

Article

How small could a pup sound? The physical bases of signaling body size in harbor seals

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Abstract

Vocal communication is a crucial aspect of animal behavior. The mechanism which most mammals use to vocalize relies on three anatomical components. First, air overpressure is generated inside the lower vocal tract. Second, as the airstream goes through the glottis, sound is produced via vocal fold vibration. Third, this sound is further filtered by the geometry and length of the upper vocal tract. Evidence from mammalian anatomy and bioacoustics suggests that some of these three components may covary with an animal's body size. The framework provided by acoustic allometry suggests that, because vocal tract length (VTL) is more strongly constrained by the growth of the body than vocal fold length (VFL), VTL generates more reliable acoustic cues to an animal's size. This hypothesis is often tested acoustically but rarely anatomically, especially in pinnipeds. Here, we test the anatomical bases of the acoustic allometry hypothesis in harbor seal pups *Phoca vitulina*. We dissected and measured vocal tract, vocal folds, and other anatomical features of 15 harbor seals post-mortem. We found that, while VTL correlates with body size, VFL does not. This suggests that, while body growth puts anatomical constraints on how vocalizations are filtered by harbor seals' vocal tract, no such constraints appear to exist on vocal folds, at least during puppyhood. It is particularly interesting to find anatomical constraints on harbor seals' vocal tracts, the same anatomical region partially enabling pups to produce individually distinctive vocalizations.

Key words: acoustic allometry, honest signaling, larynx, pinniped, vocal tract.

In recent years, the study of mammal vocal communication has greatly benefited from the source-filter theory (Taylor and Reby 2010). Originally conceived for human speech (Fant 1960), the concept of this theory has been extended to other mammals, and states that sounds are produced by the vibration of vocal folds within the larynx (the sound source), and then filtered by the vocal tract before being radiated into the environment. Within this framework,

fundamental frequency (hereafter F0) represents the rate of vibration of the vocal folds and formants represent the selectively filtered frequencies resulting from vocal tract geometry. Research relying on this framework has improved our understanding of the role played both by the sound source and the vocal tract in various communication systems, identifying the determinants and functions of specific acoustic features, such as vocal indicators of emotions (Briefer

2012), sound production mechanisms (Herbst et al. 2012), sound modulation (Pisanski et al. 2016), and transmission of information about caller-specific attributes (Fitch and Hauser 2003; Garcia et al. 2014), to name just a few.

The examination of existing correlations between morphology and acoustics, defined as acoustic allometry, has received particular attention. Initial focus has been put on the relationship between body size and source-related acoustics features (Morton 1977; Ohala 1984), with a negative correlation observed across a broad range of mammalian species (Bowling et al. 2017). However, the connections of the larynx to its surrounding anatomical structures make the larynx relatively independent from the growth of the body compared with vocal tract (Fitch and Hauser 1995). This anatomical feature potentially allows for laryngeal, and by extension vocal fold, enlargement independent from body size, as seen for instance in roaring cats (Hast 1989; Klemuk et al. 2011). In turn, this independent enlargement leads, in principle, to statistical independence between vocal fold length (VFL), a key determinant of F0 (Titze 2000), and body size. Hence, filter-related acoustic features (i.e., formants) are expected to reflect body size better than source-related acoustic features (i.e., F0). In theory, formants are then likely to be used as a more reliable external cue than F0 by conspecifics assessing body size. Various studies supported this argument in mammals, showing that an accurate cue to body size is given through formants (Fitch 1997, 2000a, 2000b; Riede and Fitch 1999; Plotsky et al. 2013; Garcia et al. 2016), and that F0 poorly predicts body size within a species (Pfefferle et al. 2007; Charlton et al. 2011; Pisanski et al. 2014). Even in species where vocal tract anatomy has been modified and leads to size exaggeration relative to other species, formants have proven reliable and honest cues to body size at the within-species level (reviewed in Charlton and Reby 2016). However, only few studies testing the vocal allometry hypothesis included actual measures of vocal tract length, hereafter VTL, and/or VFL, either using photogrammetry (Sanvito et al. 2007a, 2007b, 2007c; Casey et al. 2015), X-rays (Fitch 2000a; Fitch and Reby 2001; Plotsky et al. 2013; Garcia et al. 2016), CT-scans (Efremova et al. 2016), skeleton measures (Fitch 2000b), silicon casts (Riede et al. 2005; Gamba and Giacoma 2006; Gamba et al. 2012), and tissue dissection (Fitch and Reby 2001).

Pinnipeds are among the most vocal mammals (Schusterman et al. 2001; Schusterman 2008). They consist of three families of carnivores linked phylogenetically at the superfamily level (Riedman 1990; Bowen 1991; Higdon et al. 2007; Reichmuth and Casey 2014). Within the family Phocidae (i.e., earless seals), research on behavior and communication has mostly focused on harbor seals, possibly due to their broad geographical distribution (Riedman 1990; Härkönen and Harding 2001). In this species, individuals vocalize until weaning, which occurs at 26–40 days from birth (Cottrell et al. 2002), and then again during adulthood (Van Parijs et al. 2003; Hayes et al. 2004). However, while adult females do not seem to vocalize at all, males become again vocally active (Ralls et al. 1985; Van Parijs and Kovacs 2002; Van Parijs et al. 2003). In particular, they produce underwater song-like (sensu Fitch 2015) vocal displays during the breeding season (Schusterman et al. 1970; Hanggi and Schusterman 1994; Nikolich et al. 2016; Sabinsky et al. 2017). In addition, one adult male harbor seal has been shown capable of vocal production learning (Ralls et al. 1985). This is the ability, rare among mammals, to modify vocalizations usually based on social experience (Janik and Slater 1997); for vocal learning in pinnipeds, see Shapiro et al. (2004); Sanvito et al. (2007b); Schusterman (2008); Schusterman and Reichmuth (2008); Spasikova et al. (2008); Reichmuth and Casey (2014); Stansbury (2015)).

Harbor seals often breed in dense rookeries, entailing the risk of mothers confusing other pups for their own (Newby 1973; Khan et al. 2006). Accordingly, after only a few hours from birth (Lawson and Renouf 1985) and until weaning (Perry and Renouf 1988; Khan et al. 2006), pups of both sexes produce mother-directed calls known as “mother attraction calls” (Sauvé et al. 2015a), hereafter MAC. These calls are individually distinct (Sauvé et al. 2015a) and recognizable by the pups’ mothers (Sauvé et al. 2015b). Source-related (Khan et al. 2006; Sauvé et al. 2015a) and filter-related (Sauvé et al. 2015a) features of MAC are affected by pups’ sex, age, and/or body length. Acoustic parameters exhibit a broad variation depending on sex, age, and body length: for instance F0s range between 270 and 620 Hz, durations between 193 and 1113 ms, and the 5 maximum amplitude peaks between 129 and 9,182 Hz (Sauvé et al. 2015a). Such acoustic variation suggests potentially similar anatomical variation in the larynx, upper vocal tract, and other phonatory structures. This leads to some natural questions. What are the physical bases of the individual distinctiveness of these calls? Does body size form part of the information potentially transmitted in pups’ calls?

The present study addresses these open questions in harbor seals. Here, we test several anatomical hypotheses that may explain bioacoustics findings on MAC (Renouf 1984; Perry and Renouf 1988; Khan et al. 2006; Sauvé et al. 2015a). Namely, we aim at identifying some anatomical determinants for acoustic parameters of pup vocalizations that are most likely to carry body size information. This research should prove particularly relevant to understand the mechanistic bases of effective mother–pup acoustic recognition, a key component of harbor seals’ socio-ecology (Renouf 1984; Perry and Renouf 1988; Riedman 1990; Sauvé et al. 2015a).

The existence and form of a particular behavior can be investigated with emphasis on its function, phylogeny, ontogeny, and mechanism (Tinbergen 1963). The study of pinniped vocal behavior has mainly focused on its function and phylogeny (reviewed in Riedman 1990; Reichmuth and Casey 2014; Ravignani et al. 2016). In other words, comparison of vocal production across pinniped species and their socio-ecology has elucidated the evolutionary history of different vocal communication systems and how their distinctive features are adapted to particular species’ ecological niches. The ontogeny of vocal behavior in pinnipeds has received comparatively less attention, though recent research has focused more on developmental aspects (Khan et al. 2006; Sanvito et al. 2008; Sauvé et al. 2015a, 2015b). Finally, *mechanistic* approaches to pinniped vocal production have been historically neglected (Tyack and Miller 2002). A few relevant exceptions to this have focused on pure pinniped anatomy (Negus 1949; Schneider 1962; Schneider et al. 1964; Piérard 1969; Bryden and Felts 1974), bronchograms and tracheograms to better understand breathing (Kooyman et al. 1970) or actual sound production mechanisms (Sanvito et al. 2007a; Spasikova et al. 2008).

Within the source-filter framework, previous bioacoustics results can be translated into anatomical predictions. Source-related predictions are quite easy to formulate because previous research analyzed the fundamental or the minimum frequency produced. In particular, larger and older pups have a lower F0 and minimum frequency (Khan et al. 2006; Sauvé et al. 2015a). If we assume that in harbor seals these frequency parameters are mostly determined by the length of the vocal folds, larger and older pups should have longer vocal folds. Alternatively, larger and older pups might be endowed with a larynx comparable in size to younger conspecifics’, but still

obtain a lower F0 by exerting neuro-motor control over the laryngeal cartilages on which vocal folds attach (Harrison 1995). Although the first hypothesis is more compatible with previous findings in harbor seals' bioacoustics (Sauvé et al. 2015a), a lack of laryngeal allometric scaling (second hypothesis) dovetails with a feeble correlation between vocal folds' length and body size in several mammal species (Taylor and Reby 2010).

Filter-related predictions in light of bioacoustics findings are more problematic. Acoustic analyses of pup vocalizations have measured filter-related *proxies*, rather than measuring the location of each formant in the frequency spectrum. These filter-related proxies were, among others: energy quartiles, the frequency of amplitude peaks in the spectrum, and the ratios between these amplitudes (Khan et al. 2006; Sauvé et al. 2015a). Using proxies might be explained by duration, F0 and harmonics, rather than formants, being more accessible to measurements. In recordings, age and body length are statistically predicted by energy quartiles, the frequency of amplitude peaks in the spectrum, and the ratios between these amplitudes (Khan et al. 2006; Sauvé et al. 2015a). Assuming that, in harbor seals, the frequency of amplitude peaks in the spectrum and the ratios between these amplitudes correlate with formants' frequencies (in the hypothetical situation of a sound source producing white noise, frequency location of amplitude peaks would coincide with some of the formants. If however, the source is a mammalian larynx, the mapping between spectral peaks and formant frequency becomes more problematic), we have a simple prediction. Older and larger seal pups will show a formant shift downwards, corresponding to a longer upper vocal tract. Here, unlike the 2 alternative hypotheses for source-related features, results from both harbor seals' bioacoustics and anatomy of mammalian vocal production concur into hypothesizing an allometric relationship between body size and VTL.

Finally, the trachea has been suggested to affect vocalizations in several phocid species (Rogers T, personal communication; Piérard 1969; Bryden and Felts 1974; Boness and James 1979; Burns 1981; Ray 1981; Gailey-Phipps 1984; Miller and Job 1992; Terhune et al. 1994; Tyack and Miller 2002). However, no such data are available for harbor seals, leading us to an agnostic aptitude toward the influence of the trachea on phonation.

To summarize, and sticking to maximum parsimony, we would predict older and larger seals to have a longer upper vocal tract and longer vocal folds. Due to lack of studies on the effect of the trachea on phonation in harbor seals, no particular predictions are advanced for the trachea.

Materials and Methods

Research was performed at Sealcentre Pieterburen in The Netherlands, which rehabilitates approximately 500 phocids (family Phocidae) every year (Osinga and van Voorst Vader 2010; Rubio-García et al. 2015). The success rate of the rehabilitation center is high: Between 2010 and 2015, the average percentage of animals successfully released in the wild was 84%. This study involved 15 harbor seals (12 females) admitted at the center. Due to serious medical conditions, these animals could not be successfully rehabilitated and died naturally at arrival (3 animals), during rehabilitation (10 animals), or were humanely euthanized (1 animal). One animal was found freshly dead in the wild. Their age was estimated by experienced veterinarians and ranged from 10 to 108 days (median age: 48 days) at the moment of death (Table 1). Age in new-born harbor seal pups was estimated from the appearance of the umbilical cord and the closing of the umbilicus. For older seal pups, we assumed June as the month of birth, as most harbor seal pups are born during this month (Reijnders et al. 2010; Osinga et al. 2012). Body weight (scale accuracy: ± 0.1 kg) was measured before necropsy (Table 1). No animals were harmed or sacrificed for the purpose of the present study.

Dead bodies were stored in a cooler and none was frozen after death. Necropsies were performed within 3 days. Body length and axillary girth were measured post-mortem during the necropsy (Table 1). Standard (as opposed to curvilinear) body length was measured as the distance between nose and tip of the tail, and axillary girth was measured as the body girth directly behind the front flippers. The accuracy of the sewing measuring tape was ± 1 mm, although length measures might be less accurate than this (see Table 1 for the standard deviation of this and other measures). The vocal apparatus (including larynx and upper vocal tract), from the first tracheal rings to the end of the tongue, was extracted and frozen at

Table 1. Individual values, mean, and standard deviations of all variables used in our analyses

| Sex | Length (mm) | Girth (mm) | Weight (kg) | Age (days) | Trachea (mm) | VFL (mm) | VTL (mm) |
|------|-------------|------------|-------------|------------|--------------|----------|----------|
| F | 870 | 60 | 16.4 | 108 | 15.25 | 11.63 | 81.1 |
| F | 820 | 72 | 21.4 | 95 | 14.6 | 10.19 | 87.325 |
| F | 720 | 58 | 11.9 | 10 | 14.45 | 10.04 | 79.99 |
| F | 740 | 46 | 8.1 | 45 | 14.12 | 11.73 | 88.52 |
| M | 800 | 53 | 12.7 | 63 | 16.51 | 11.045 | 83.22 |
| F | 800 | 51 | 20.1 | 49 | 14.77 | 9.965 | 82.455 |
| F | 690 | 51 | 10 | 46 | 14.11 | 11.565 | 75.78 |
| F | 790 | 45 | 8.7 | 10 | 14 | 9.195 | 79.46 |
| F | 800 | 48 | 9.5 | 30 | 14.84 | 9.165 | 80.12 |
| F | 650 | 39 | 6.4 | 10 | 11.13 | 10.335 | 73.895 |
| M | 790 | 45 | 9.4 | 55 | 14.46 | 10.675 | 81.295 |
| F | 810 | 56.5 | 11.7 | 102 | 14.3 | 9.49 | 83.28 |
| F | 790 | 54 | 13.14 | 45 | 14.57 | 9.83 | 83.205 |
| M | 840 | 55 | 14.1 | 90 | 14.44 | 10.545 | 88.415 |
| F | 790 | 51 | 10.7 | 48 | 15.24 | 11.11 | 92.08 |
| Mean | 780 | 52.30 | 12.28 | 53.73 | 14.45 | 10.43 | 82.68 |
| SD | 57.32 | 7.81 | 4.26 | 32.68 | 1.07 | 0.85 | 4.86 |

Notes: Age in days was obtained by adding the veterinarian estimate for a particular seal at arrival to the time that individual spent at the center before dying.

–20 degrees immediately after necropsy. All vocal apparatus samples were in conditions comparable to each other (and to vocal tracts depicted in Schneider 1962), independently of the cause of death.

Samples were slowly thawed under refrigeration at 8 degrees. VFL, VTL, and trachea diameter (accuracy of measuring calliper: ± 0.01 mm) were measured as follows. First, the epiglottis was removed (Alipour et al. 2011). Then, samples were cut medially, resulting in 2 hemi-vocal tracts (Schneider 1962; Alipour et al. 2011). VTL was measured as the distance from the cranial end of the tongue muscle (point T in Figure 1A) to the anterior commissure (i.e., cranial end of the plica vocalis, point C in Figure 1A). VFL was measured as the distance from the anterior commissure to the vocal process of the arytenoid (i.e., caudal end of the plica vocalis, point P in Figure 1B). VTL was measured twice, once for each hemi-vocal tract. Vocal folds were measured twice for each hemi-larynx. This was done as the size of measurement error is potentially negligible for TC (distance between points T and C) but not for CP (distance between points C and P), so more measurements were performed on CP to increase its accuracy. The dorsoventral diameter of the trachea was measured on the third visible tracheal ring (black arrows in Figure 1A).

Statistical analyses were performed in R version 2.15.1 (R Team 2014). The Shapiro–Wilk test was used to test the assumption that each variable was normally distributed. The coefficient of variation was calculated as the ratio between standard deviation and sample mean. The parametric Pearson’s correlation coefficient was only performed after ascertaining the normality of the data (all cases). Correlations were performed as exploratory, rather than strongly inferential. Because of this, our results put less emphasis on the significance of correlations and more on their magnitude (notice that here the *P*-value and correlation coefficient are mathematically redundant with respect to each other). To avoid biased optimal stopping

and p-hacking (Bastardi et al. 2011; Head et al. 2015), we only started analyzing the data once all available vocal tract samples had been measured.

Results

All VTL and VFL measures were normally distributed (Shapiro–Wilk normality test: all W , $0.93 < W \leq 0.99$, all $P > 0.28$), justifying use of Pearson’s correlation coefficient. The correlation between repeated measures of VTL was significant ($r = 0.71$, $P < 0.01$). The correlations between repeated measures of folds were significant ($r \geq 0.77$, $P < 0.001$, for both left and right hemi-larynx). All correlations among the 4 measures of vocal folds (2 sides and 2 repeated measures per side) were significant ($r \geq 0.56$, $P < 0.05$). For each individual, further analyses were performed on the median of the 2 VTL measurements and the median of the 4 VFL measurements (Yurkowski et al. 2011). Table 1 shows all measures for each individual, together with variables mean and standard deviation.

VFL and VTL across individuals exhibited similar coefficients of variation: 0.08 versus 0.06. Their ratio was 1.38, meaning that cross-individual variation in VFL measurements was 38% higher than variation in VTL measurements. This suggests that coefficients of variation are moderately comparable between VTL and VFL. As folds are shorter than vocal tracts, hypothesizing a normal distribution for both populations but a higher measuring precision for higher orders of magnitude, the coefficient of variation for VFL might have been much larger than for VTL. This result shows this was in fact not the case. This however does not exclude a measurement error or bias.

Before calculating parametric statistics, we tested that all variables were normally distributed. Normality of all variables (Shapiro–Wilk normality test: all W , $0.90 < W \leq 0.96$, all $P > 0.10$) licensed the use of Pearson’s correlation coefficient.

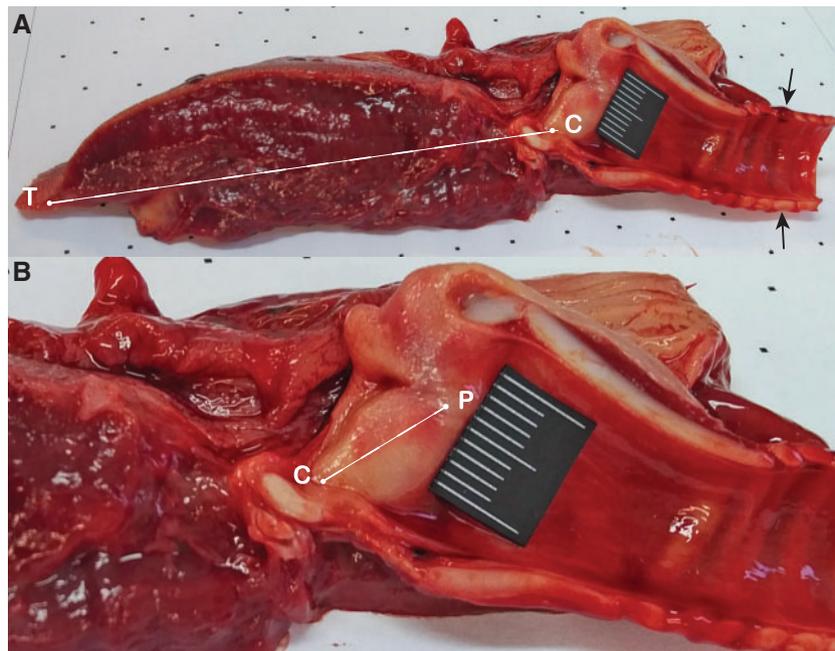


Figure 1. Hemi-vocal (A) tract and hemi-larynx (B). VTL was measured as the distance TC in panel A. Trachea diameter was measured on the third tracheal ring, shown by the black arrows in panel A. VFL was measured as the distance CP in panel B. Notice that both our linear measure TC and the curved surface of the tongue above it are only approximations to the actual VTL. For alternatives see for example, Garcia et al. (2016). The scale shown in both figures corresponds to 1 mm (distance between any adjacent white line) and 1 cm (distance between the two longest white lines at the edges).

Table 2. Correlation coefficients among measured variables

| | VTL | Body length | Girth | Weight | Trachea |
|-------------|------|-------------|-------------|-------------|-------------|
| VFL | 0.21 | -0.11 | -0.02 | -0.03 | 0.17 |
| VTL | | 0.54 | 0.39 | 0.33 | 0.50 |
| Body length | | | 0.52 | 0.59 | 0.68 |
| Girth | | | | 0.80 | 0.47 |

Notes: For our sample size and an alpha level equal to 0.05, bi-directional significance is reached for correlations $\geq .514$. Correlations significant at $P < 0.05$ are highlighted in bold. However, notice that our correlation analysis is meant to be exploratory (as opposed to strongly inferential), and that for a given sample size and beta level, P -values are solely dependent on the magnitude of Pearson's correlation coefficient.

There were high correlations among all body size measures unrelated to vocal production (lower entries in the girth and weight columns of Table 2, namely between girth and length, length and weight, girth and weight). As acoustic allometry results from a special case of general body allometry, these correlations provide a sanity check: harbor seal pups do exhibit some degree of physical allometry.

There was a positive correlation between VTL and body length (Table 2 and Figure 2). The length of the vocal tract, an important parameter in determining formants in the acoustic signal, correlated with body size, hence harbor seal pups possess the physical predisposition for formants to provide a potential cue to body size.

There was little or negative correlation between VFL and all other measures (first row in Table 2), crucially a feeble and negative correlation between VFL and body length. Interestingly, the lowest correlations are all, and only, those between VFL and any other parameter. The length of the vocal folds, an important parameter in determining the F_0 of vocalizations, did not predict body size, which suggests that F_0 is unlikely to provide a cue to body size.

There was a strong correlation between body length and the diameter of the trachea (Figure 3). This suggests that, similarly to the upper vocal tract and unlike vocal folds, the trachea cross-section is anatomically constrained by body growth.

Finally, to enable comparability with previous acoustic studies, we plot the VFL and estimated age of the harbor seal pups in Figure 4: VFL does not give an indication of the body size, at least in the age class investigated here.

Discussion

This research provides three main contributions to the study of harbor seal pups' vocal communication. We show that an animal's body length: (i) correlates with its upper VTL; (ii) does not appear to correlate with the length of its vocal folds at rest; (iii) strongly correlates with the diameter of its trachea. Although point (i) supports previous bioacoustics findings in harbor seals, point (ii) contrasts with positive correlations found between body size and acoustic parameters influenced by the vocal folds. Finally, point (iii) represents a novel anatomical observation, potentially relevant to this species' sound production. In particular, it implies that, if the trachea affects some parameters of phonation in harbor seals, information about its size is likely to be communicated.

Previous research has shown that proxies for the filter, that is, the upper vocal tract, statistically predict pups' age and body length. These proxies are the frequency of amplitude peaks in the spectrum, the ratios between these amplitudes, and energy quartiles (Sauvé et al. 2015a). While these features also partly capture effects of the

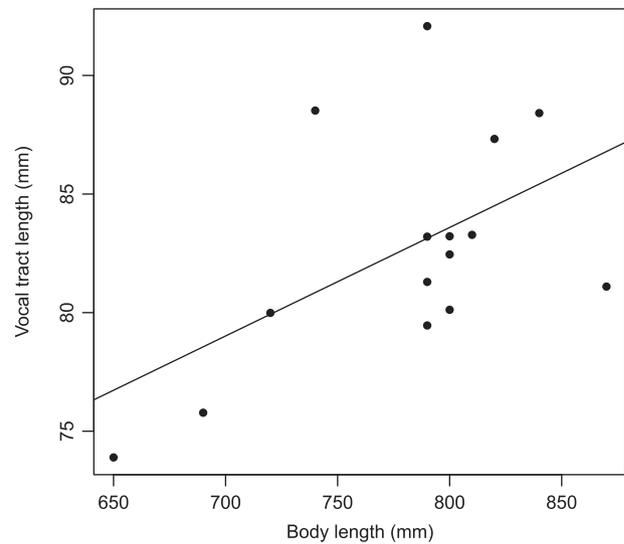


Figure 2. Plot of body length and VTL. The best fit line resulting from the significant correlation is included for purely illustrative purposes.

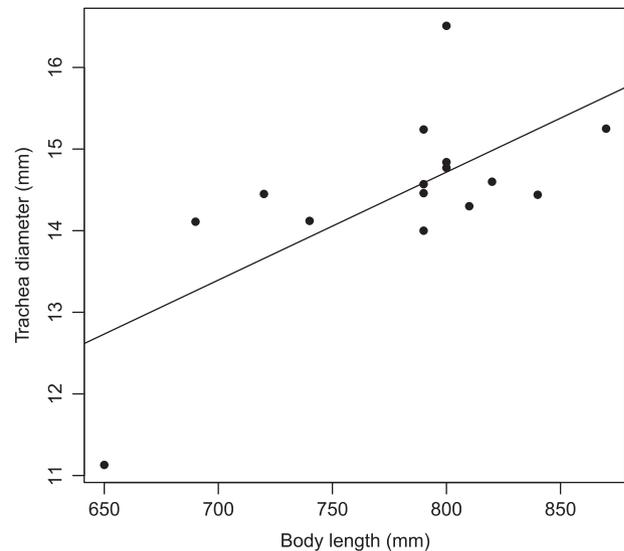


Figure 3. Correlation of body length and diameter of the trachea. The best fit line resulting from the significant correlation is included for purely illustrative purposes.

sound source, combining these with our own findings (stated in the previous paragraph), we suggest that filter-related acoustic features making calls individually distinctive may be a partial by-product of animals' body length and VTL.

Previous research found that source-related features also encode individual signatures in vocalizations (Khan et al. 2006; Sauvé et al. 2015a). In particular, F_0 was shown to decrease as body length increases in some harbor seal pups vocalizations (Sauvé et al. 2015a). However, a solid candidate for the physiological bases of this correlation, VFL, did not strongly or significantly correlate with body length in our sample (Table 2). This finding, and hence the partial inconsistency with previous literature, could be due to a number of factors. First, it could be a type II error: we failed, with our data, to detect an existing effect. Second, previous research might have run into a type I error, that is, a false positive. This seems unlikely, as a positive relation between source-related acoustic parameters and

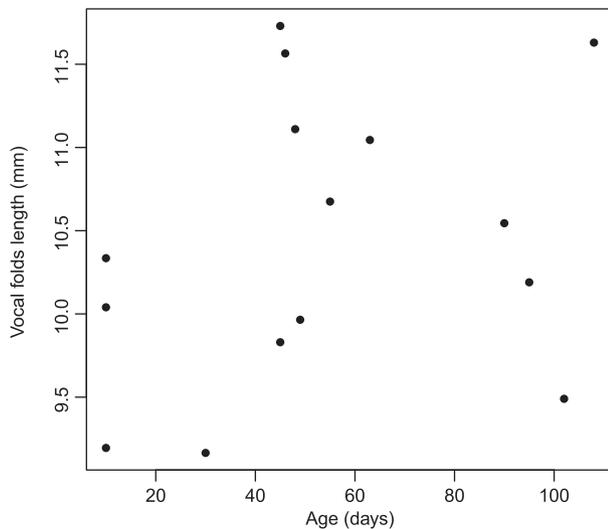


Figure 4. Plot of estimated age and vocal folds length.

body size appears robust both within and between studies (Khan et al. 2006; Sauvé et al. 2015a). Third, the mismatch could be quantitative rather than qualitative. The reason behind this observation lies in the anatomy of mammal vocal production (Reby and McComb 2003; Pisanski et al. 2014). While laryngeal dimensions are not strongly limited by their soft surrounding structures (Fitch and Hauser 1995), the vocal tract is more anatomically constrained as mostly encompassed by the skull, whose dimensions correlate positively with body size (Fitch 2000b). Formants are thus a more reliable indicator of body size than F0 in various species (Reby and McComb 2003; Pfefferle et al. 2007; Charlton et al. 2011; Pisanski et al. 2014), which harbor seals likely join, given the anatomical correlations observed in this study. This framework does not exclude that, in some species, F0 could still predict body size, although it will do so less reliably than formants. As our correlations were intended to be exploratory and descriptive, their cut-off *P* values have limited strength. Hence, finding a higher correlation between VTL and body length than VFL and body length may be considered consistent with the theoretical framework predicting prominence of formants over F0. Fourth, our mismatch with previous research could be due to other static features of vocal folds not measured here, such as tissue composition or tensile stress (Titze 2000). Fifth, individual distinctiveness of F0 found in other studies may be produced by dynamic reconfiguration of the vocal folds *in vivo*, rather than their anatomy *in vitro*. Sixth, together with a decrease in F0 with increasing body length (Sauvé et al. 2015a), previous research found male pups have a higher F0 than female pups, which seems counterintuitive. The interaction between these 2 factors, individual distinctiveness and sexual dimorphism, might have resulted in the lack of correlation we find here between VFL and body length (due to insufficient statistical power required for this more structured hypothesis). Seventh, both studies reporting a statistical effect of body size on F0 (Khan et al. 2006; Sauvé et al. 2015a) analyzed calls of pups within 42 days of age. However, the mean and median ages of the seals in our data are higher than this (Table 1). This suggests that previous findings can only be applied to few data points in our sample (see age distribution in Figure 4). It might be that the growth rate of seals' vocal folds is linear during the first weeks of life (in principle consistent with Khan et al. 2006 and Sauvé et al. 2015a), but then reaches a plateau, also explaining the lack of correlation

found here (see Figure 4). Eight, individuality in F0 might be found elsewhere in the lower vocal tract, somewhere beyond the vocal folds. This suggestion derives from the observation that other pinniped species use sub-laryngeal mechanisms for sound production (Boness and James 1979; Gailey-Phipps 1984; Miller and Job 1992; Terhune et al. 1994; Tyack and Miller 2002). This could also be true for harbor seals. To sum up, the reasons why our results are contradictory to the previously found correlation of F0 and body length can be caused by a large number of factors. Other factors affect sound production beyond the anatomical structures investigated here. For one, our dataset cannot capture dynamic variations of vocal folds and vocal tract, which nonetheless constitute an important dimension of sound production.

We find a strong correlation between trachea diameter and body length (Figure 3). This is, to our knowledge, the first finding of an allometric relationship between body length and trachea diameter in non-human animals. Together with the involvement of the trachea, contradicting results exist on whether harbor seals' vocalization exhibit similar (Sauvé et al. 2015a) or different (Perry and Renouf 1988) acoustic features between aerial and underwater production. On the one hand, this allows us to tentatively apply our findings to underwater natural communication. On the other hand, this partial matching raises the question of which additional mechanisms might be used in underwater vocalizations. Amphibious calls in harbor seals have an underwater component, likely radiated through the tissues close to the larynx (Renouf 1984) and the animal's neck (Sauvé et al. 2015a). This, together with the possibility that these animals might be re-circulating and re-using air while vocalizing underwater, brings as to speculate about the role of the trachea as a third component possibly affecting vocalizations in this species. Here, we show that trachea diameter strongly predicts body size. The trachea could affect sound production, and hence the correlations found elsewhere between acoustic traits and age (Khan et al. 2006) or body length (Sauvé et al. 2015a). Mechanistically, trachea diameter partially predicts turbulence for large airflows (Van den Berg et al. 1957), and hence trachea diameter may predict spectral characteristics of the emitted signal (at least in humans for which these studies have been performed). Similarly, in humans, body height correlates with tracheal sounds (Sanchez and Pasterkamp 1993). Comparatively, a large number of phocid pinnipeds may use the trachea for sound production (Piérard 1969; Bryden and Felts 1974; Boness and James 1979; Burns 1981; Ray 1981; Gailey-Phipps 1984; Miller and Job 1992; Terhune et al. 1994; Tyack and Miller 2002). The re-use of tracheal structures for phonation might be a by-product of functional adaptations of the respiratory tract to diving (Kooyman and Andersen 1969; Bryden and Felts 1974; Kooyman 1981; Ray 1981; Tyack and Miller 2002). Our data show that an anatomical structure, the trachea, has the potential to convey information about the caller's body size if used in phonation. Future research in harbor seals should investigate the involvement of the trachea in underwater vocalizations, and how different tracheal dimensions relate to potential acoustic information on body size.

Can our results be extrapolated to a broader age range? Here, we only provide a snapshot of harbor seals' vocal anatomy during early development: We show that for a limited age cohort VTL correlates with body size. In mammals, VTL depends on body growth rates (Fitch and Hauser 2003; Sauvé et al. 2015a), so we would predict its increase until the onset of adulthood. Conversely, the larynx might, depending on the species, be constrained over the lifespan (Fitch 1997), but experience a sudden change under the influence of sex steroids during particular life periods [and possibly differentially

between males and females (Sauvé et al. 2015a)]. Sex hormones have indeed been shown to act upon laryngeal development and the visco-elastic properties of vocal fold tissue (Beckford et al. 1985; Abitbol et al. 1999; Fitch and Hauser 2003) and suggested to affect laryngeal descent at puberty in human males (Fitch and Giedd 1999). Acoustic analyses of harbor seal pups found source-related acoustic differences between males and females calls (Khan et al. 2006; Sauvé et al. 2015a). As our sample was not balanced across sexes and ages, we did not test for the effect of either of those factors on vocal anatomy. Future research investigating vocal tract anatomy in harbor seals should therefore use larger samples to test for differences in VFL and VTL between males and females matched in body length and age. Only then will we be able to test the hypothesis that hormones act differently on vocal apparatuses depending on age, sex, and sizes.

Pinnipeds intriguingly exhibit a large variability in (i) socio-ecological factors (Riedman 1990), (ii) vocal production learning capacities (Reichmuth and Casey 2014), and (iii) laryngeal configuration, in particular the angle of the vocal folds with respect to the tracheal airstream (Schneider 1962). Adopting a broad comparative approach, future research should aim at increasing anatomical descriptions and understanding the relationship between anatomy and sound production, as these data are lacking for pinnipeds. Studies similar to ours in other pinniped species will enable systematically relating socio-ecology, vocal flexibility, and laryngeal configuration to possibly find common evolutionary patterns (Nowicki and Searcy 2014; Ravignani et al. 2016; Belyk and Brown 2017).

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