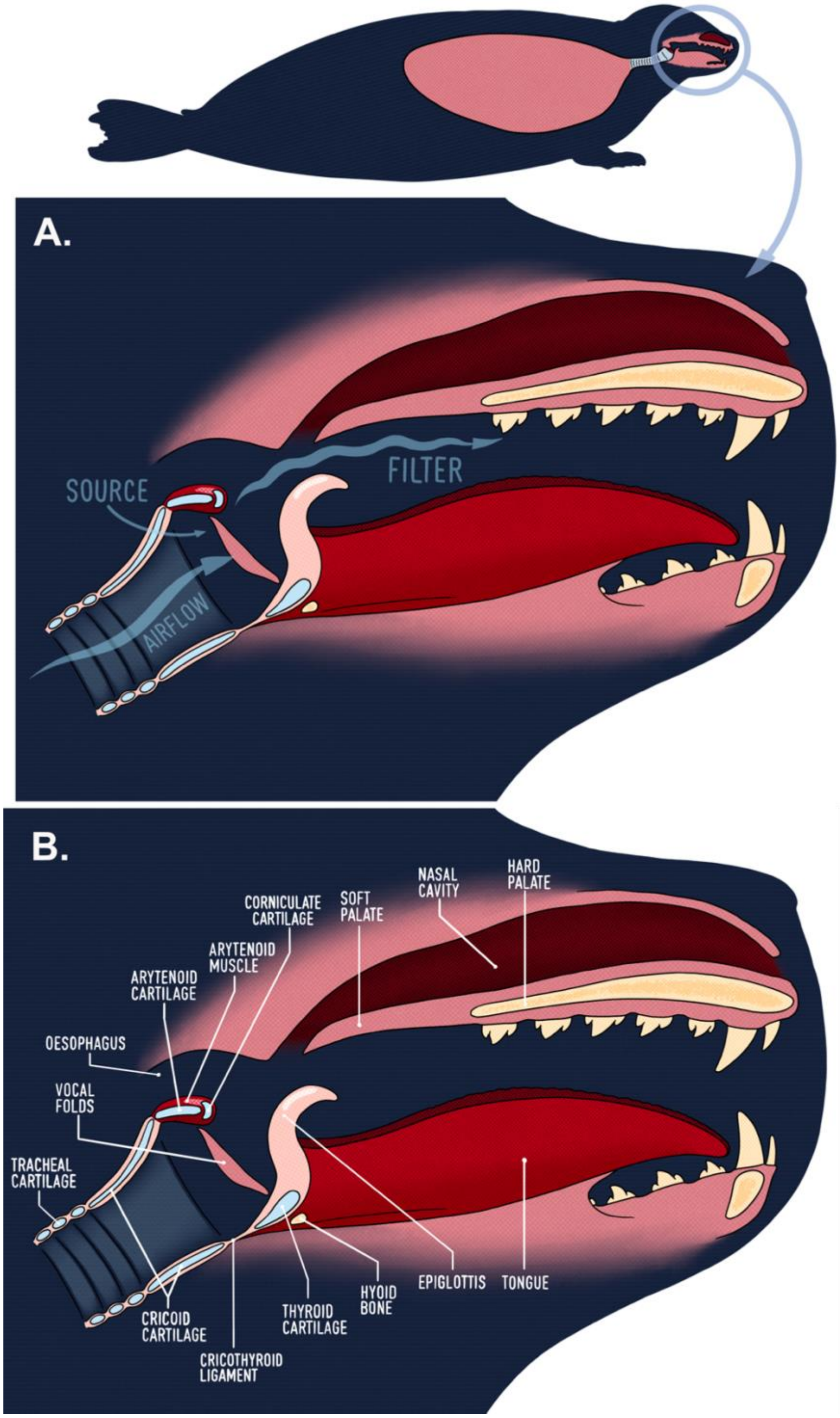

Figure 1. Vocal anatomy of the harbour seal. Panel A illustrates the source-filter theory of sound production. Panel B shows the main anatomical structures making up the vocal tract.

Bioacoustics studies often investigate allometric relationships between acoustic signal features and body size, without consideration of the underlying allometric scaling between body size and vocal anatomy. Mammals often show allometry between body size and upper vocal tract length because the upper vocal tract is constrained by bony structures (Fitch, 1997; Fitch & Giedd, 1999; Fitch, 2000; Plotsky, Rendall, Riede, & Chase, 2013; Garcia et al., 2016). However, allometry between body size and the size of the vocal folds is less common: the larynx is surrounded by cartilaginous structures and is thus less constrained, suggesting that vocal fold length can be decoupled from overall body size, as found in nonhuman primates (Fitch & Hauser, 1995; Fitch, 1997; Garcia, Herbst, Bowling, Dunn, & Fitch, 2017). In mammals, formants, the acoustic proxy of vocal tract length, are thus a stronger body size predictor than F0, the acoustic proxy of vocal fold length (Fitch, 1997; Garcia et al., 2016). These findings indicate that, if animals do not have particular anatomical adaptations, their upper vocal tract should scale with body size, but their vocal folds should not.

Within the larger framework of the hypothesis above, this study tests for allometric relationships between body size and vocal anatomy measurements in young harbour seals and tests how these relationships vary with sex and age. Preliminary work found that harbour seals' body length correlates with upper vocal tract length and tracheal diameter, but not with vocal fold length (Ravignani et al., 2017). Here, we aim to expand on these findings by using a larger sample size (353% increase), adding refined anatomical measurements, and comparing different age classes (to test for developmental effects). Based on previous literature, we expect to find allometry between body size and vocal tract structures that are surrounded - and hence constrained - by bony structures, such as vocal tract length. However, based on harbour seals' vocal learning abilities (Janik & Slater, 1997), we expect their vocal flexibility to offer favourable grounds to find deviations from body size allometry for vocal tract components surrounded by cartilage, such as the trachea and vocal fold length.

Results

Inter-rater reliability for VTL, VFL, VFT, and both STDVs was evaluated using Pearson correlations. VTL ($r = 0.94$), VFL ($r = 0.88$), STDV1 ($r = 0.97$) and STDV2 ($r = 0.93$) showed high inter-rater reliability. VFT ($r = 0.59$) showed lower inter-rater reliability and was consequently excluded from further analysis. All correlations were significant at $p < 0.001$.

All Spearman correlations between body size and vocal anatomy measurements showed positive relationships and significance at the 0.05 level (see Table 1). There were high correlations between weight and length ($r_s = 0.70$), and between weight and girth ($r_s = 0.86$). Other notable correlations included those between VTL and VFL ($r_s = 0.72$), VTL and STDV1 ($r_s = 0.70$), VFL and STDV1 ($r_s = 0.82$), VFL and STDV2 ($r_s = 0.76$). Spearman correlations for pups and weaners can be found in Tables 2-3 of the Supplement.

Table 1

Pairwise Spearman correlations

Variable	Length (cm)	Weight (kg)	Girth (cm)	VTL (mm)	VFL (mm)	VFT (mm)	STDV1 (mm)
Weight (kg)	0.70						
Girth (cm)	0.53	0.86					
VTL (mm)	0.62	0.69	0.63				
VFL (mm)	0.73	0.79	0.65	0.72			
VFT (mm)	0.44	0.67	0.68	0.52	0.60		
STDV1 (mm)	0.63	0.78	0.65	0.70	0.82	0.69	
STDV2 (mm)	0.58	0.72	0.62	0.67	0.76	0.60	0.81

Note. All correlations were significant at $p < 0.05$ after correcting for multiple comparisons using the Holm-Bonferroni method.

All anatomical measurements were non-normally distributed but showed equal variances across age and sex groups. A Mann Whitney U-test was used to test for group differences in both variables as only the assumption for homogeneity of variance was satisfied. All anatomical measurements were significantly larger in weaners than in pups ($p < 0.001$; see Table 2 and Figure 3). No significant sex differences were found when considering pups and weaners together ($p > 0.05$). When considering pups alone, both the normality and homoscedasticity assumptions were met. A two-tailed independent samples t-test found significant sex differences for vocal tract length ($t = -3.416$, $p < 0.05$; see Figure 4A). Male pups (86.03 mm ± 2.92) had a larger mean VTL than females (79.80 mm ± 3.68). When considering weaners alone, variables showed non-normal distribution, but equal variances. A series of Mann Whitney U-tests found that only the first subglottic-tracheal dorsoventral distance was significantly different across sexes ($U = 218$, $p < 0.05$; see Figure 4B). Weaned males (25.12 mm ± 1.50) had a wider mean STDV1 compared to weaned females (24.22 mm ± 1.24).

Table 2

Means and standard deviations

	All		Pups		Weaners	
Variable	Mean	SD	Mean	SD	Mean	SD
Length (cm)	88.07	8.03	79.86	4.79	90.19	7.32
Weight (kg)	14.53	3.92	9.81	1.46	15.75	3.39
Girth (cm)	57.55	9.62	46.54	5.66	60.41	8.30
VTL (mm)	91.43	6.77	83.36	4.48	93.53	5.60
VFL (mm)	10.92	1.16	9.26	0.90	11.35	0.77
VFT (mm)	5.15	0.61	4.50	0.43	5.35	0.51
STDV1 (mm)	23.97	1.95	21.35	1.37	24.65	1.43
STDV2 (mm)	17.43	1.82	15.17	1.04	18.02	1.49

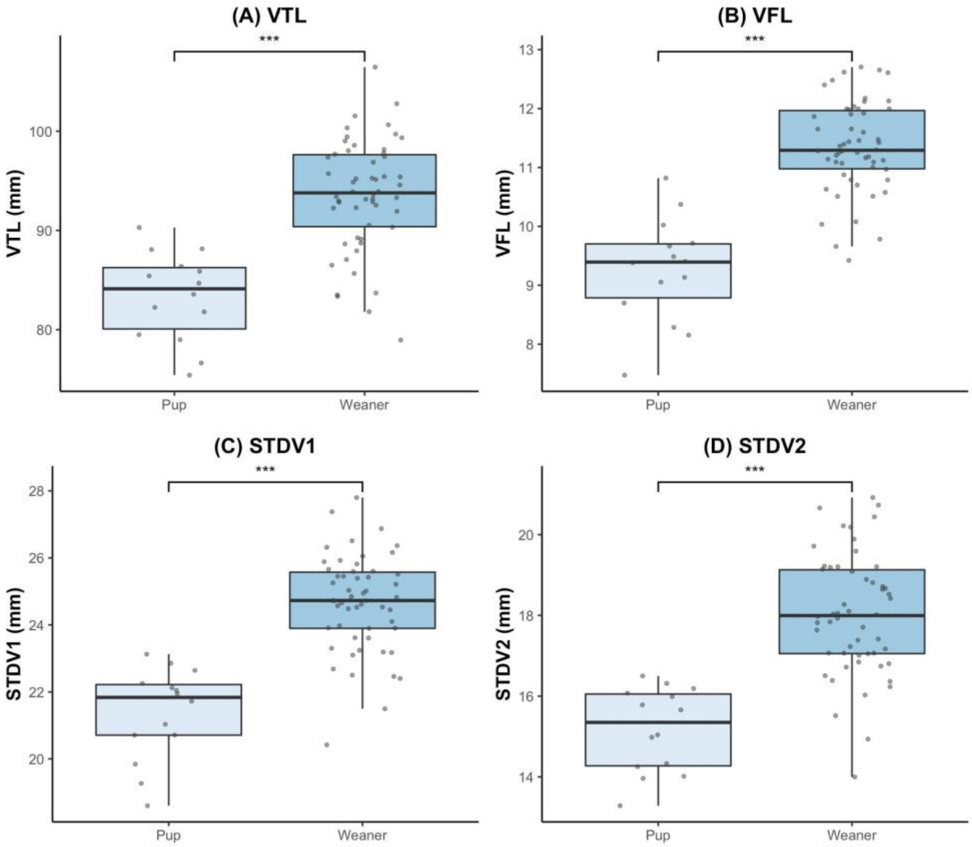

Figure 3. Boxplots illustrating the significant age differences between pups and weaners for (A) VTL, (B) VFL, (C) STDV1, and (D) STDV2. The level of significance is denoted by asterisks, where *** = 0.001.

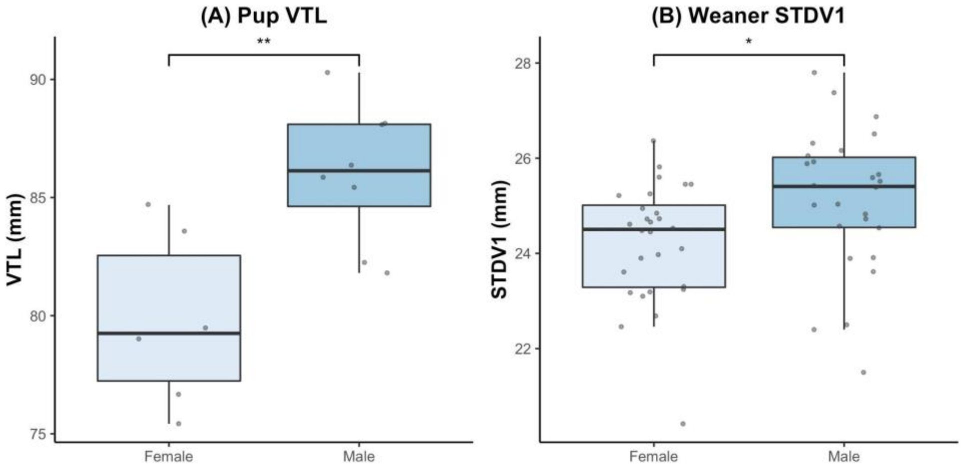

Figure 4. Boxplots illustrating the significant sex differences for (A) VTL in pups and (B) STDV1 in weaners. The level of significance is denoted by asterisks, where * = 0.05 and ** = 0.01.

A reduced GLM, obtained by stepwise regression based on AIC values, was produced for every vocal tract measurement with high inter-rater reliability, including VTL, VFL, STDV1 and STDV2. All VIF scores were lower than 5 suggesting that multicollinearity was not problematic in the selected models. All model assumptions were satisfied. Moreover, ANOVA testing indicated that the reduced models did not perform significantly worse than the full models ($p > 0.90$). GLM results showed that most vocal tract dimensions were best explained by length, weight, age, and sex (see Table 3). Girth was not retained as a predictor term in any of the selected models. For each model, the predictor estimates with their confidence intervals can be found in Tables 4-7 of the Supplement and plots of the predicted effects can be found in Figures 1-5 of the same document. Significant interaction effects are shown in Figure 5.

Table 3

Selected models for each vocal tract structure

Measurement	Selected model	Deviance explained (%)
VTL	$\underline{L} + \underline{W} + \underline{A} + \underline{S} + \underline{L*S}$	59.30
VFL	$\underline{L} + W + A + \underline{S} + A*W + \underline{A*S}$	74.89
STDV1	$\underline{L} + \underline{W} + \underline{A} + \underline{S}$	69.99
STDV2	$L + \underline{W} + \underline{A} + S$	58.38

Note. Models included the predictors length (L), weight (W), age class (A) and sex (S). Predictor terms joined by an asterisk denote an interaction effect. Significant predictor terms are shown as underlined.

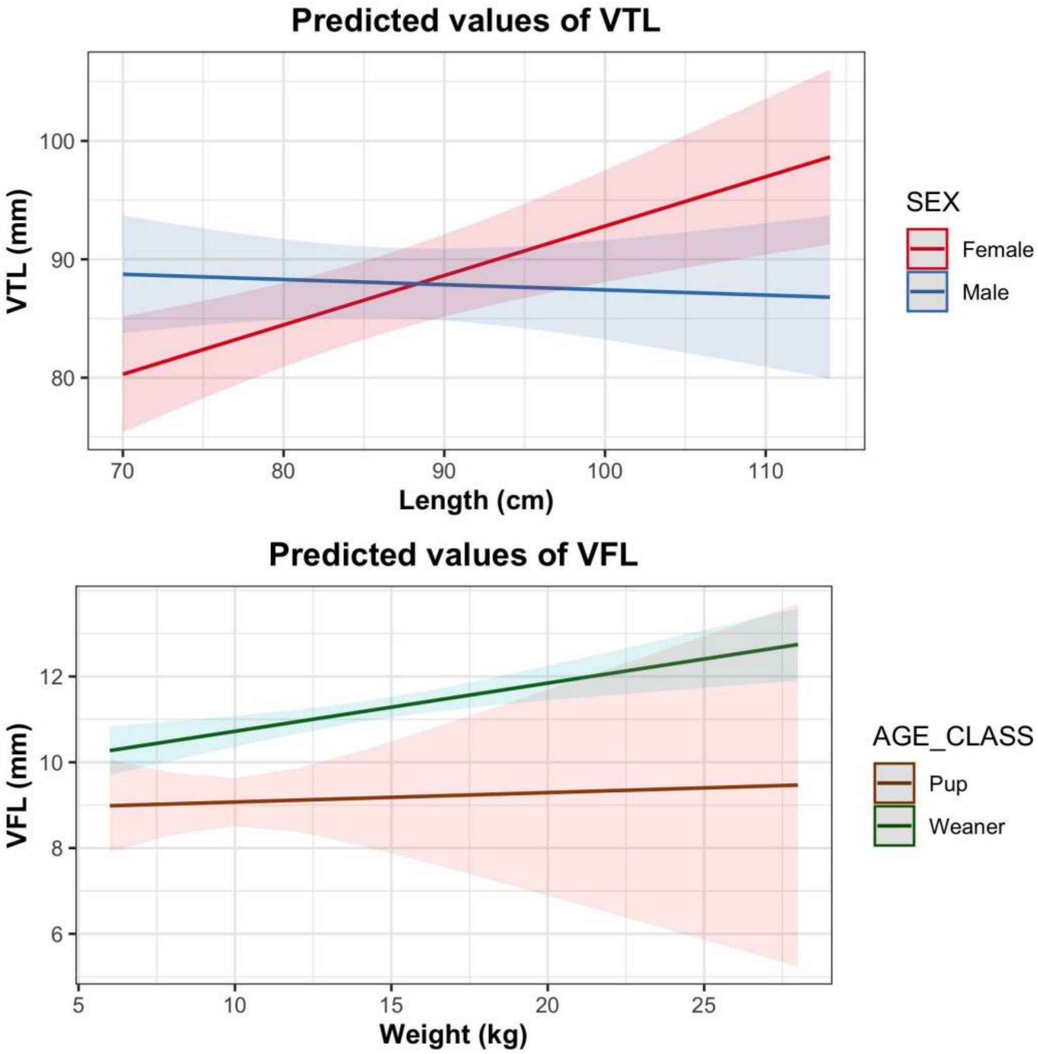

Figure 5. Predicted effects of the body length and sex interaction for VTL including both age classes (top), and the weight and age interaction for VFL including both sexes (bottom). The shading around each line of best fit indicates the 95% confidence interval.

Discussion

This study reports on the allometric relationships between body size and vocal tract dimensions in harbour seals. It shows that body length accurately predicts VTL, VFL, and STDV1, and body weight predicts VTL and both tracheal measurements (STDVs). We also find age and sex to be important predictors for the size of vocal tract structures. This is evidenced by significant differences in measurements between age classes and significant sexual differences within age classes.

Previous work showed that upper vocal tract (i.e., filter) dimensions in mammals are predicted by body size measurements (Fitch, 1997; Fitch & Giedd, 1999; Fitch, 2000; Plotsky, Rendall, Riede, & Chase, 2013; Garcia et al., 2016, Ravignani et al., 2017) and our results provide additional evidence to support such allometry. Although most studies have used body length as a proxy for body size, we find that body weight can also be used to predict VTL in harbour seals. In the first years of life, harbour seals show a linear growth rate for both length (Haukkson, 2006) and weight (Markussen, Bjørge, & Øritsland, 1989), suggesting that VTL may develop in a similar fashion during this period. Acoustic proxies for the filter could thus provide a good estimation of a harbour seal's size. In mammals, formant frequencies and formant spacing can be predicted from VTL and vice versa (see Reby & McComb, 2003). Other acoustic proxies include energy quartiles, the frequency of amplitude peaks, and the ratios between these amplitudes (Sauvé et al., 2015). These parameters also encode individual signatures, suggesting that acoustic individuality may partially be an allometric by-product (Ravignani et al., 2017). Harbour seals have the vocal tract predispositions to produce vocalisations that accurately reflect body size whilst also sharing individual-specific information, suggesting that learning does not need to be invoked to explain individuality.

Across mammals, source-related features such as F0 can sometimes predict body size despite showing weaker allometric scaling than filter-related features (Reby & McComb, 2003; Charlton et al., 2011; Pfefferle et al., 2007; Charlton & Reby, 2016); it was unclear whether this holds for harbour seals (Ravignani et al., 2017; Bowling et al., 2017). Our findings indicate that vocal fold length, which may be used to approximate F0, can be predicted by body size in harbour seals. Moreover, Sauvé and colleagues (2015) reported a decrease in F0 with an increase in body length of harbour seal pups. Taken together, this suggests that a harbour seal's F0 can be predicted from vocal anatomy. Previous evidence against allometric scaling for VFL could be explained by low statistical power or lack of testing for age effects on vocal tract measurements (Ravignani et al., 2017). It is indeed notable that age is included in both interactions which were retained in the selected VFL model. Our results, including both pups and weaners, show that allometric scaling between body size and VFL only emerges after weaning, suggesting that VFL may not be constrained in harbour seal pups (see bottom panel of Figure 5). This begs the following question: how would escaping acoustic allometry for source-related features be beneficial for pups? Broadcasting honest body size information may be detrimental for harbour seal pups as they are significantly more likely to be displaced by larger conspecifics during agonistic interactions (Neumann, 1999). However, pups may be able to benefit from lowering the F0 (Borda et al., in press) of their calls to create an impression of size exaggeration. On the other hand, pups may also benefit from increasing the F0 of their calls to create an impression of distress to the mother (Briefer, 2012). Future playback studies could contrast these hypotheses.

Several phocid species use the trachea for sound production (Bryden & Felts, 1974), but this could be a by-product of adaptive modifications to the respiratory tract required for diving (Kooyman & Andersen, 1969; Tyack & Miller, 2002). Our results support the correlation between tracheal diameter and body length found by Ravignani and colleagues (2017), but also provide evidence that tracheal dimensions can be predicted by body weight. Previous literature found that the trachea may potentially convey body size information if its size influences acoustic call features (Ravignani et al., 2017). In humans, a wider tracheal diameter partially predicts turbulence (i.e., unsteady air movements) for large airflows (Van den Berg, Zantema, & Doornenbal, 1957). Applying the same logic to other mammals, larger seals would have wider tracheal dimensions which, in turn, would make vocalisations noisier. This could explain, for instance, why the signal-to-noise ratio decreases as harbour seals get older (de Reus, 2017; de Reus, Jadoul, & Ravignani, in prep.). Future work on sound production in this species could test this prediction using sound-anatomy correlations and excised larynx set-ups. Moreover, playback experiments could test whether adding noise to vocalisations alters interactive behaviour to determine if signal-to-noise ratio may encode body size information. Understanding whether the trachea is actively involved in sound production will thus require further research.

As expected, all anatomical measurements are larger for weaners than they are for pups. In Ravignani and colleagues (2017), animals up to 108 days old were classified as pups. However, in the wild, the lactation period for harbour seals ranges from 23 to 42 days, after which the pups are weaned (Renouf, 2012). Hence, for the sake of simplicity, we consider animals up to one month old as pups and animals older than one month as weaners. Thanks to this categorical classification, we were able to identify how allometric trends develop over the harbour seal's early life. Future research including larynges from subadults and adults will further extend our knowledge of how vocal allometry develops in harbour seals.

There were no sexual differences when considering the sample size as a whole, but significant sexual differences existed within age classes. These differences could be attributed to differing levels of steroid hormones acting on the laryngeal structures in males and females (Aufdemorte, Sheridan, & Holt, 1983; Sauvé et al., 2015). In some mammals, sex hormones affect the structural development of the larynx and the viscoelastic properties of the vocal fold tissue (Fitch & Giedd, 1999; Beckford, Schaid, Rood, & Schanbacher, 1985). At puberty, the male larynx descends in the vocal tract causing an elongation of the length of the upper vocal tract, allowing males to convey an exaggerated impression of size (Fitch &

Giedd, 1999; Fitch & Reby, 2001). In harbour seal pups, males have larger VTLs than females, suggesting that laryngeal descent in males possibly occurs early in life. Once weaned, however, females show a clear increase in VTL whereas it remains relatively constant in males (see top panel of Figure 5), suggesting that VTL differences across sexes may become less pronounced over time. In mammalian males, sex hormone action also causes a rapid increase in cartilage size leading to an enlarged larynx and an increase in the vibrating portion of the vocal folds (Fitch & Hauser, 2003). This could explain why, in weaners, STDV1 is larger in males than in females. Nevertheless, these findings are somewhat surprising as young harbour seals normally show little sexual dimorphism (Le Boeuf, 1991). In particular, there is a lack of evidence for sexual differences regarding birth mass and growth rates among harbour seal pups (Bowen, Oftedal, Boness, & Iverson, 1994). In our sample, there are no significant body size differences between sexes ($p > 0.05$), but male pups are slightly larger than female pups in both body length (M = 81.63 cm ± 4.44, F = 77.50 cm ± 4.51) and weight (M = 9.82 kg ± 1.49, F = 9.78 kg ± 1.55). It is important to note that the sampled animals were sick and/or in poor condition; hence weight values are not representative of healthy individuals and should be interpreted with caution. In short, based on these observed differences in vocal anatomy across sexes, formants are expected to differ in pups and signal-to-noise ratio is expected to differ in weaners. The anatomical structures that determine these acoustic features both show strong allometric scaling, hence these parameters may provide distinct body size cues across age classes, potentially facilitating the discrimination of male and female conspecific calls. Sexual selection could thus play a role in driving size differences in VTL and STDV1, but it is yet unknown if these differences are maintained throughout developmental life stages. Future research should aim to investigate the elastic properties of harbour seal laryngeal tissues and to understand how sex hormones affect these properties. Hormone levels can be measured by taking blood samples from healthy male and female seals at different developmental stages, and results can be combined with magnetic resonance imaging (MRI) mapping of laryngeal tissue elasticity.

The high inter-rater reliability observed for VTL, VFL and both STDVs demonstrates that these quantities can be measured and replicated easily, making them reliable landmarks for vocal tract measurements. However, tissue properties such as the viscoelasticity of certain vocal tract structures, like the vocal folds, are significant obstacles to getting accurate measurements. Indeed, raters struggled to produce precise data for VFT. Future research in the field of pinniped vocal anatomy would benefit from improved measuring techniques using 2D pictures, radiography, MRI and computed tomography (CT) scans as this would enable more accurate measurements for structures that are difficult to handle. Finally, future similar studies should include measurements of another vocal tract structure: the corniculate cartilage. Although widely absent in terrestrial carnivores, harbour seals have rather large corniculate cartilage that help close the trachea together with the epiglottis (Adams et al., 2020). These cartilages are located close to the vocal folds and are possibly innervated by the same nerves and controlled by the same muscles. It may be possible that these cartilages play a role in sound production by, for example, lowering the F0 by adding weight to the vocal folds. Taken together, these suggestions will provide a more precise and detailed picture of the harbour seal's vocal anatomy.

Observed species-specific vocalisations are determined by both the species' vocal anatomy and their capacity for vocal learning (Garcia & Ravignani 2020; Ravignani & Garcia 2021). The vocal anatomy generates vocal predispositions by imposing biomechanical constraints, whereas neural processes determine the degree of control species have over their vocal organs (Garcia & Manser, 2020). Particularly, vocal learners, like the harbour seal, are capable of actively modulating sounds, suggesting that they are less constrained by anatomy and have a refined capacity for vocal motor control. Unfortunately, the relative contribution of both sound production mechanisms is unclear. Here, we provide support for a hypothesis trying to segregate anatomical vs. learning mechanisms (Garcia & Ravignani, 2020; Ravignani & Garcia 2021). We find that harbour seals are mechanistically constrained by their vocal anatomy, and their large vocal flexibility (Ralls et al., 1985; Borda et al., in press) thus points towards extensive volitional control over their vocalisations. As shown here, by testing for allometric relationships between body size and vocal tract structures, one can start to disentangle the respective contributions of vocal anatomy and vocal motor control in shaping acoustic signals.

Conclusion

In sum, we provide evidence of allometry between body size and vocal tract measurements in harbour seals. Body length is a strong predictor for VTL, VFL, and STDV1, and body weight is a strong predictor for VTL and both tracheal measurements (STDVs). Age and sex are also important in predicting the dimensions of these anatomical structures. Taken together, the combined findings demonstrate that harbour seal vocal tracts do indeed scale with body size, although allometry between VTL and body size may only emerge after weaning. Inferences, based on their vocal tract or body size, can now be made about the vocal predispositions of harbour seals (e.g., F0, formants, signal-to-noise ratio). Comparing these predictions to data obtained from observed natural vocalisations will shed light on the range of vocal flexibility resulting from their extensive vocal motor control. Moreover, connecting harbour seals' vocal anatomy measurements to the vocalisations they produce while alive is a critical next step to directly relate acoustic features to sound production structures. Integrating such results with investigations of call function will eventually inform on which vocal structures are responsible for generating the individual- and species-specific information encoded in harbour seals' vocalisations.

Methods

Sample collection

Larynges were collected during necropsies on 68 harbour seals (35 males). 52 samples came from seals that stranded on the Dutch coastline, the rest from animals found on the German coastline (Schleswig-Holstein). Forty-two animals died in captivity at Sealcentre Pieterburen, Pieterburen, the Netherlands, either naturally during rehabilitation or by means of euthanasia, and 26 animals died in the wild, either naturally or by means of mercy killing (see Table 1 of the Supplement). No animals were harmed for the purpose of this study.

At the time of death, the seals studied were aged between 9 days and 12 months (median 6 months). The age of new-born individuals was estimated in number of days by expert seal veterinarians based on the condition of the umbilical cord or the umbilicus. Older individuals with a closed umbilicus were assigned June as their birth month, which is consistent with the majority of harbour seal births in the Wadden Sea (Osinga, Nussbaum, Brakefield, & de Haes, 2012; Reijnders, Brasseur, & Meesters, 2010). Animals aged 1 month or younger were classified as pups, while those between 1 and 12 months in age were classified as weaners, making age a binary variable. Of the 68 individuals included in this study, 14 (8 males) were classified as pups and 54 (26 males) were classified as weaners. A Fisher's exact test showed no significant associations between age and sex ($\chi^2 = 0.7652$, $p > .05$), suggesting our sample is balanced between sexes and ages.

Sample treatment and measurements

Post-mortem examinations were performed by trained veterinarians. Dutch seals were examined at Sealcentre Pieterburen and German seals were necropsied at the Institute for Terrestrial and Aquatic Wildlife Research (ITAW), Büsum, Germany. Necropsies were performed on either cooled or defrosted carcasses. Body weight, body length and axillary girth were all measured prior to the start of the necropsy. Body length was measured from the tip of the nose to the end of the tail in a non-curvilinear fashion, while the animal was in supine position, and axillary girth was measured as the body circumference directly caudal to the front flippers. The vocal apparatus including the upper vocal tract, the larynx, and part of the trachea was then removed and immediately frozen at -20°C. All samples were in a similar condition (i.e., none presented signs of decomposition), comparable to pinniped vocal tracts in Schneider (1962) and Ravignani and colleagues (2017).

Prior to measurement, samples were thawed in a refrigerator at 8°C and each larynx was cut medially to produce two hemi-vocal tracts. The measurements taken on these hemi-vocal tracts include vocal tract length (VTL), vocal fold length (VFL), vocal fold thickness (VFT), and tracheal measurements in the form of subglottic-tracheal dorsoventral distances (STDVs) (called subglottic-tracheal anterior-posterior distance, STAP, in Roers, Mürbe, & Sundberg, 2009) using a calliper to an accuracy of ±0.01 mm (see Figure 2). Although the vocal tract can be divided into lower (below larynx) and upper (above larynx) sections, formants (the resonant frequencies which often encode information about body size) are only determined by the upper vocal tract (Lester & LaGasse, 2008). VTL will henceforth refer to the length of the upper vocal tract. VTL was measured as the linear distance from the caudal end of the epiglottis to the rostral end of the tongue muscle while the tongue was kept straight. VFL was measured as the distance from the ventral attachment of the vocal fold on the thyroid cartilage to the dorsal attachment of the vocal fold on the arytenoid cartilage. VFT was measured as the distance between the anterior and posterior sides of the vocal folds. The first STDV was measured as the distance between the cricothyroid ligament and the caudal end of the arytenoid. The second STDV was measured as the diameter of the first tracheal ring. All measurements were performed independently by two researchers. For both researchers, VTL, VFL, and VFT were measured twice for each hemi-vocal tract, and STDVs were taken once for each hemi-larynx because the start and end measuring points were composed of cartilage (as opposed to soft tissue) and hence, we assumed that the inter-rater reliability for STDVs would be higher than for other measurements.

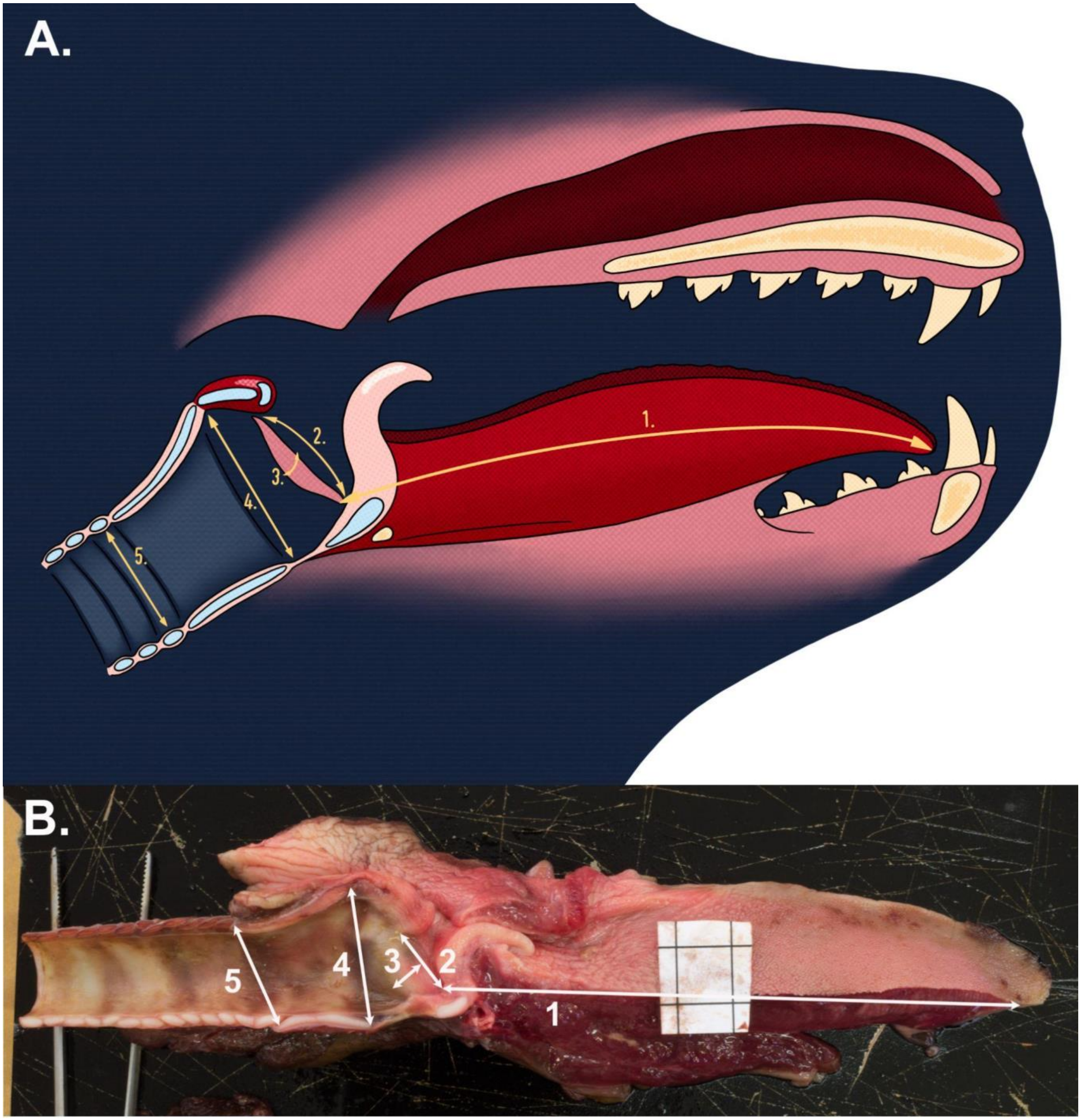

Figure 2. Images depicting the measurements taken using (A) a digital rendering of the vocal tract and (B) one hemi-larynx of a harbour seal pup. The vocal tract measurements taken include (1) vocal tract length (VTL), (2) vocal fold length (VFL), (3) vocal fold thickness (VFT), (4) subglottic-tracheal dorsoventral distance 1 (STDV1), and (5) subglottic-tracheal dorsoventral distance 2 (STDV2). In panel B, the black square outlined on the white paper serves as reference and is exactly 1 cm^2.

Statistical analysis

Statistical analyses were performed in RStudio version 1.1.463 (R version 4.0.4). First, for both raters, the medians for VTL, VFL and VFT were computed from all values reported for every right and left hemi-larynx. Second, using the medians from the first step, the median values for all measurements including STDV1 and STDV2 were computed for each larynx. This provided, for each larynx and rater, five measurements: VTL, VFL, VFT, STDV1 and STDV2. The inter-rater reliability for VTL, VFL, VFT, STDV1 and STDV2 was evaluated using Pearson's correlations. Finally, the overall median values between raters were computed for all measurements. Using these new values, Spearman's correlations between body size and vocal anatomy measurements were then calculated (see Table 1). For each measurement, normality was assessed using the Shapiro-Wilk test and homogeneity of variance was assessed using an F-test. If both assumptions were met, a two-tailed independent samples t-test was computed to check for age and sex differences. When variables were not normally distributed, but samples had equal variance, a Mann Whitney U-test was performed to assess group differences instead.

Predictive modelling was done using generalised linear models (GLMs) with the *stats* package (R Core Team, 2013). A series of models were produced for all anatomical measurements with high inter-rater reliability ($r > 0.70$). For every response variable, the full model included the fixed effects length, weight, girth, sex, age and the interaction effects of sex with all body size predictors, age with all body size predictors and the interaction of age and sex. The reduced model was then obtained through stepwise regression based on Akaike Information Criterion (AIC) values. An analysis of variance (ANOVA) test was performed to ensure that the reduced model was not performing significantly worse than the full one. Variance inflation factors (VIF) scores were calculated for all predictors included in the reduced models using the *car* package (Fox & Weisberg, 2019). Multicollinearity was considered problematic for subsequent model selection if VIF scores were greater than 5 (Akinwande, Dikko, & Samson, 2015). For all selected models, deviance explained was calculated from the model output (1 – residual deviance / null deviance) and expressed as a percentage. Plots displaying the predicted effects of every predictor retained in the final models were produced to assess their relationship with the response variable. Diagnostic residual plots were used to verify the model assumptions. Independence of residuals was tested using a Durbin Watson test (Fox & Weisberg, 2019). Normality of residuals was assessed visually by plotting model fit against the observed data. Homoscedasticity (i.e., constant variance) of residuals was also assessed visually using quantile-quantile plots. Finally, influential data points were assessed by calculating Cook's distance.

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Competing interests

The authors declare no competing interests.

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Supplementary material

Koen de Reus, Daryll Carlson, Alice Lowry, Stephanie Gross, Maxime Garcia, Ana Rubio-Garcia, Anna Salazar-Casals, Andrea Ravignani

Table 1

List of sampled animals

ID	Age class	Where from	Sex	Length (cm)	Weight (kg)	Girth (cm)	Cause of death
1	weaner	NL	F	86	15.6	90	Euthanised
2	weaner	NL	F	99	17.3	83	Died during rehab
3	weaner	NL	M	96	26.8	76	Found dead in the wild
4	weaner	NL	M	96	22.9	71	Euthanised
5	weaner	NL	M	86	19.2	71	Died before rehab
6	weaner	NL	M	84	18.2	69	Euthanised
7	weaner	NL	M	89	19.9	66	Euthanised
8	weaner	NL	F	94	14.2	66	Euthanised
9	weaner	NL	F	92	15.8	65	Euthanised
10	weaner	NL	F	94	14.9	65	Died during rehab
11	weaner	NL	M	104	20.8	64	Died before rehab
12	weaner	NL	F	86	16.5	63	Died during rehab
13	weaner	NL	F	86	15.7	63	Euthanised
14	weaner	NL	M	100	18.37	62	Euthanised
15	weaner	NL	F	93	16.8	62	Euthanised
16	weaner	NL	M	114	18.8	61	Died before rehab
17	weaner	NL	F	87	15.8	61	Euthanised
18	weaner	NL	F	93	17.8	60.5	Euthanised
19	weaner	NL	M	96	16.3	60	Died during rehab
20	weaner	NL	F	82	15.3	60	Euthanised
21	weaner	NL	M	80	14.3	60	Died before rehab
22	weaner	NL	F	88	16.1	59	Died during rehab
23	weaner	NL	F	89	16.9	58	Euthanised
24	weaner	NL	F	71	10	58	Euthanised
25	weaner	NL	M	92	17	57	Euthanised
26	weaner	NL	F	94	14.5	57	Died during rehab
27	weaner	NL	M	86	13.9	57	Died before rehab
28	weaner	NL	F	79	11.9	56.5	Euthanised
29	weaner	NL	M	85	14.6	56	Died during rehab
30	weaner	NL	M	94	14.6	55	Euthanised

31	weaner	NL	F	92	13.7	55	Died during rehab
32	weaner	NL	M	80	13.1	55	Died during rehab
33	pup	NL	F	75	11.9	55	Euthanised
34	pup	NL	M	84	11.79	54	Found dead in the wild
35	weaner	NL	F	93	14	53	Died during rehab
36	pup	NL	M	83	11.47	52	Euthanised
37	weaner	NL	F	93	13.9	51.5	Euthanised
38	weaner	NL	M	86	13	51.5	Died before rehab
39	weaner	NL	F	87	12.4	51	Died before rehab
40	pup	NL	M	86	10.6	51	Euthanised
41	pup	NL	F	81	11.37	49.5	Euthanised
42	pup	NL	F	82	9.3	49	Found dead in the wild
43	pup	NL	M	80	9.46	47	Euthanised
44	pup	NL	M	73	8.6	46	Found dead in the wild
45	pup	NL	F	77	8.5	44.5	Died before rehab
46	pup	NL	F	80	9.63	44	Found dead in the wild
47	weaner	NL	M	87	9.3	44	Died during rehab
48	pup	NL	F	70	8	44	Found dead in the wild
49	pup	NL	M	87	9.43	41	Found dead in the wild
50	weaner	NL	F	77	7.47	40	Euthanised
51	pup	NL	M	80	7.28	38.5	Died during rehab
52	pup	NL	M	80	9.95	36	Died before rehab
53	weaner	DE	M	85.5	19.2	67.5	Mercy killed
54	weaner	DE	F	98.5	17	66	Mercy killed
55	weaner	DE	M	90	14.6	65	Mercy killed
56	weaner	DE	M	101	20.8	64	Mercy killed
57	weaner	DE	M	90	17	63	Found dead in the wild
58	weaner	DE	F	92	20.4	62	Found dead in the wild
59	weaner	DE	F	99	17.8	60.5	Mercy killed
60	weaner	DE	M	86	16	60.5	Found dead in the wild
61	weaner	DE	M	90	16.6	59	Found dead in the wild
62	weaner	DE	F	94	17	58	Found dead in the wild
63	weaner	DE	M	90	14.6	57	Mercy killed
64	weaner	DE	M	96	13.4	56	Found dead in the wild
65	weaner	DE	F	82	11.6	55.5	Found dead in the wild
66	weaner	DE	F	97	14.8	54	Mercy killed
67	weaner	DE	M	81	10.2	51	Mercy killed
68	weaner	DE	F	88.5	12	50	Mercy killed

Note. Seals were from the Netherlands (NL) or Germany (DE). Sex is denoted as F for females and M for males.

Table 2

Pairwise Spearman correlations for pups

Variable	Length (cm)	Weight (kg)	Girth (cm)	VTL (mm)	VFL (mm)	VFT (mm)	STAP1 (mm)
Weight (kg)	0.40						
Girth (cm)	0.23	0.72					
VTL (mm)	0.23	-0.03	-0.04				
VFL (mm)	0.22	0.08	-0.01	0.49			
VFT (mm)	-0.21	0.11	0.42	0.01	0.16		
STAP1 (mm)	0.20	0.18	0.08	0.49	0.79*	0.55	
STAP2 (mm)	0.35	0.36	0.38	0.26	0.71	0.57	0.76*

Note. * indicates $p < .05$ after correcting for multiple comparisons using the Holm-Bonferroni method.

Table 3

Pairwise Spearman correlations for weaners

Variable	Length (cm)	Weight (kg)	Girth (cm)	VTL (mm)	VFL (mm)	VFT (mm)	STAP1 (mm)
Weight (kg)	0.51*						
Girth (cm)	0.28	0.76*					
VTL (mm)	0.39*	0.48*	0.39*				
VFL (mm)	0.58*	0.64*	0.41*	0.54*			
VFT (mm)	0.16	0.48*	0.50*	0.22	0.34		
STAP1 (mm)	0.38*	0.61*	0.38*	0.46*	0.67*	0.47*	
STAP2 (mm)	0.32	0.48*	0.31	0.47*	0.57*	0.30	0.66*

Note. * indicates $p < .05$ after correcting for multiple comparisons using the Holm-Bonferroni method.

Table 4

GLM estimates for vocal tract length (VTL)

Effect	Estimate	Std.Error	2.5%	97.5%	p
Intercept	42.4788	9.2445	23.9898	60.9678	< 0.001
Age Class-Weaner	4.6695	1.7801	1.1093	8.2297	< 0.05
Length	0.4170	0.1184	0.1802	0.6538	< 0.001
Weight	0.5933	0.2182	0.1569	1.0297	< 0.01
Sex-Male	40.7192	12.1685	16.3822	65.0562	< 0.01
Length*Sex-Male	-0.4610	0.1379	-0.7368	-0.1852	< 0.01

Note. The reference level for Age Class is ‘Pup’ and the reference level for Sex is ‘Female’.

Table 5

GLM estimates for vocal fold length (VFL)

Effect	Estimate	Std.Error	2.5%	97.5%	p
Intercept	9.1651	3.0598	3.0455	15.2847	< 0.01
Age Class-Weaner	-2.7492	3.2348	-9.2188	3.7204	0.399
Length	-0.0189	0.0396	-0.0981	0.0603	0.635
Weight	0.1050	0.0301	0.0448	0.1652	< 0.001
Sex-Male	1.0070	0.3670	0.2730	1.7410	< 0.01
Age Class-Weaner*Length	0.0562	0.0411	-0.026	0.1384	0.177
Age Class-Weaner*Sex-Male	-1.1833	0.4071	-1.9975	-0.3691	< 0.01

Note. The reference level for Age Class is ‘Pup’ and the reference level for Sex is ‘Female’.

Table 6

GLM estimates for subglottic tracheal dorsoventral distance 1 (STDV1)

Effect	Estimate	Std.Error	2.5%	97.5%	p
Intercept	15.389	1.6827	12.0236	18.7544	< 0.001
Age Class-Weaner	1.7474	0.4352	0.8770	2.6178	< 0.001
Length	0.0472	0.0236	0.0000	0.0944	< 0.05
Weight	0.1887	0.0533	0.0821	0.2953	< 0.001
Sex-Male	0.5956	0.2754	0.0448	1.1464	< 0.05

Note. The reference level for Age Class is ‘Pup’ and the reference level for Sex is ‘Female’.

Table 7

GLM estimates for subglottic tracheal dorsoventral distance 2 (STDV2)

Effect	Estimate	Std.Error	2.5%	97.5%	p
Intercept	9.9726	1.8526	6.2674	13.6778	< 0.001
Age Class-Weaner	1.5194	0.4792	0.5610	2.4778	< 0.01
Length	0.0427	0.0260	-0.0093	0.0947	0.105
Weight	0.1560	0.0587	0.0386	0.2734	< 0.01
Sex-Male	0.4523	0.3032	-0.1541	1.0587	0.141

Note. The reference level for Age Class is ‘Pup’ and the reference level for Sex is ‘Female’.

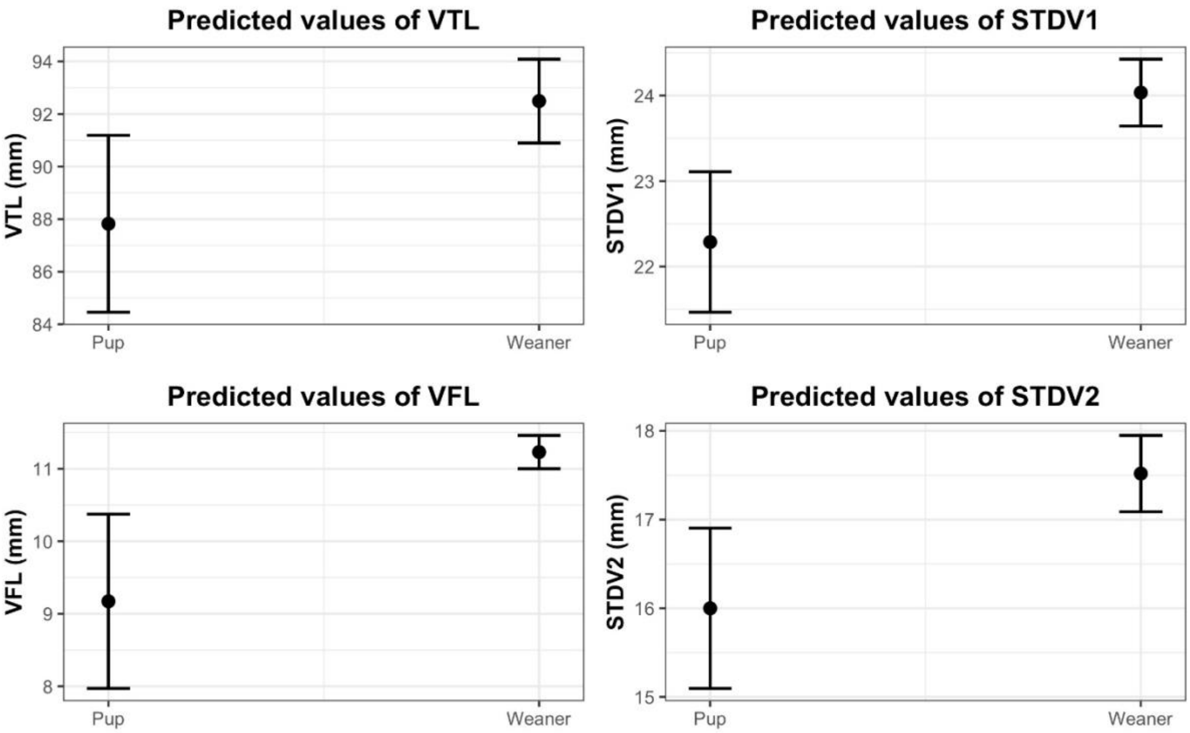

Figure 1. Predicted effects of age class (A) in each of the GLM models.

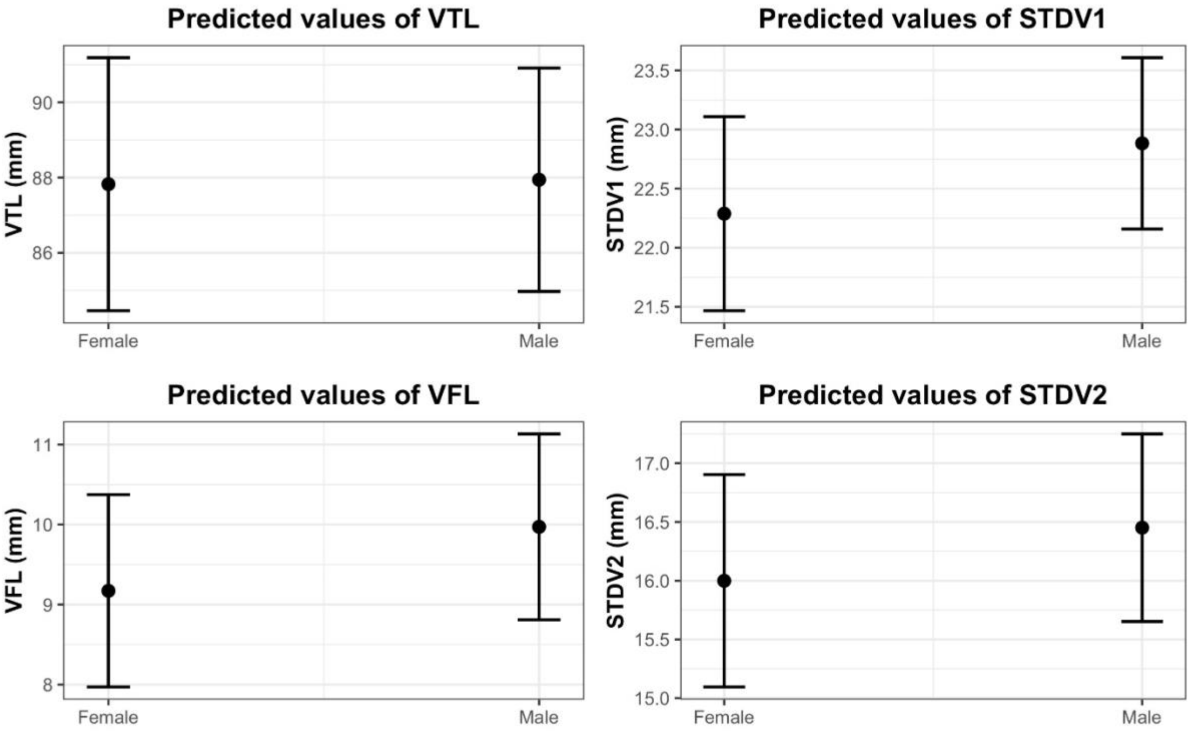

Figure 2. Predicted effects of sex (S) in each of the GLM models.

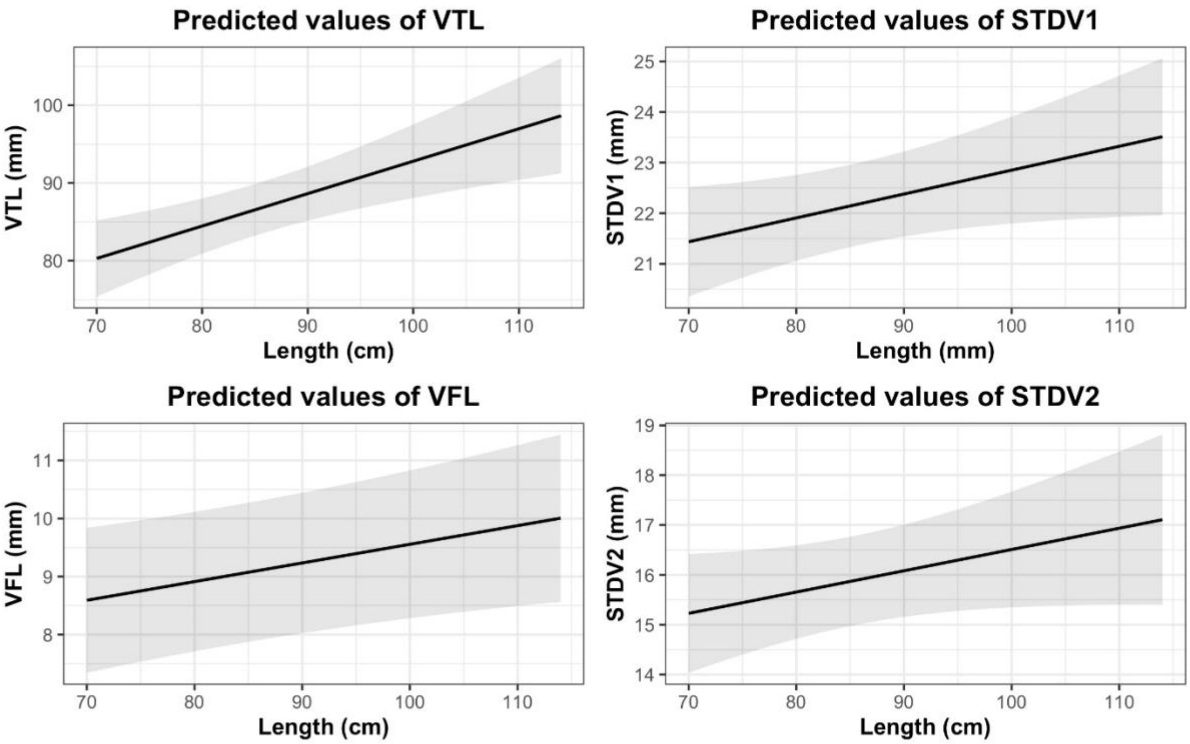

Figure 3. Predicted effects of length (L) in each of the GLM models.

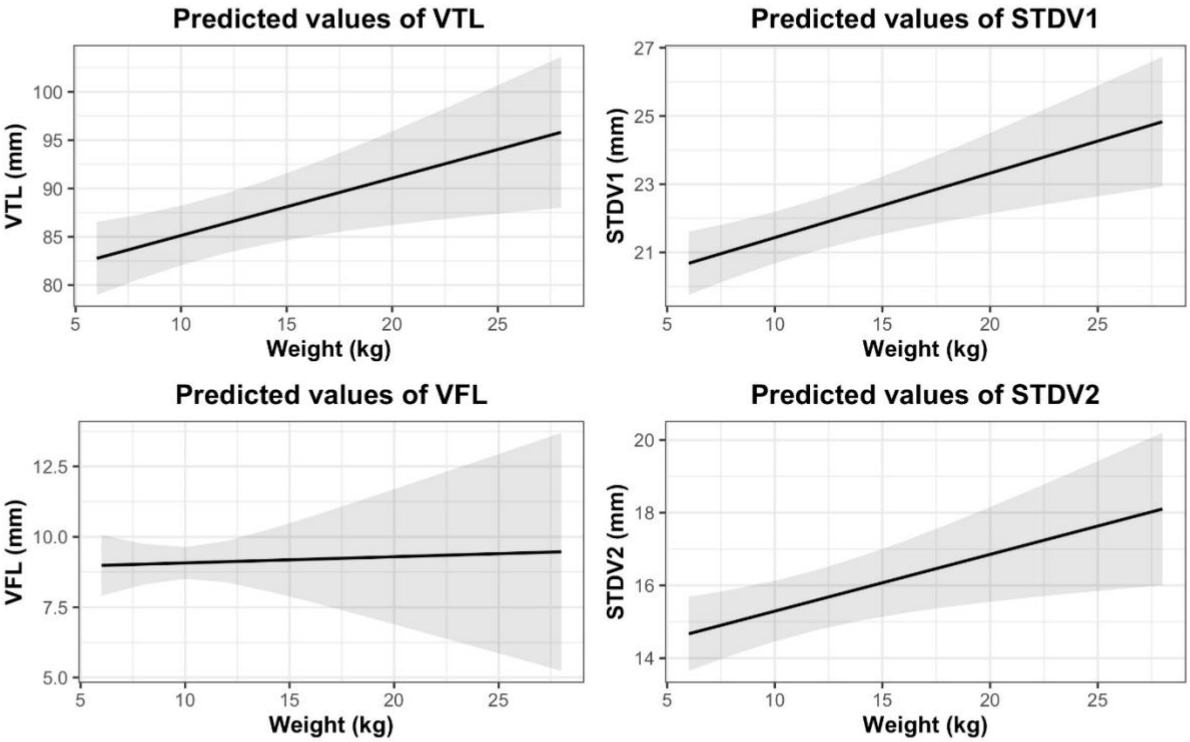

Figure 4. Predicted effects of weight (W) in each of the GLM models.

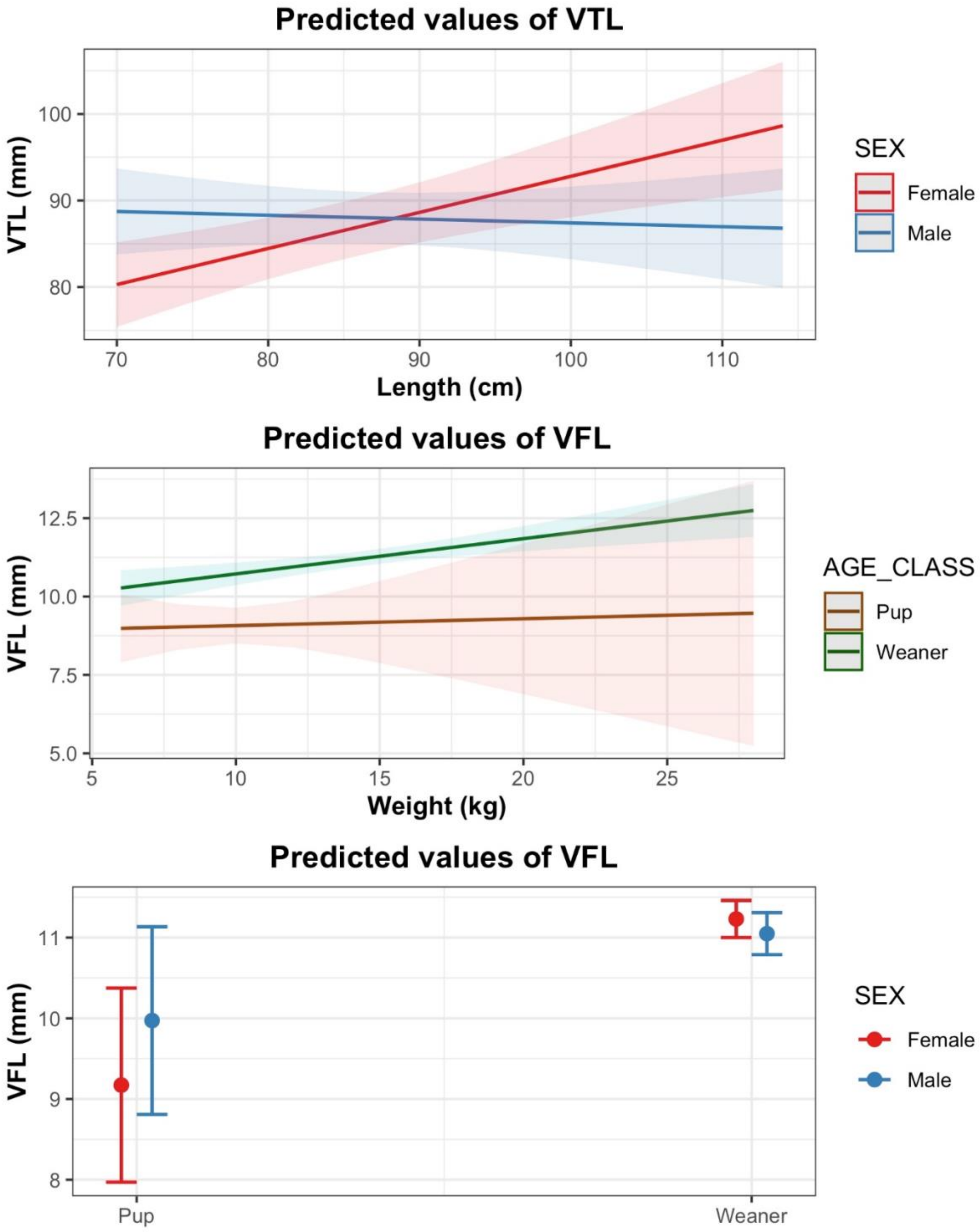

Figure 5. Predicted effects of the length and sex interaction for VTL (top), the weight and age interaction for VFL (middle) and the age and sex interaction for VFL (bottom).