Assessing auditory evoked potentials of wild harbor porpoises (*Phocoena phocoena*)

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Testing the hearing abilities of marine mammals under water is a challenging task. Sample sizes are usually low, thus limiting the ability to generalize findings of susceptibility towards noise influences. A method to measure harbor porpoise hearing thresholds *in situ* in outdoor conditions using auditory steady state responses of the brainstem was developed and tested. The method was used on 15 live-stranded animals from the North Sea during rehabilitation, shortly before release into the wild, and on 12 wild animals incidentally caught in pound nets in Denmark (inner Danish waters). Results indicated that although the variability between individuals is wide, the shape of the hearing curve is generally similar to previously published results from behavioral trials. Using 10-kHz frequency intervals between 10 and 160 kHz, best hearing was found between 120 and 130 kHz. Additional testing using one-third octave frequency intervals (from 16 to 160 kHz) allowed for a much faster hearing assessment, but eliminated the fine scale threshold characteristics. For further investigations, the method will be used to better understand the factors influencing sensitivity differences across individuals and to establish population-level parameters describing hearing abilities of harbor porpoises. © *2016 Acoustical Society of America*. [http://dx.doi.org/10.1121/1.4955306]

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I. INTRODUCTION

Anthropogenic noise has received much attention as a potential factor negatively affecting marine fauna (Huddleston, 2010; Slabbekoorn *et al.*, 2010). In Europe, the most significant contributions of anthropogenic noise originate from shipping, seismic exploration, dredging, military exercises (Wright *et al.*, 2013) and, in recent years, pile driving for offshore wind farms (Götz *et al.*, 2009). The presence of high-amplitude impulsive sounds, common to pile driving and reflection seismology in northern Europe, has triggered a number of studies to assess the potential impact of ongoing construction work of offshore wind farms on harbor porpoises (*Phocoena phocoena*), an ubiquitous marine mammal species in these waters (e.g., Brandt *et al.*, 2011; Dähne *et al.*, 2013; Dähne *et al.*, 2014; Kastelein *et al.*, 2012a; Kastelein *et al.*, 2013b; Tougaard *et al.*, 2009a). Two general categories of impacts are of major concern: direct damage to the auditory system by intense sound in the vicinity of active pile driving (Lucke *et al.*, 2009; Tougaard *et al.*, 2015); and disturbance effects that potentially lead to behavioral alterations such as stress, loss of foraging opportunities or reduced foraging efficiency, disruption of social or breeding behavior, and other possible responses (Dähne *et al.*, 2014).

Early assessments of the hearing abilities of harbor porpoises using behavioral methods revealed that this species hears best between 8 and 32 kHz and that sensitivity declines sharply between 140 and 150 kHz (Andersen, 1970). Over 30 yr later, the hearing ability of a harbor porpoise in human care was reassessed by using psychoacoustic methods, to aid the design of acoustic alarms meant to prevent porpoise bycatch in gillnets (Kastelein *et al.*, 2010). Porpoise hearing was also assessed using electrophysiological methods (Bibikov, 1992; Popov *et al.*, 1986). In these studies, hearing sensitivity was found to be more sensitive around 130 kHz,

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at a much higher frequency range than previously reported by Andersen (1970). Thus, only a low number of hearing measurements have been made so far on harbor porpoises. The methods and results vary considerably between the studies making it difficult to determine whether or not the collective hearing threshold data is representative of harbor porpoises as a species. This subsequently impedes the ability of regulators to confidently target frequencies of sound most likely to affect harbor porpoises. Furthermore, how hearing abilities vary with age, gender, and across individuals is not fully understood, yet it is fundamental to understanding the potential impact of anthropogenic induced sound on harbor porpoise populations.

Psychophysical studies conducted with marine mammals typically rely on a limited number of animal subjects. This is due to the small number of animals in human care available for research, as well as the amount of time required for training the specific behaviors required for such studies. The limited number of marine mammals that can be tested via psychophysical means make it difficult to account for variability on population-level (e.g., age, gender, etc.). Electrophysiological hearing tests can be performed in many marine mammals with little to no training and the hearing test data can be collected quickly (minutes to hours). The approach has provided good estimates for the best frequency range of hearing when compared to behavioral assessments in the same bottlenose dolphin subjects (*Tursiops truncatus*; Houser and Finneran, 2006a; Schlundt *et al.*, 2007). However, there are differences in sensitivity estimates (or thresholds) with the greatest differences typically occurring at the highest and lowest limits of the frequency range of hearing. Furthermore, the magnitude of the differences may vary with the methods used (Finneran and Houser, 2006; Houser and Finneran, 2006a; Schlundt *et al.*, 2007; Yuen *et al.*, 2005). Nevertheless, electrophysiological approaches have enabled large scale studies to be undertaken in odontocete species that demonstrate population-level variability in hearing, including changes in hearing sensitivity and the frequency range of hearing associated with age and gender (Houser and Finneran, 2006b; Popov *et al.*, 2005).

In the study described here, electrophysiological measurements of hearing were made on 27 harbor porpoises with the goal of better quantifying variation in the range of hearing and hearing sensitivity in this species. Testing was done opportunistically, utilizing porpoises either incidentally caught in pound nets or animals undergoing rehabilitation following a live stranding. Trials were conducted on animals from the North and Baltic Seas, regions that are subject to increased anthropogenic noise activity due to wind farm construction and operation, shipping, and seismic exploration.

II. MATERIALS AND METHODS

Electrophysiological measurements of hearing abilities in harbor porpoises were performed on wild animals from the inner Danish waters (Fig. 1), which were incidentally caught in Danish pound nets, and on live-stranded animals rehabilitated at the SOS Dolfijn (Harderwijk, Netherlands). For each animal an electrophysiological procedure in which auditory evoked potentials (AEPs) were recorded in response to varying levels of acoustic stimuli was performed.

A. Animal subjects and study locations

The locations where wild porpoises were caught in static pound nets were spread along the inner Danish waters, within the range of the population residing in the Belt Sea and adjacent waters (Fig. 1). The animals can swim freely in those pound nets. When discovered, Danish fisherman reported the presence of a by-caught porpoise to the investigators at Aarhus University. Upon notification, equipment and personnel were gathered and the research team travelled to the site of the pound net. The earliest arrival at the study site was approximately 5 h after the notification that an animal had been caught, the latest after 24 h, depending on

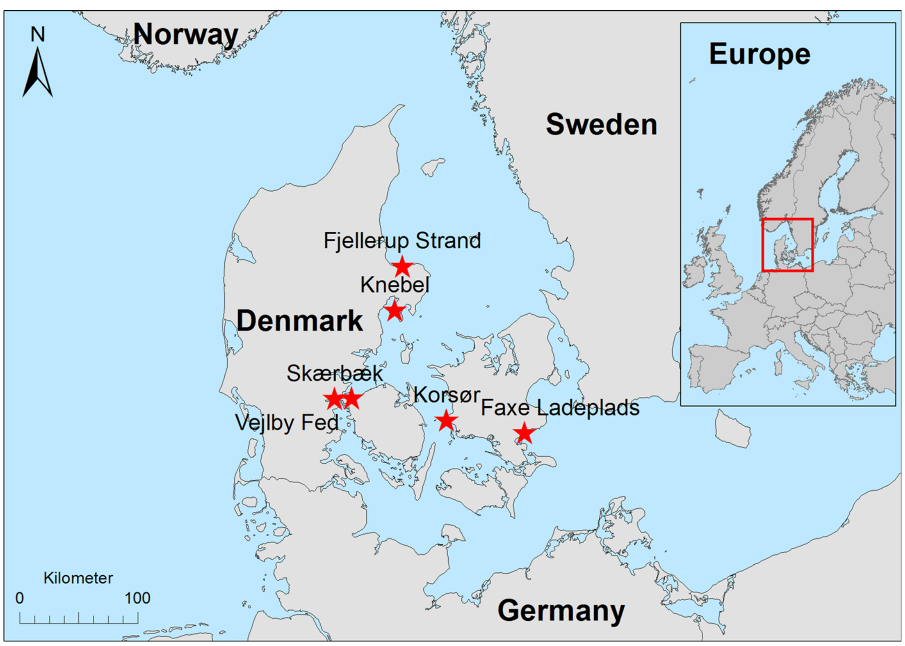

FIG. 1. (Color online) Locations of porpoise ABR-hearing trials in the inner Danish waters.

TABLE I. Overview of porpoises assessed in the inner Danish waters. Age was estimated using methods described in Benke *et al.* (1998).

Date	Id	Location	Gender	Weight (kg)	Length (cm)	Age estimated
Jul. 2011	wild_01	Fjellerup	male	24	117	subadult
Jul. 2011	wild_02	Fjellerup	female	28	128	subadult
Aug. 2011	wild_03	Knebel	female	44	141	adult
Sep. 2011	wild_04	Knebel	female	39	147	adult
Aug. 2012	wild_05	Skærbæk	male	39	145	adult
Apr. 2013	wild_06	Korsør	male	54	141	adult
May 2013	wild_07	Korsør	male	51	149	adult
May 2013	wild_08	Skærbæk	male	31	116	subadult
Aug. 2013	wild_09	Faxe	male	38	146	adult
Mar. 2014	wild_10	Vejlby Fed	female	36	122	subadult
Apr. 2014	wild_11	Fjellerup	male	19	115	subadult
Apr. 2014	wild_12	Fjellerup	male	36	127	subadult

daylight, wind, wave height, and availability of personnel. After arrival of the team, the bottom of the net was lifted up and the porpoise was placed on the fisherman's boat for an initial health check by the attending veterinarian. Standard biological parameters such as length and weight were recorded and blood and blow samples were collected. A good health condition determined by the veterinarian was required before a porpoise was placed into the custom-built stretcher used for the hearing test. Throughout the health assessment and the following hearing tests, the health condition of the porpoise was continuously monitored by the attending veterinarian. Weather was a limiting factor for experiments in the wild; strong wind and waves as well as rain often made hearing tests impossible and not all by-caught animals were used in hearing test procedures. From 2011 until 2014, 12 harbor porpoises were fully assessed (Table I).

Measurements on rehabilitating porpoises were performed at the SOS Dolfijn in Harderwijk as part of standard medical evaluations conducted prior to the animals being released in the North Sea. In principle, the experimental set-up was the same as in the wild, the distance of the transducers presenting the stimulus to the porpoise and the position of the hydrophone to control the sound pressure level (SPL) were similar. The animal was held by a caretaker during the measurement procedure; the caretaker stood lateral to the animal and out of the direct sound path from the transducer to the animal. From 2012 to 2014, the hearing ability of 15 rehabilitated harbor porpoises was assessed (Table II).

TABLE II. Overview of porpoises assessed in rehabilitation at the SOS Dolfijn (Harderwijk, NL). Age was estimated using methods described in Benke *et al.* (1998).

Date	Id	Gender	Weight (kg)	Length (cm)	Age estimated
Apr. 2012	rehab_01	female	22	105	subadult
Apr. 2012	rehab_02	male	27	112	subadult
Jun. 2012	rehab_03	female	31	121	subadult
Jun. 2012	rehab_04	male	22	103	subadult
Jun. 2012	rehab_05	female	27	116	subadult
Jun. 2012	rehab_06	male	20	100	subadult
May 2013	rehab_07	female	31	125	subadult
May 2013	rehab_08	female	50	148	adult
May 2013	rehab_09	male	23	107	subadult
May 2013	rehab_10	female	31	124	subadult
Jun. 2014	rehab_11	male	28	108	subadult
Jun. 2014	rehab_12	male	27	115	subadult
Jun. 2014	rehab_13	male	24	108	subadult
Jun. 2014	rehab_14	female	32	125	subadult
Jun. 2014	rehab_15	male	46	146	adult

B. Experimental setup and stimulus presentation

The setup for the measurements in the wild and at the rehabilitation center was similar. In both locations, the porpoise was kept at the surface so that the blowhole was just above the water surface to allow the porpoise to breathe freely. The stimulus sound projector was placed 1 m in front of the porpoise at a depth of 50 cm (Fig. 2). The receiving hydrophone, used to determine the actual SPL at the porpoise, was placed 30 cm lateral to the middle of the lower jaw. This was as close to the porpoise that the hydrophone could be placed without causing irritation and stress to the animal. At the SOS Dolfijn, all anticipated sources of acoustic and electromagnetic interference under facility control (e.g., lights, pumps) were turned off during the trials.

Sinusoidal amplitude modulated (SAM) tones were used as stimuli to produce an auditory steady state response (ASSR), the amplitude and phase of which was used in the hearing threshold determination. Stimuli were digitally generated with a Panasonic Toughbook CF30, converted to analog with a 1 MHz update rate and 16-bit resolution (NI USB 6251, National Instruments, USA), band pass filtered (100 Hz–250 kHz, 24 dB/octave; Krohn-Hite, USA), and attenuated before being applied to a TC4033 transducer (Teledyne Reson, DK) in the frequency range from 10 to 160 kHz. Stimulus levels were manipulated using a combination of a digitally controlled analog attenuator (0–70 dB in

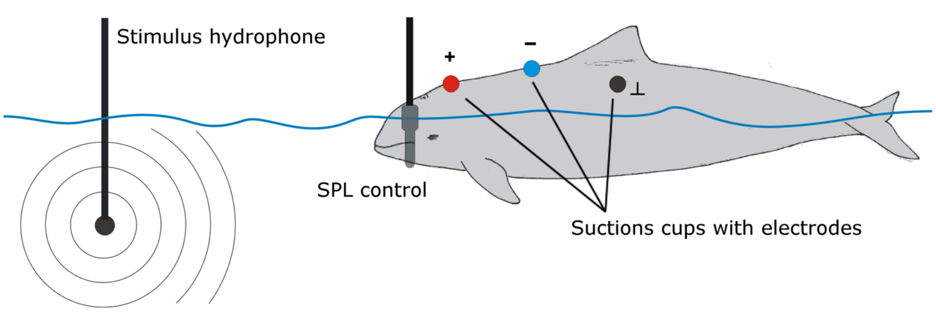

FIG. 2. (Color online) Experimental setup.

10 dB steps) and varying the voltage output of the USB-6251. Each test stimulus consisted of four SAM tones presented simultaneously with different amplitude modulation (AM) rates (Finneran and Houser, 2007), and are thus termed a 4-component SAM stimulus (4-SAM). SAM tones elicit an ASSR, which is a periodic neural signal that occurs at the frequency of amplitude modulation. The ASSR may be analyzed in the frequency domain using established techniques for objective, statistically based response detection methods (Dobie and Wilson, 1996; Stapells *et al.,* 1987).

When multiple SAM tones are combined, the ASSR of each carrier frequency (tone) can be independently analyzed providing different modulation rates (see Table III for the AM frequencies used) are used for each. Thus, as has been performed with bottlenose dolphins, amplitude modulation rates of the individual carrier frequencies within a 4-SAM were varied so that component signals could be individually analyzed within the frequency domain (Finneran and Houser, 2007). The AM frequencies used here are based on different studies on the so-called modulation rate transfer function (Linnenschmidt *et al.,* 2013; Lucke, 2008; Lucke *et al.,* 2007). Each carrier frequency within a 4-SAM stimulus was 100% amplitude modulated. Signals were 60 ms in duration, including a 1-ms cosine envelope rise and fall with a total epoch length of 71 ms (i.e., 9 ms of silence followed each stimulus).

When the investigations started in 2011, hearing thresholds were obtained by conducting four measurements, each with different 4-SAM stimuli (Table III, Set A, starting 2011). The tested frequencies within a 4-SAM stimulus were separated by 40 kHz. Between the four 4-SAM stimuli used, differences in the lowest frequency tested differed by integer multiples of 10 kHz. This allowed the hearing range between 10 and 160 kHz to be covered in 10 kHz steps. A total of 12 porpoises were tested using this configuration of stimuli (six at the SOS Dolfijn, six in the wild). As testing progressed it was deemed necessary to reduce the time required for the procedure and the combination of frequencies constituting the 4-SAM stimuli was changed to octave steps (when appropriate and feasible) in 2013. This allowed for testing one-third octave band intervals covering the frequency range of interest with only three different 4-SAM stimuli (Table III). A total of 15 porpoises were tested with this SAM stimulus configuration (nine at the SOS, six in the wild).

TABLE III. Test frequencies and associated amplitude modulation (AM) rates for 4-component SAM stimuli in the used hearing threshold assessment. The frequency spacing of the component frequencies were changed closest to octave steps starting in 2013 to decrease the data collection time necessary for audiogram determination.

Set A Starting 2011	Test frequencies (kHz)	AM rates (kHz)	Set B Starting 2013	Test frequencies (kHz)	AM rates (kHz)
SAM stimulus #1	**10**	0.90	SAM stimulus #1	**16**	1.10
	50	1.10		**32**	1.15
	90	1.16		**64**	1.20
	130	1.23		**128**	1.25
SAM stimulus #2	**20**	0.90	SAM stimulus #2	**20**	1.10
	60	1.10		**40**	1.15
	100	1.16		**80**	1.20
	140	1.23		**150**	1.25
SAM stimulus #3	**30**	0.90	SAM stimulus #3	**25**	1.10
	70	1.10		**50**	1.15
	110	1.16		**100**	1.20
	150	1.23		**160**	1.25
SAM stimulus #4	**40**	0.90			
	80	1.10			
	120	1.16			
	160	1.23			

The received levels of test stimuli were measured with a hydrophone placed near the porpoise or attached to the frame holding the porpoise (TC4014 or TC4013, respectively; Teledyn Reson, DK). Signals were amplified by 20 dB and band pass filtered from 1 to 180 kHz (ETEC B 1501, DK) and then digitized at 500 kHz with a 16-bit DAQ-card (NI USB 6251, National Instruments, USA), which was part of the Evoked Response Study Tool (EVREST; Finneran, 2009; Finneran *et al.,* 2008). All hearing test results presented in this work were performed at the surface in different environments. The lower jaw of the porpoise was situated around 10–20 cm below the surface. Therefore the received SPLs had to be measured during all trials and for all frequencies. The variability of the SPLs over the full frequency range at the different locations was within ±3 dB, when the environmental conditions were good. The SPL control measurements for the hearing tests on wild porpoises at sea were strongly influenced by the wave height resulting in movements of the measuring platform. Due to the changes in the position of the hydrophone in the water column, SPLs were underestimated often with large deviations from the mean. Two different placements of hydrophones were tested to counteract these procedural variations: A TC4014 placed 20–30 cm distally to the porpoise and/or a smaller TC4013 was directly attached to the construction holding the porpoise in position. All hearing threshold measurements were corrected with the mean value of the SPLs measured under good conditions.

C. Evoked response measurement

Both stimulus presentation and ASSR recordings were collected from each porpoise with the EVREST system. The EVREST software was run on the same PC previously described for stimulus presentation (Panasonic Toughbook CF30). Brainstem responses were recorded using 10 mm gold-plated electrodes imbedded in suction cups and placed at three positions between the blowhole and the dorsal fin of the porpoise, as previously reported (Lucke *et al.,* 2007). The active (+) electrode was placed 7 cm behind the blowhole, the inverting electrode (−) along the dorsal midline of the porpoise between the blowhole and dorsal fin, and the ground electrode (⊥) on the left or right side of the dorsal fin (Fig. 2). The ASSRs measured at the electrodes were amplified (100-dB gain), and filtered (0.3–3 kHz) with a bio-potential amplifier (CP511, Grass Technologies, USA), then digitized at 50 kHz and 16-bit resolution via the USB-6251 data acquisition board. The reject level used in EVREST to exclude electrical responses not evoked by the hearing was 20 μV. Recordings were synchronized to the stimulus onset and averaged until 1024 evoked response epochs were obtained, at which point the next stimulus level was tested. Collection of the evoked response for a single stimulus sound pressure level took 71 s, and overall, six to 10 different SPLs were tested by an automated staircase routine in order to determine the hearing threshold using magnitude-squared coherence (MSC) calculation with 16 sub-averages and $\alpha = 1$ (Dobie and Wilson, 1989; Finneran *et al.*, 2007).

D. Background noise recordings

Noise levels were recorded with a hydrophone (TC4032, Teledyn Reson, DK), amplifier (+20 dB) with band pass filter (100 Hz–180 kHz, ETEC 1501, DK), and a DAQ-card (NI USB 6251, National Instruments, USA) with a sample rate of 200 kHz at the SOS Dolfijn and 400 kHz in the wild. One-third octave levels (in dB re 1 μPa) of background noise were measured before and after each hearing test. Background noise at sea changed during the course of testing; noise conditions at sea changed within short time periods (e.g., passing ships), or slowly during the trial (changing weather). To account for these circumstances, an automated routine was initiated in 2014 to record 10-s samples at regular time intervals of 2 min to allow for the evaluation of noise variability throughout the trial.

E. Data analysis

Signals were analyzed during the trials using a staircase procedure based on a magnitude squared coherence test (MSC) and controlled afterwards by post-filtering the ABR response (band pass filtered 0.3–3 kHz, 72 dB/octave). When an ABR response was obviously disturbed (waveform, amplitude, and phase) due to, e.g., changes of the environment (rain or upcoming stronger waves), the background noise level or movements of the animal, results for the individual transmitted sound pressure levels were neglected in post analysis. This resulted in a higher deviation for the threshold determined or an omission of the full threshold measurement. The thresholds were defined as the midpoint between the lowest stimulus level corresponding to the last MSC response detected and the highest stimulus level where no response was detected. Following the determination of frequency-specific thresholds, the median, the quantiles (0.25 and 0.75), the whiskers and outliers according to Borcard *et al.* (2011) were calculated using the R environment (R Core Team, 2014) to visualize thresholds determined for the two locations (Fig. 6) as well as for the two SAM stimulus sets (Set A and Set B from Table III in Fig. 7).

F. Ethics statement

Auditory threshold measurements conducted on harbor porpoises in Danish waters were conducted under permission issued to Jonas Teilmann, Aarhus University by the Danish Nature Agency (Danish Ministry of Environment, NST-3446-0016) and the Animal Experiments Inspectorate (Danish Ministry of Food, Agriculture and Fisheries, 2010/561-1801). Auditory threshold measurements on porpoises in rehabilitation were conducted at the SOS Dolfijn under a permit to rehabilitate small cetaceans (exemption of articles 9 and 13.1 of "Flora en Fauna wet") and issued by the Dutch Ministry of Economic Affairs to SOS Dolfijn. The measurements at SOS Dolfijn were part of the regular medical screening conducted during the rehabilitation process (i.e., adequacy of hearing determined before a release determination was made). All trials were conducted adhering to the respective ethical principles as well as to the relevant international and national guidelines for animal experiments and under constant supervision of an experienced veterinarian. Condition and potential stress of the animal from handling and testing was monitored by the attending veterinarian and testing was immediately halted if observed. One test was aborted based on the veterinarian's assessment of the animal.

III. RESULTS

Hearing tests were completed with six animals at the SOS Dolfijn rehabilitation facility using 10 kHz frequency spacing and nine with a one-third octave frequency spacing (Fig. 4) Similarly, hearing tests conducted within the Danish Baltic Sea were completed with six animals using 10 kHz frequency spacing and six with a one-third octave frequency spacing (Fig. 5). The audiograms were typically odontocete in shape, showing a skewed U-shape when hearing was tested at the lowest frequency of 10 kHz; the U-shape was not obvious in animals tested at one-third octave steps where the lowest tested frequency was 16 kHz. The results of tests using both frequency spacing showed high variation for the hearing thresholds between the individuals.

The median thresholds for the Baltic Sea porpoises in the frequency range from 25 to 110 kHz were 70–75 dB re 1 μPa [Fig. 6(a)] and the lowest value of ~63 dB re 1 μPa was found at 128 kHz. In comparison, the median thresholds of the rehabilitated porpoises were 5 to 10 dB lower for frequencies between 25 and 130 kHz with a best hearing value of ~56 dB re 1 μPa at 128 kHz [Fig. 6(b)]. For all animals in which a full range of hearing was tested, a slight decrease in

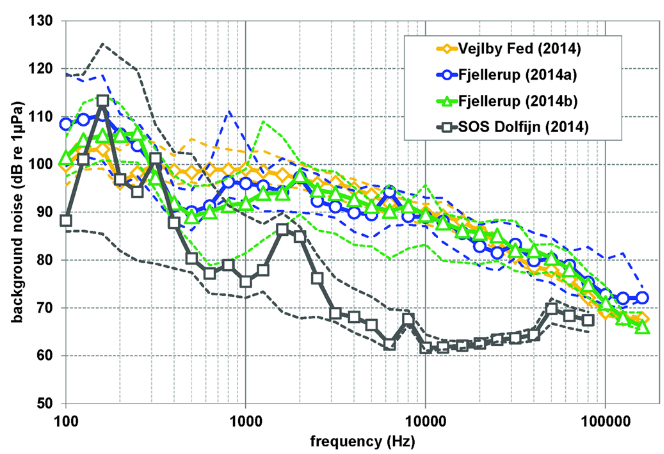

FIG. 3. (Color online) Mean one-third octave background noise level (solid lines with symbols) for the indoor (at the SOS Dolfijn, NL) and outdoor trials (Vejlby Fed and Fjellerup, DK) in 2014. The dotted lines indicate the maximum and minimum values of the spectra used for averaging.

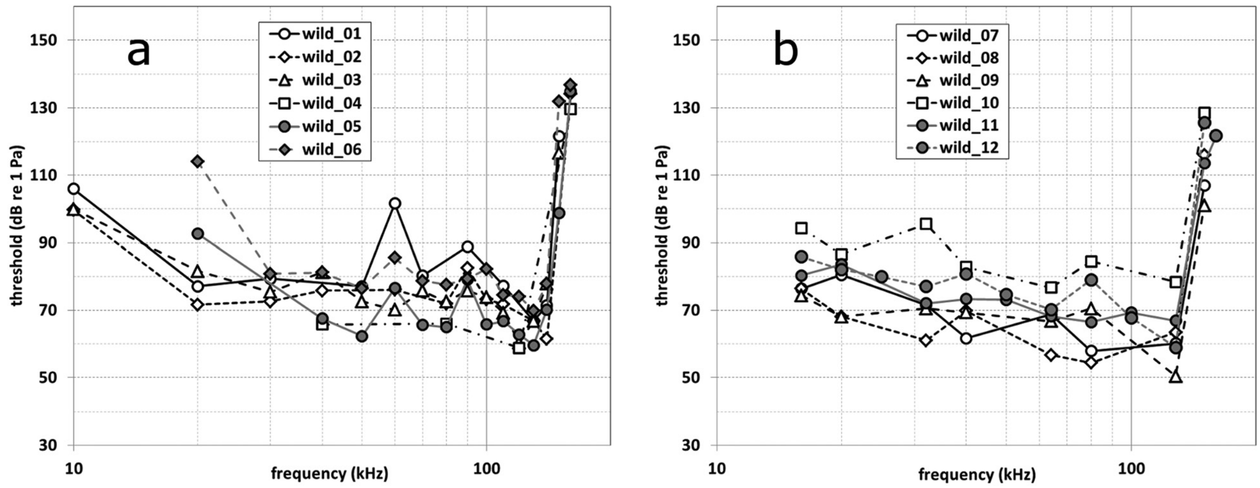

FIG. 4. Hearing thresholds for the porpoises in rehabilitation [(a) 10 kHz frequency spacing, (b) one-third octave frequency spacing].

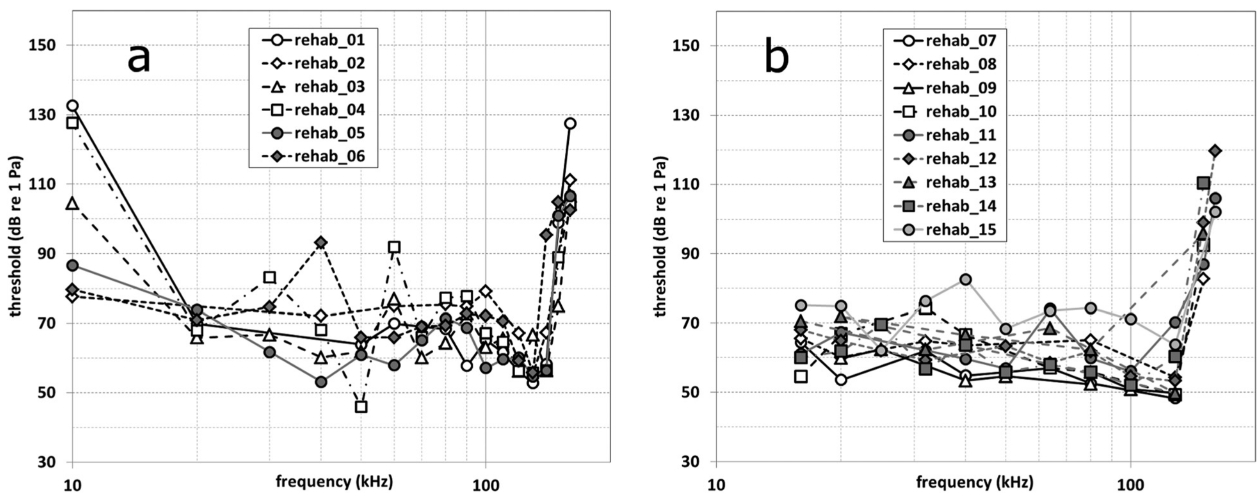

FIG. 5. Hearing thresholds for the porpoises from the inner Danish waters [(a) 10 kHz frequency spacing, (b) one-third octave frequency spacing].

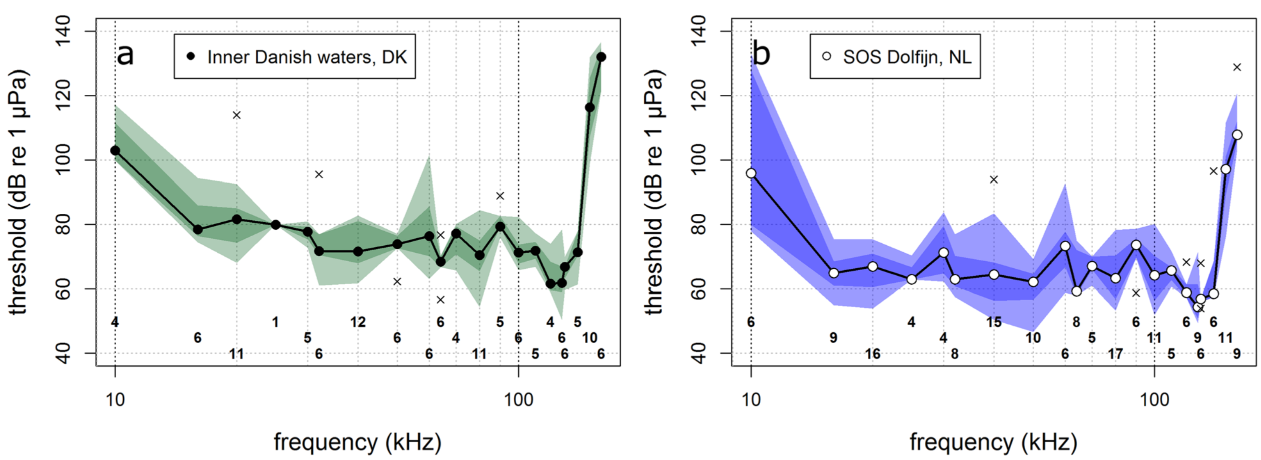

FIG. 6. (Color online) Thresholds for the porpoises (a) from the Danish Baltic Sea and (b) the rehabilitated animals at the SOS Dolfijn [median: black filled circles connected with a black line, quantiles 0.25 and 0.75: dark colored area, whiskers: light colored area according to Borcard *et al.* (2011)]. The numbers within the plot area representing the thresholds determined at the certain frequencies.

hearing sensitivity at frequencies below 25 kHz and a sharp decline in sensitivity for frequencies ≥140 kHz was detected.

Noise levels calculated across one-third octave bands, both at-sea and at the SOS Dolfijn, are presented in Fig. 3. Background noise levels at the SOS Dolfijn, especially for frequencies <4 kHz, were strongly affected by sounds coming from the nearby Dolfinarium (Harderwijk, NL) with which it is associated. Even though all pumps and lights of the SOS Dolfijn facility were turned off for the hearing tests, some modest electrical noise spikes at 2, 8, and 50 kHz were observed. The noise levels below 10 kHz sometimes increased during the measurements by 10–20 dB for several minutes and then went back to normal, presumably due to operations at the adjacent Dolfinarium (Harderwijk, NL). The background noise measurements at sea (Vejlby Fed and Fjellerup; Fig. 3) showed maximum mean differences from 0.1 to 2 kHz of 10–20 dB within the one-third octave bands. Above 10 kHz, the background noise levels recorded at sea were quite similar regardless of location (within ∼2 dB, except the 130 and 160 kHz values for Fjellerup, 2014a in Fig. 3). In comparison to the background noise measured at the SOS Dolfijn, the outdoor noise levels are ≥20 dB higher in the frequency range of 400 Hz–40 kHz. Although noise levels decreased with increasing frequency, the noise levels recorded at sea generally did not approximate that of the SOS Dolfijn until the highest frequencies recorded. Nevertheless, for the frequencies of interest, the background noise at the SOS Dolfijn provided the better of the test environments.

A. Comparison of the frequency-sets used

The differences in the tonal frequency intervals used during testing (see Sec. II B) and the effect on the shape of the audiograms of the porpoises are shown in Fig. 7 and Fig. 8. For frequency interval Sets A and B (refer to Table III), the hearing sensitivity showed a sharp decrease at frequencies ≥140 kHz. Between 30 and 130 kHz, the median sensitivity for both test sets showed a slightly different shape. The audiogram resulting from use of the Set B intervals was associated with a more "flattened" sensitivity curve. The one-third octave frequency steps for Set B eliminated the dip in the frequency range from 120 to 130 kHz, which is clearly observable for the 10 kHz frequency steps used in Set A. Tests conducted with Set A showed a decrease in hearing sensitivity at 10 kHz that was not observed with the Set B spacing (10 kHz was not sampled with Set B). The 10 kHz threshold was also associated with a large variation in sensitivity estimates. The differences in determined hearing thresholds at 10 kHz observed with Set A in this study are big and the decrease of hearing sensitivity is possibly not conclusive.

IV. DISCUSSION

Historically, knowledge about the hearing ability of harbor porpoises was limited to a small number of animals. Andersen (1970) first determined an audiogram on a female porpoise using behavioral methods, although noise limitations to the thresholds estimates obtained during the study could not be determined, as the background noise was not reported. On two later occasions, Kastelein *et al.* (2010); Kastelein *et al.* (2015) determined behavioral audiograms on a 1.5 and a 3 yr old male porpoise. These latter two studies, which were conducted throughout a period of 1–1.5 yr, were performed in low ambient noise and provide greater confidence in the threshold estimates. Popov *et al.* (2005) used evoked potential methods to determine the audiograms of the related Yangtze finless porpoise (*Neophocaena phocaenoides asiaorientalis*); two animals were studied, an 8 yr old male and a 5 yr old female, but background noise levels were again not reported.

This is the first study to test the hearing of such a large number of wild porpoises. We could show that harbor porpoises have a broad hearing range between 16 to 140 kHz with the highest sensitivity at ∼130 kHz. For frequencies above 140 kHz a sharp decline in sensitivity was detected. Despite some variability in sensitivity between individuals, equivalent audiogram shapes (Fig. 4 and Fig. 5) were observed.

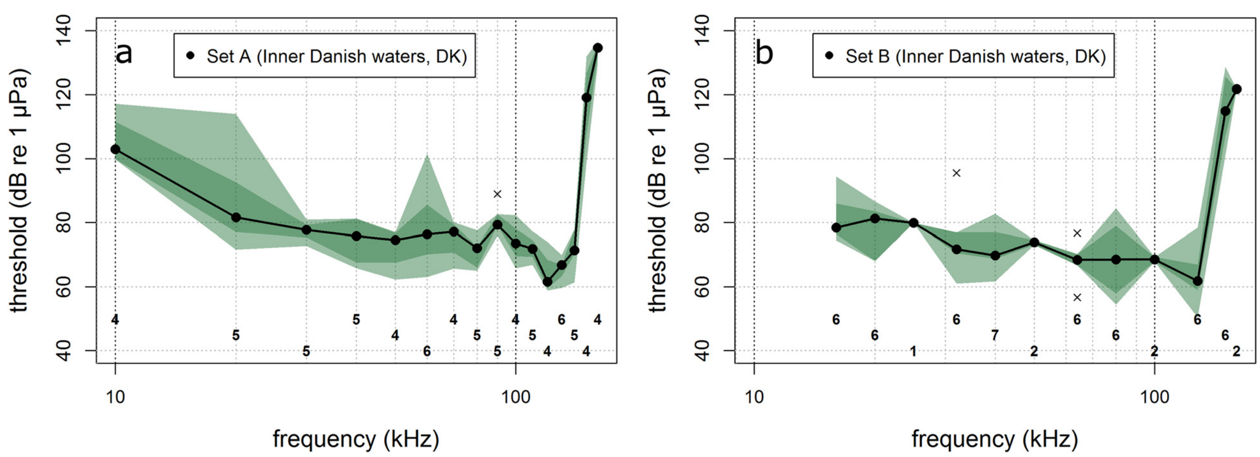

FIG. 7. (Color online) Thresholds for the porpoises from the inner Danish waters split for the hearing thresholds determined with (a) SAM Set A and (b) SAM Set B described in Table III [median: black filled circles connected with a black line, quantiles 0.25 and 0.75: dark colored area, whiskers: light colored area according to Borcard *et al.* (2011)]. The numbers within the plot area representing the thresholds used at the certain frequencies.

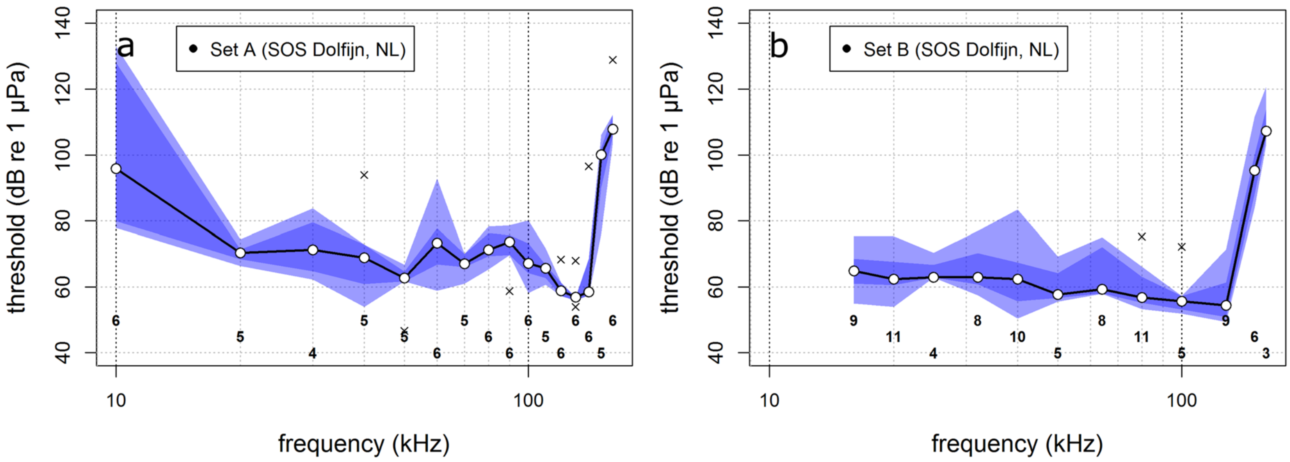

FIG. 8. (Color online) Thresholds for the porpoises from the rehabilitated animals at the SOS Dolfijn split for the hearing thresholds determined with (a) SAM Set A and (b) SAM Set B described in Table III [median: black filled circles connected with a black line, quantiles 0.25 and 0.75: dark colored area, whiskers: light colored area according to Borcard *et al.* (2011)]. The numbers within the plot area representing the thresholds used at the certain frequencies.

A comparison of prior audiograms collected via behavioral methods with the results presented here illustrates some distinct differences (Fig. 9). Thresholds determined by behavioral methods are lower than the results of this study, but the form of the curve is comparable with respect to the limits of hearing and the frequency range of best hearing sensitivity. These types of differences are not uncommon when comparing AEP and behavioral thresholds. Prior comparisons of AEP and behavioral methods under various test conditions within the same subject have demonstrated that AEP threshold estimates can differ from behavioral thresholds by up to 20 dB, depending upon the method used for estimating threshold (Houser and Finneran, 2006a; Yuen *et al.*, 2005). Furthermore, the frequency of the auditory test stimuli as well as the proximity/distance of the subject to acoustic boundaries (surface) during the measurements affects the resulting thresholds. Consequently, when behavioral and AEP methodologies are more harmonized, these differences can be substantially minimized (Schlundt *et al.*, 2007). Differences in the low and high frequency tails of the audiograms are also consistent with prior comparisons of AEP and behavioral methods in odontocetes, as approaching the low and high frequency limits of hearing generally result in greater differences in thresholds estimated with the two methods (Finneran and Houser, 2006; Houser and Finneran, 2006a; Schlundt *et al.*, 2007; Yuen *et al.*, 2005). Detailed statistical comparison was not deemed useful due to the different methods used and number of animals tested.

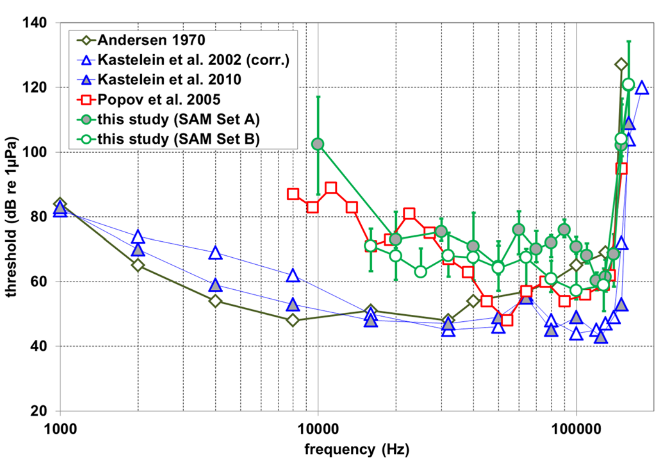

FIG. 9. (Color online) Audiograms on three harbor porpoises using behavioral methods (Andersen, 1970; Kastelein *et al.*, 2002; Kastelein *et al.*, 2010), an evoked potential audiogram on a finless porpoise (Popov *et al.*, 2005) and the median thresholds with the lower and upper quantiles (0.25 and 0.75) as error bars for the SAM Sets A and B for all thresholds determined in this study.

The audiogram of the finless porpoise taken from Popov *et al.* (2005) is the only other study using AEP in a porpoise and shows a comparable form and similar slopes in the ranges of decreasing sensitivity. It is possible that slight differences in the audiograms might be due to true differences in the thresholds of the individuals or species. However, methodological explanations and differences in sample size and analytical methods are also likely contributors. For example, threshold estimates for the Yangtze River porpoise were obtained by establishing a regression line describing the relationship between the spectral amplitude of the ASSR at its modulation frequency to the stimulus level, and tone pips were used instead of SAM tones (Popov *et al.*, 2005). The regression line was then extrapolated to the zero-amplitude crossing of the amplitude axis to obtain an estimate of the threshold. This approach should result in a lower threshold estimate than the method used in this study, which estimated threshold as the midpoint between the lowest stimulus level at which an ASSR was detected and the highest stimulus level at which no ASSR was detected. Based on the two approaches, it might reasonably be expected that if the estimate procedures were consistent that the ranges of best sensitivity might be in better agreement. It should also be noted that greater variability in the audiogram should be expected based on the small sample size (n = 2) of Popov *et al.* (2005).

The use of AEP methods to test the hearing of wild harbor porpoises produced similar results to those obtained from rehabilitating porpoises under more controlled conditions. In contrast to the results of Mann *et al.* (2010), which found that a number of stranded odontocetes showed hearing deficits, no obvious hearing deficits of harbor porpoises were detected in either the stranded and rehabilitated animals or the animals assessed in the wild. The tested porpoises showed wide variability in hearing thresholds under both conditions, but the audiograms showed gross similarity in the overall range of hearing and patterns of sensitivity. The differences in the spacing of the tested frequencies (in Set A and Set B) likely contributed to the differences observed in the two resulting audiograms. On the one hand, the finer 10-kHz spacing of Set A enabled a finer resolution of the audiogram and it may be that the dip in hearing sensitivity at 120–130 kHz was not well characterized by the overall one-third octave spacing of frequencies in Set B. Conversely, in both sets, 4-SAM stimuli existed with frequency spacing that were less than an octave apart at the highest frequency groupings. This can potentially lead to the influence of an individual component over the ASSR produced by closely spaced neighboring components, particularly if it is presented at high amplitude while the neighboring components are at low amplitude (e.g., near thresholds). Presumably, interactions should be minimized if components are separated by greater than the cochlear filter bandwidth, but high amplitude signals increase the bandwidth of the cochlear filter making interactions more likely (Lins and Picton, 1995). Another factor potentially contributing to the differences in sensitivity is the amplitude modulation rate. The modulation rate transfer function has been assessed in the harbor porpoise (Linnenschmidt *et al.,* 2013; Lucke *et al.,* 2007) and this information was used in establishing the modulation rates used for multiple-SAM tones in this study. In addition, some of the porpoises in this study were used to verify that differences in threshold estimation were not caused by differences in the modulation rate. However, Set A utilized used a lower modulation rate for the testing of some frequencies (900 Hz), which may have resulted in a suboptimal ASSR amplitude and affected the threshold estimate. Finally, the decrease in hearing sensitivity at 10 kHz (Set A only) is potentially due to a reduction in the effectiveness of the ASSR method at lower frequencies. Threshold estimates were the most variable at 10 kHz, which contributed to an elevated mean threshold; thus, the threshold at 10 kHz should be interpreted with caution. Additional care should be given to the background noise in the wild, as this was higher with decreasing frequency in comparison to the trials at the rehabilitation center (Fig. 3). It seems conceivable that the thresholds measured outdoors are more prone to masking.

Rapid development of renewable energy infrastructures in the seas of northern Europe has been a growing concern with respect to potential impacts on harbor porpoises. In general, construction of wind farms or the emission of low frequency sound by operational wind turbines have dominated the concern as to how and to what degree harbor porpoises might be impacted (Brandt *et al.,* 2011; Dähne *et al.,* 2013; Scheidat *et al.,* 2011; Tougaard *et al.,* 2009b). Impacts due to sound exposure are likely within the hearing range of the porpoise and are potentially more severe at frequencies of greatest hearing sensitivity, although recent work in humans suggests that even sound outside the hearing range might potentially impact hearing abilities (Kugler *et al.,* 2014). Although concerns about higher frequency noise from ships have also been speculated as potentially problematic (Hermannsen *et al.,* 2014).

With respect to auditory physiology, sufficient data has been collected that indicates harbor porpoises have a greater susceptibility to auditory fatigue relative to other odontocetes, such as the bottlenose dolphin. Sound exposure levels (dB re 1 μPa^2.s) required to induce the onset of temporary threshold shifts (TTS) in harbor porpoises at frequencies below 10 kHz can be tens of decibels less than that observed in bottlenose dolphins (Finneran *et al.*, 2005; Finneran *et al.*, 2015; Kastelein *et al.*, 2014; Kastelein *et al.*, 2012b; Kastelein *et al.*, 2013a; Lucke *et al.,* 2009). However, sample sizes for TTS and basic hearing studies have been limited and need to be increased in order to address variability in TTS onset and thresholds of hearing. This is true for all hearing related studies (e.g., masking), and is necessary to provide confidence in acoustic impact predictions. As exploration into differences in sensitivity across the range of hearing between porpoises and other cetaceans continues, understanding variability in the range of hearing and hearing sensitivity of the harbor porpoise will be essential for contextualizing behavioral and physiological observations rooted in the porpoises' detection and perception of anthropogenic sound. This is an essential prerequisite in order to enunciate future management strategies and environmental law in relation to noise pollution in the marine environment.

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