The Company of **Biologists**

SHORT COMMUNICATION

Lateralized sound production in the beluga whale (*Delphinapterus leucas*)

Audra E. Ames[1],*, Kristian Beedholm[2] and Peter T. Madsen[2]

ABSTRACT

Like other toothed whales, belugas produce sound through pneumatic actuation of two phonic lip pairs, but it is unclear whether both pairs are actuated concurrently to generate a single sound (the dual actuation hypothesis) or laterally in the production of their rich vocal repertoires. Here, using suction cup hydrophones on the head of a trained beluga whale, we measured seven different communication signal types and echolocation clicks in order to test the hypothesis that belugas produce distinct sounds unilaterally. We show that, like other delphinoids, belugas produce echolocation clicks with the right phonic lips and tonal sounds from the left. We also demonstrate for the first time that the left phonic lips are responsible for generating communication signals other than tonal sounds. Thus, our findings provide empirical support for functionalized laterality in delphinoid sound production, in keeping with the functional laterality hypothesis of vocal-motor control in toothed whales.

KEY WORDS: Phonic lips, Toothed whales, Echolocation, Communication, Lateral

INTRODUCTION

Beluga whales, *Delphinapterus leucas* (Pallas 1776), are renowned for their loquacious nature, producing a diverse repertoire of tonal and pulsed sounds for communication (e.g. Sjare and Smith, 1986; Karlsen et al., 2002; Chmelnitsky and Ferguson, 2012; Vergara and Mikus, 2019) and echolocation clicks for navigation and foraging (e.g. Rutenko and Vishnyakov, 2006; Roy et al., 2010). Like all delphinoids, belugas generate sounds in the nasal system through pneumatic actuation of two phonic lip pairs, located on the underside of the vestibular air sacs below the blowhole (Cranford et al., 1996; Cranford, 2000). The dual actuation hypothesis proposes that the two pairs work concomitantly in producing a single sound (Cranford et al., 1996, 2011; Cranford, 2000), but there is a growing body of evidence indicating that delphinoid sound production is in fact unilateral (e.g. Madsen et al., 2010, 2013).

Studies of other delphinoid species (harbor porpoises, *Phocoena phocoena*: Madsen et al., 2010; bottlenose dolphins, *Tursiops truncatus*: Dormer, 1979; MacKay and Liaw, 1981; Cranford et al., 2011; Madsen et al., 2013; Ridgway et al., 2015; and false killer whales, *Pseudorca crassidens*: Madsen et al., 2013) have shown that the right pair of phonic lips is responsible for the production of echolocation clicks while tonal sounds, when produced, originate from the left pair. A previous study on echolocation reported

findings that were inconsistent with such unilateral sound production within the beluga, however (Lammers and Castellote, 2009). The authors of this study interpreted the divergence of individual on-axis clicks into two separate clicks off-axis as evidence for dual actuation of the phonic lip pairs in click generation (Lammers and Castellote, 2009). Subsequent studies (Madsen et al., 2010, 2013) have since offered alternative explanations to these findings, thus prompting the need for direct measurement of beluga sound production to resolve these different interpretations.

We hypothesized that a trained female beluga would produce tonal sounds with her left set of phonic lips and echolocate with her right, consistent with sound production in other delphinoid species. Such unilateral signal production in bottlenose dolphins corresponds with structural asymmetries in the dolphin brain, suggesting that lateralized signal production and processing may be based on function: the functional laterality hypothesis (Ridgway et al., 2015; Wright et al., 2018). As such, we also investigated the production of pulsed sounds believed to be used for communication to determine whether these sounds were produced similarly to (1) echolocation clicks, an indicator of laterality in similar sound production, or (2) tonal sounds, an indicator of laterality in social sound production. Finally, we hypothesized that during biphonation production, mixed call pulsed components would originate from the right phonic lip pair while tonal components would simultaneously be generated from the left.

MATERIALS AND METHODS
Subject and sampling

Trials were conducted at Oceanogràfic (Valencia, Spain) with Yulka, the female beluga subject of the previous Lammers and Castellote (2009) study. Yulka was believed to be around 22 years of age at the time of the current study. At the start of each trial, Yulka was asked to station pool-side and submerge her head as trainers placed suction cup hydrophones in a configuration consisting of one hydrophone on each side of her head, aligned with the blowhole (and hence her phonic lip pairs), and a third hydrophone on the front of her melon (i.e. configuration A; Madsen et al., 2013). Hydrophones that were aligned with the blowhole (i.e. lateral hydrophones) were placed 15 cm from the medial nasal midline on either side of the head (Fig. 1). Once hydrophone placement was complete, Yulka was allowed to freely move her head during the remainder of the trial. A total of 12 trials were conducted on 11 days over a 3 week period (23 February to 14 March 2018), with one trial excluded later when it was determined by video inspection that hydrophone placement had been incorrect during recording.

Suction cup hydrophones were custom built and molded in degassed silicone with 50 mm diameter. Hydrophone sensitivity was -210 dB re. 1 V µPa^{-1}, and the hydrophones were connected to a custom-built conditioning box with 40 dB gain and band-pass filters between 1 and 160 kHz (one pole high-pass, four pole low-pass). Recordings were made with three channels of a National Instruments (USB-6356, Austin, TX, USA) box simultaneously

[1]Fundación Oceanogràfic de la Comunitat Valenciana, 46013 Valencia, Spain. [2]Zoophysiology, Department of Biology, Aarhus University, 8000 Aarhus, Denmark.

*Author for correspondence (aames@oceanografic.org)

A.E.A., 0000-0002-0760-9025
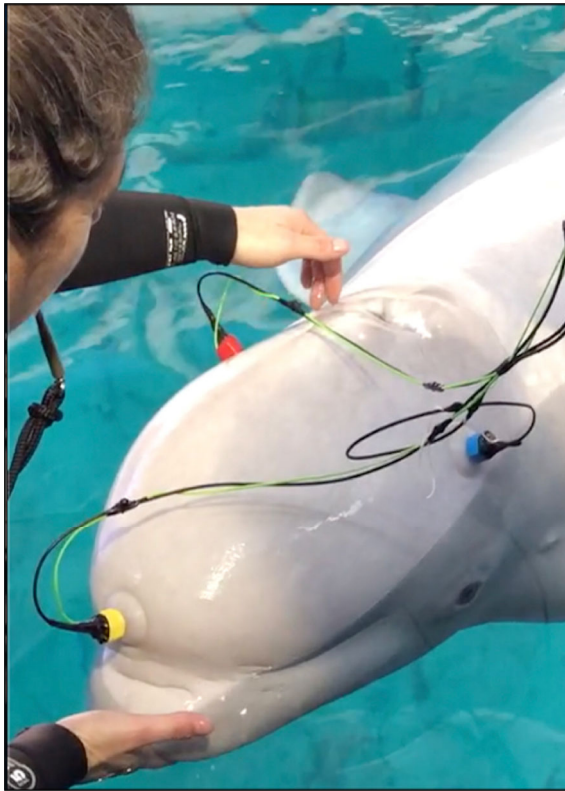
Fig. 1. Yulka receiving a hand signal. The image shows the configuration of the suction cup hydrophones (configuration A; Madsen et al., 2013): two lateral (aligned with the blowhole) and one on the melon. The hand signal featured is a light finger tapping motion adjacent to Yulka's blowhole requesting that she produce one of the sound types presented in the current study.

sampling 400 kHz per channel with 16-bit resolution, segmented into 30 s wave files written to disk.

This study was approved by the Animal Care and Welfare Committee of Oceanogràfic (project reference: OCE-15-18).

Sound types

Prior to the current study, Yulka was trained to produce seven sound types by reinforcing sounds from the naturally occurring vocal repertoire of Yulka's social group (Ames and Vergara, 2020) to cue with a food reward; each sound type had its own discriminatory hand signal. During trials, trainers presented Yulka with these hand signals, eliciting production of a series of the associated sound type (Fig. 1). Hand signals were given in varied order across sessions so that no two session orders were the same. Each of the different hand signals prompted production of one of the following sound types. (1) Burst pulse (BP): packets of individual pulses produced at a rapid rate of repetition (usually >200 pulses per second). (2) Pulse tone (PT): pulsed sounds of extremely high pulse repetition rate (1000–4000 pulses per second), resulting in visible sideband intervals (Watkins, 1967) and an aurally tonal sound. The aural tonal quality is harsher than the pure tone sound of beluga whistles. Modulation in the harmonic structure of these sounds is indicative of increasing or decreasing pulse repetition rate. (3) Pulse 1 (P1): a pulse train (a series of pulses produced with a definable repetition rate) determined to be non-echolocation based on the lower frequency limits of the pulse band (i.e. <20 kHz) and longer intra-pulse duration [see mean root mean square (rms) duration in Table 1] not usually characteristic of beluga echolocation clicks.

Additionally, these pulses accompany other communication sounds during social interactions of this beluga group. (4) Pulse 2 (P2): a second pulse train type also determined to be non-echolocation based on the above criteria for P1 pulses; however, the intra-pulse duration was slightly longer (Table 1). (5) Abbreviated tonal sweep series (ATSS; Ames and Vergara, 2020) 1: a series of truncated tonal sounds. (6) ATSS 2: similar to ATSS 1, but intra-tonal durations were slightly shorter (Table 1). (7) Mixed call (MC): unstereotyped biphonations composed of overlapping pulsed and tonal components (i.e. whistles with fundamental frequencies >5 kHz).

Echolocation was recorded as Yulka naturally produced echolocation clicks throughout each trial. Fig. S1 provides example spectra for each sound type.

Analysis

Signal detection and analyses were completed using a custom-written MATLAB script (2015b and 2018b, MathWorks, Natick, MA, USA). In order to yield signals with sufficient signal-to-noise ratios, detection thresholds were determined per signal type based on recordings from the melon-positioned hydrophone (Madsen et al., 2010, 2013). Detection thresholds of 10 dB below the maximum signal peak for echolocation clicks and 20 dB below the maximum for non-echolocation pulses provided the highest quality signals. For the remaining communication signals, detection thresholds per signal type were derived from first calculating the rms value of the entire 30 s wave file on the melon hydrophone and then multiplying the rms value by a constant threshold value determined to eliminate the most noise or low-quality signals for each sound type. These threshold values were 14 (ATSS 2), 5 (PT) and 7 (all other signals), and resulted in a relatively inclusive signal detection threshold for each sound type. Signal peaks that were detected above the threshold for each sound type were then extracted using a time window based on the general duration of the sound (determined from visual inspection of signal spectrograms in Raven Pro 1.5, Cornell Lab of Ornithology) in order to eliminate secondary peaks which may belong to the same signal. Finally, all detected signals were visually verified in MATLAB to eliminate false positives.

Signal time of arrival differences (TOADs, μs) were analyzed from the lateral hydrophones and evaluated differently based on the sound type. For transient signals, we formed the mean sound envelope for each lateral hydrophone as the arithmetic mean of the analytical envelopes of each individual signal. We then subtracted the onset of the right mean envelope from that of the left. The onset of the mean envelope was defined as the point in time when the mean sound envelope in a 100 μs interval crossed 50% of the range between the baseline level (ending 50 μs before the peak) and the peak of the mean envelope. This method resulted in a mean TOAD (mTOAD) for all pulses within a train or echolocation clicks within a 30 s wave file. During previous detection experiments (Madsen et al., 2013), two delphinoid species used echolocation inspection times lasting only a few seconds, so from the viewpoint of the animals, 30 s seems to be much longer than what could be considered an independent perceptual unit. TOADs for the remaining sound types (i.e. ATSS 1 and 2, BP and PT sounds) were evaluated by subtracting the peak sample of a signal's autocorrelation on the right hydrophone from the peak sample of the signal's cross-correlation between the right and left hydrophones with the right hydrophone as reference.

Jarque–Bera goodness of fit tests (MATLAB, 2018b) were used to determine normality in the distribution of the TOADs calculated for each sound type. Extreme values were considered a result of

Table 1. Summary table of descriptive and *t* statistics for each sound

Sound type	Detections (*N*)	mTOAD (*N*)	Mean rms duration (ms)[a]	Mean arrival difference (μs)[b]	Median arrival difference (μs)[b]	s.d.	*t*	d.f.	*P*
ATSS 1	41	–	52.1	−98.5	−90.0	296.7	−2.1	40	**0.040**
ATSS 2	210	–	3.2	−258.8	−230.0	278.7	−13.5	209	**<0.001**
P1	999	16	0.6	−8.9	−12.5	66.2	−0.5	15	0.599
P2	178	10	2.3	−52.0	−57.5	58.0	−2.8	9	**0.020**
BP	18	–	1.38E+03	−5.8	−1.3	26.3	−0.9	17	0.359
PT	32	–	103.6	−41.6	−5.0	69.6	−3.4	31	**0.002**
Echolocation clicks	11,473	56	0.1	17.6	17.5	7.3	18.2	55	**<0.001**
MC pulses	730	11	–	41.1	45.4	24.1	5.7	10	**<0.001**

ATSS, abbreviated tonal sweep series; P1 and P2, pulse 1 and 2; BP, burst pulse; PT, pulse tone; MC, mixed call pulses. [a]There was no rms duration calculated for individual mixed call pulses as calculations would have been skewed by the overlapping tonal component. [b]Negative values indicate arrival on the left hydrophone first, while positive values indicate arrival on the right first. Bold *P*-values indicate significance.

large variability between the cross-correlated real signals of communication sounds or peaks of the mean sound envelopes for transient signals and were consequently removed as outliers. One-sample (two-tailed) *t*-tests (MATLAB, 2018b) were used to determine whether the average TOAD for each sound type was significantly different from zero.

RESULTS AND DISCUSSION

A total of 13,681 sounds were analyzed. Table 1 summarizes descriptive and *t* statistics for each sound type. Jarque–Bera goodness of fit tests indicated that the distribution of TOADs for all sound types was normal. Table S1 provides outlying values that were removed.

Echolocation and tonal production

Lammers and Castellote (2009) concluded that Yulka produced two pulses, one at each phonic lip pair, that joined to form a single sonar click. Our findings with the same whale do not support that interpretation; we show that echolocation clicks were generated unilaterally by Yulka's right phonic lip pair. Echolocation clicks arrived first on the right hydrophone, and all but one mTOAD (which showed a zero difference in arrival time) fell in a range of 5 to 32.5 μs (Fig. 2), corresponding to a sound path that was 1–5 cm shorter. These values were well within the range of variability expected from small differences in hydrophone placement between sessions (Madsen et al., 2013) or variations in click origin along the right phonic fissure (Cranford et al., 2011). Thus, consistent with
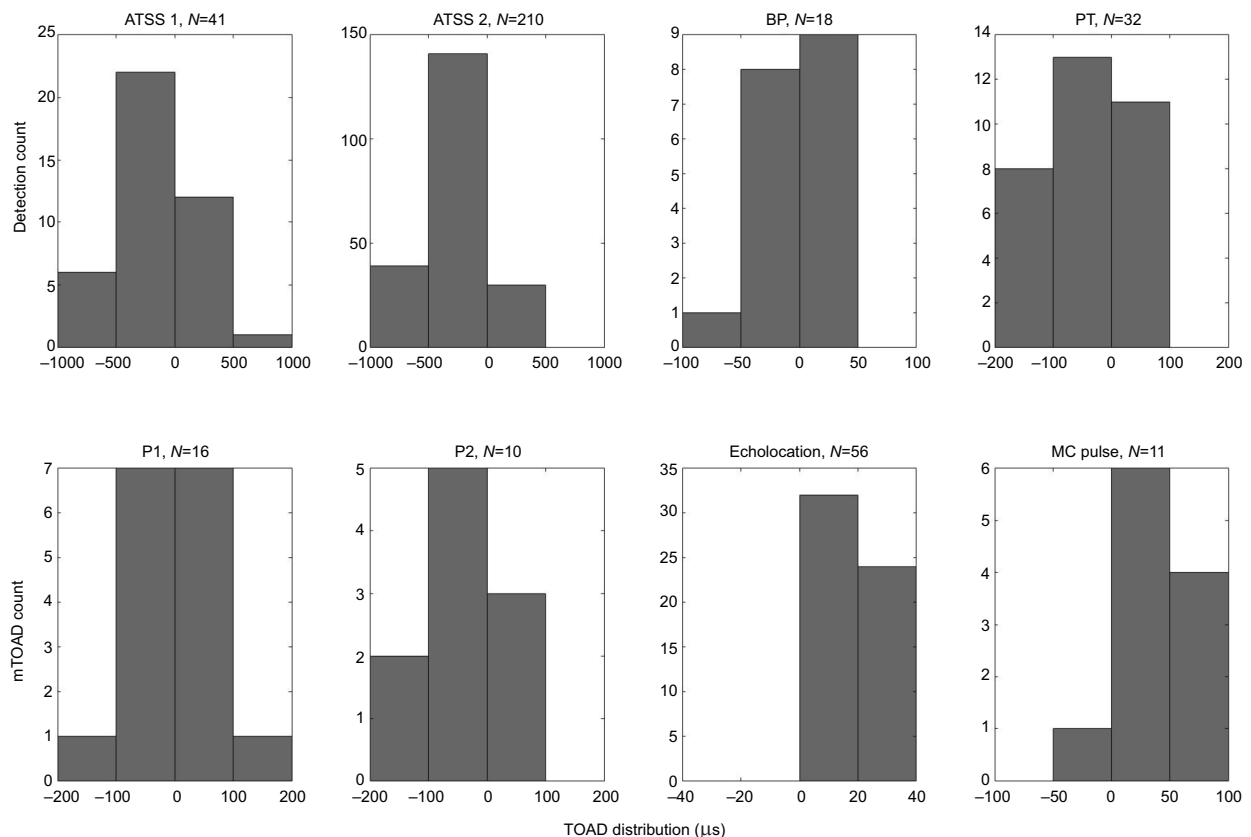
Fig. 2. Distribution of time of arrival differences (TOADs) per sound type. Positive values indicate arrival on the right hydrophone prior to the left. For non-transient signals, *N* is the count of individual signals detected for each sound type; for transient signals, *N* is the count of mean TOADS (mTOADs) calculated for each sound type. ATSS, abbreviated tonal sweep series; BP, burst pulse; PT, pulse tone; P1 and P2, pulse 1 and 2; MC, mixed call pulses.

other delphinoid species studied with similar methods (e.g. Madsen et al., 2010, 2013), we found that beluga echolocation clicks, also with bi-modal spectra (Fig. S2), are produced unilaterally by the right phonic lip pair, supporting the growing notion of this being a commonality (or perhaps even a universality, we posit) in toothed whale sound production.

Our study also corroborates findings of unilateral tonal production in other delphinoid species (Dormer, 1979; MacKay and Liaw, 1981; Cranford et al., 2011; Madsen et al., 2013; Ridgway et al., 2015) as we found that Yulka predominantly produced tonal sounds with her left phonic lip pair. TOADs between the left and right hydrophone were significant for both tonal series types (i.e. ATSS 1 and 2). Fig. 2 illustrates the distribution of TOADs per sound type.

Pulsed sound production

PT and P2 sounds showed clear unilateral production, arriving on the left hydrophone significantly prior to the right. PT production from the left phonic lips is congruent with movement on the left side of the blowhole described by Ridgway and Carder (1988) during beluga 'whistle' production, as the 'whistle' depicted in Ridgway and Carder's (1988) study appears structurally similar to defined PTs in the current study. Thus, it seems that sounds with aurally tonal presentation are produced predominantly from the left phonic lips in addition to other social pulsed sounds.

Unfortunately, BP and P1 sounds did not exhibit consistent arrival on either lateral hydrophone, so we were unable to determine the phonic lip pair responsible for generating these sound types as a result. Mean and median difference values for both sound types indicated production from the left phonic lips, but first arrival of these sounds on either hydrophone was no better than chance overall. We interpret these results to be a limitation of the methods we used to analyze these sounds and not an indication of a lack of laterality in sound production, however. While the analyses we employed in this study are the most effective tools for determining time delays (Madsen et al., 2010), they often yielded TOADs that were greater than what could be biologically possible (Table 1). For example, TOADs for ATSS 1 and 2 were far longer than expected, but the cross-correlation function of tonal sounds is inherently broad, offering very noisy estimates of TOADs as a result of their narrow-band, albeit shallowly frequency modulated characteristics. Despite the broad distributions of TOADs for the sound types featured in this study, our methods of analysis were still accurate in statistically resolving the side of origin for all sounds except for BP and P1 communication pulses. Thus, alternative methods, such as endoscopy, glottography, ultrasound Doppler or accelerometry seem necessary in determining whether all pulsed sounds that belugas produce socially originate from the left phonic lip pair. Additional testing of other belugas would also help to elucidate whether Yulka's laterality is consistent for this species.

MC production

Pulse production defaulted to the right phonic lip pair when pulses accompanied a tonal component. During MC production, pulse mTOADs showed arrival on the right hydrophone prior to the left. Unfortunately, laterality in MC tonal production could not be adequately evaluated by the cross-correlation method we employed for tonal signal analyses. It was not possible to measure the delay in the tonal peak sample between hydrophones as MC peaks were influenced by the pulsed component. We infer, however, that Yulka likely produced the tonal component of her MCs from the left phonic lips as pulse production originated in the right and both

phonic lip sets are likely employed during biphonation emission (e.g. Murray et al., 1998; Cranford et al., 2011; Madsen et al., 2013).

Conclusion

We found that the studied beluga unilaterally produced echolocation and tonal signals, contrary to what was originally reported for sound production in this beluga (Lammers and Castellote, 2009). In bottlenose dolphins, unilateral sound production of these signals corresponds with structural asymmetries in the brain, suggesting a lateralization of signal production and processing based on function (Ridgway et al., 2015; Wright et al., 2018). We also demonstrate for the first time that the left phonic lips are responsible for generating communication signals other than tonal sounds, offering further support for the functional laterality hypothesis in delphinoid sound production. The complexities of some delphinoid vocal repertoires like the beluga's and the rigidity of functionalized laterality deserve further exploration, however, as pulse generation by the left phonic lip pair also shows that both phonic lip pairs are capable of broadband sound production, and likely narrowband sound production as well (Murray et al., 1998; Cranford et al., 2011; Madsen et al., 2010, 2011, 2013). It is possible that similar signals provide alternative information to a signal recipient when produced variably along the phonic fissure or by the opposing pair of phonic lips. Future studies of signal production and processing involving contextual clues or behavioral responses from signal recipients could lead to exciting revelations regarding specific signal parameters or the use of particular sounds in delphinoid communication.

References

Ames, A. E. and Vergara, V. (2020). Trajectories of vocal repertoire development in beluga (Delphinapterus leucas) calves: insights from studies a decade apart. *Aquat. Mamm.* **46**, 344-366. doi:10.1578/AM.46.4.2020.344

Chmelnitsky, E. G. and Ferguson, S. H. (2012). Beluga whale, *Delphinapterus leucas*, vocalizations from the Churchill River, Manitoba, Canada. *J. Acoust. Soc. Am.* **131**, 4821-4835. doi:10.1121/1.4707501

Cranford, T. W. (2000). In search of impulse sound sources in odontocetes. In *Hearing by Whales and Dolphins* (ed. W. W. L. Au, A. N. Popper and R. R. Fay), pp. 109-155. New York, NY: Springer Verlag.

Cranford, T. W., Amundin, M. and Norris, K. S. (1996). Functional morphology and homology in the odontocete nasal complex: implications for sound generation. *J. Morphol.* **228**, 223-285. doi:10.1002/(SICI)1097-4687(199606)228:3<223:: AID-JMOR1>3.0.CO;2-3

Cranford, T. W., Elsberry, W. R., Van Bonn, W. G., Jeffress, J. A., Chaplin, M. S., Blackwood, D. J., Carder, D. A., Kamolnick, T., Todd, M. A. and Ridgway, S. H. (2011). Observation and analysis of sonar signal generation in the bottlenose

dolphin (Tursiops truncatus): evidence for two sonar sources. *J. Exp. Mar. Biol. Ecol.* **407**, 81-96. doi:10.1016/j.jembe.2011.07.010

Dormer, K. J. (1979). Mechanism of sound production and air recycling in delphinids: cineradiographic evidence. *J. Acoust. Soc. Am.* **65**, 229-239. doi:10.1121/1.382240

Karlsen, J., Bisther, A., Lydersen, C., Haug, T. and Kovacs, K. (2002). Summer vocalisations of adult male white whales (*Delphinapterus leucas*) in Svalbard, Norway. *Polar Biol.* **25**, 808-817. doi:10.1007/s00300-002-0415-6

Lammers, M. O. and Castellote, M. (2009). The beluga whale produces two pulses to form its sonar signal. *Biol. Lett.* **5**, 297-301. doi:10.1098/rsbl.2008.0782

Mackay, R. S. and Liaw, H. M. (1981). Dolphin vocalization mechanisms. *Science* **212**, 676-678. doi:10.1126/science.212.4495.676

Madsen, P. T., Wisniewska, D. and Beedholm, K. (2010). Single source sound production and dynamic beam formation in echolocating harbour porpoises (Phocoena phocoena). *J. Exp. Biol.* **213**, 3105-3110. doi:10.1242/jeb.044420

Madsen, P. T., Wisniewska, D. M. and Beedholm, K. (2011). Response to 'biosonar sources in odontocetes: considering structure and function'. *J. Exp. Biol.* **214**, 1404-1405. doi:10.1242/jeb.055798

Madsen, P. T., Lammers, M., Wisniewska, D. and Beedholm, K. (2013). Nasal sound production in echolocating delphinids (Tursiops truncatus and Pseudorca crassidens) is dynamic, but unilateral: clicking on the right side and whistling on the left side. *J. Exp. Biol.* **216**, 4091-4102. doi:10.1242/jeb.091306

Murray, S. O., Mercado, E. and Roitblat, H. L. (1998). Characterizing the graded structure of false killer whale (Pseudorca crassidens) vocalizations. *J. Acoust. Soc. Am.* **104**, 1679-1688. doi:10.1121/1.424380

Ridgway, S. H. and Carder, D. A. (1988). Nasal pressure and sound production in an echolocating white whale, *Delphinapterus leucas*. In *Animal Sonar: Processes and Performance* (ed. P. Nachtigall and P. Moore), pp. 53-60. New York, NY: Plenum.

Ridgway, S., Dibble, D. S., Van Alstyne, K. and Price, D. (2015). On doing two things at once: dolphin brain and nose coordinate sonar clicks, buzzes and emotional squeals with social sounds during fish capture. *J. Exp. Biol.* **218**, 3987-3995. doi:10.1242/jeb.130559

Roy, N., Simard, Y. and Gervaise, C. (2010). 3D tracking of foraging belugas from their clicks: Experiment from a coastal hydrophone array. *Appl. Acoust.* **71**, 1050-1056. doi:10.1016/j.apacoust.2010.05.008

Rutenko, A. N. and Vishnyakov, A. A. (2006). Time sequences of sonar signals generated by a beluga whale when locating underwater objects. *Acoust. Phys.* **52**, 314-323. doi:10.1134/S1063771006030122

Sjare, B. L. and Smith, T. G. (1986). The vocal repertoire of white whales, *Delphinapterus leucas*, summering in Cunningham Inlet, Northwest Territories. *Can. J. Zool.* **64**, 407-415. doi:10.1139/z86-063

Vergara, V. and Mikus, M. A. (2019). Contact call diversity in natural beluga entrapments in an Arctic estuary: Preliminary evidence of vocal signatures in wild belugas. *Mar. Mammal Sci.* **35**, 434-465. doi:10.1111/mms.12538

Watkins, W. A., (1967). The harmonic interval: fact or artifact in spectral analysis of pulse trains. In *Marine Bio-Acoustics* (ed. W. N. Tavolga), pp. 15-43. Oxford, UK: Pergamon Press.

Wright, A. K., Theilmann, R. J., Ridgway, S. H. and Scadeng, M. (2018). Diffusion tractography reveals pervasive asymmetry of cerebral white matter tracts in the bottlenose dolphin (*Tursiops truncatus*). *Brain Struct. Funct.* **223**, 1697-1711. doi:10.1007/s00429-017-1525-9