Raising your voice: Evolution of narrow band high frequency signals in toothed whales (Odontoceti)

Short title: Evolution of narrow band high frequency signals

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ABSTRACT

Cetaceans use sound for communication, navigation and finding prey. Most extant odontocetes produce broadband (BB) biosonar clicks covering frequency ranges from tens to 150-170 kHz. In contrast, the biosonar clicks of some odontocetes are unique, being narrow in bandwidth with high centroid frequency (NBHF), peak frequencies being at 125-140 kHz and bandwidths of 11-20 kHz. Thirteen species within four families (Phocoenidae, Pontoporiidae, Kogiidae, Delphinidae) are known to produce these signals, implying convergent evolution under strong selective drivers. Several hypotheses have been proposed, including acoustic cryptsis to escape killer whale predation, but none have provided comprehensive explanation of the timing of NBHF evolution and the pressures driving sound production to such extremes. Using molecular phylogenetics and cochlea anatomy of extinct and extant taxa, we demonstrate that early NBHF adaptations occurred at least 10 Ma, and possibly up to 18 Ma, indicating that killer whales cannot have been the sole driving force of NBHF, but that now extinct odontocetes may have provided similar pressures. Using palaeoclimatic modelling, we further demonstrate that the upper advantageous spectral window for NBHF signals at around 130 kHz has persisted throughout most of the global sea area since the mid-Miocene, covering all known instances of NBHF evolution.

KEY WORDS

Anatomy, Biosonar, Climate, Hearing, Palaeontology, Phylogeny
INTRODUCTION

Hearing is critical for cetaceans. They use sound for communication, navigation, finding prey and avoiding predators. The first cetaceans, the pakicetids, that appeared about 54 million years ago (Ma) were already adapted for underwater hearing. Near-modern underwater hearing mechanisms had evolved about 10 Ma later (Nummela, Thewissen, Bajpai, Hussain & Kumar, 2004). Modifications of hearing and sound production resulting in a biosonar system probably arose about 32 Ma in odontocetes (Churchill, Martinez-Caceres, de Muizon, Mnieckowski & Geisler, 2016; Geisler, Colbert & Carew, 2014), allowing the use of sound for locating prey and underwater orientation.

Most extant odontocetes produce broadband (BB) biosonar clicks covering a frequency range from a few tens of kHz to about 150-170 kHz. In contrast, the biosonar clicks of some odontocetes have evolved to an extreme, resulting in a narrow band high frequency (NBHF) biosonar with peak frequencies at 125-140 kHz and rms bandwidth of about 11-20 kHz (Kyhn, 2010). Presently, thirteen species within four families (Delphinidae (Kyhn, Tougaard, Jensen, Wahlberg, Stone, Yoshinaga, Beedholm & Madsen, 2009; Kyhn, Jensen, Beedholm, Tougaard, Hansen & Madsen, 2010), Phocoenidae (Kyhn, Tougaard, Beedholm, Jensen, Ashe, Williams & Madsen, 2013; Li, Wang, Wang & Akamatsu, 2005; Miller & Wahlberg, 2013; Villadsgaard, Wahlberg & Tougaard, 2007), Pontoporiidae (Melcon, Failla & Iniguez, 2012) and Kogiidae (Madsen, Carder, Beedholm & Ridgway, 2005)) are known to produce NBHF signals. Consequently, there appears to have been at least four instances of convergent evolution, implying the presence of strong selective drivers for NBHF biosonar. Although several hypotheses have been proposed (Andersen & Amundin, 1976; Au, 1993; Kyhn, 2010; Madsen et al., 2005; Miller & Wahlberg, 2013; Morisaka & Connor, 2007), none have provided a comprehensive explanation of the timing of NBHF evolution and selection pressures driving sound production to such extremes.

The transmission properties of a biosonar system are dictated by the size of the sound producing organ and the spectral properties of the transmitted sound (Au, 1993). Thus, the high
centre frequency is likely contingent on the small sizes of NBHF species and it is not surprising that they use centre frequencies above 100 kHz to achieve directivity comparable to larger species (Au, 1993; Au, Kastelein, Rippe & Schooneman, 1999; Au, Pawloski, Nachtigall, Blonz & Gisner, 1995; Koblitz, Wahlberg, Stilz, Madsen, Beedholm & Schnitzler, 2012; Kyhn et al., 2013; Kyhn et al., 2009; Kyhn, 2010; Kyhn et al., 2010). It is therefore the narrow bandwidth of just 11-20 kHz in combination with a high centre frequency that make NBHF signals unique (Götz, Antunes & Heinrich, 2010; Kyhn et al., 2009; Kyhn, 2010; Kyhn et al., 2010; Li et al., 2005; Madsen et al., 2005; Villadsgaard et al., 2007).

Most studies have argued that the selective pressure driving signals above 100 kHz was killer whale (*Orcinus orca* Linneaus, 1758) predation (Andersen & Amundin, 1976; Kyhn et al., 2009; Kyhn, 2010; Kyhn et al., 2010; Madsen et al., 2005; Miller & Wahlberg, 2013; Morisaka & Connor, 2007). Killer whales have poorer hearing above 100 kHz (Branstetter, Leger, Acton, Stewart, Houser, Finneran & Jenkins, 2017), allowing extant NBHF species acoustic crypsis (Morisaka & Connor, 2007). However, such predation could only have been a selective pressure for NBHF signals after the appearance of killer whales. The crown groups of several NHBF species (e.g. Phocoenidae and Pontoporiidae) are much older (Steeman, Hebsgaard, Fordyce, Ho, Rabosky, Nielsen, Rahbek, Glenner, Sorensen & Willerslev, 2009), but it is unclear whether NBHF evolved in these groups before or after the appearance of killer whales.

A narrow bandwidth is also advantageous for detecting a signal in masking noise (Møhl, 1973). In this regard, mean noise level at Beaufort sea states 3-5 meets thermal noise at about 130 kHz forming a minimum in ambient ocean noise (Madsen et al., 2005; Madsen & Surlykke, 2014; Mellen, 1952; Miller & Wahlberg, 2013; Wenz, 1962), theoretically providing superior echo-to-noise ratio and setting the upper frequency limit for NBHF signals. To date however, there is a lack of palaeoclimatic data on wind speed and sea states, which could support the hypothesis of ambient ocean noise as a driver of NBHF evolution.
We aim to unravel the selection pressures leading to NBHF evolution using molecular phylogenomics and cochlear anatomy of extinct and extant taxa to narrow the potential time windows during which NBHF signals evolved. We further consider whether extinct odontocete predators could have exerted selection pressure for acoustic crypsis in the past. Finally, we use palaeoclimate modelling through millennia to assess whether ambient ocean noise could have provided an advantageous spectral window for NBHF signals.

MATERIALS AND METHODS

Phylogenetic relationships
The phylogenetic context of NBHF evolution and updated estimates of divergence times for odontocetes were explored by a phylogenetic analysis based on mitogenome sequence data from 41 odontocetes and one mysticete (*Balaena mysticetus* Linneaus, 1758). Data were obtained from NCBI’s Genbank and comprised the 13 protein-coding genes of the mitochondrial genome. Intergene regions and the d-loop were omitted due to missing data and to minimize mutation saturation, repetitive sequences and/or alignment ambiguities. Further, the ND6 gene was reverse-complemented, overlapping regions in the ND5, ND6, ATP6, COX3, ATP8 and ATP6 genes were duplicated, and all indels were removed to obtain an 11,388 base pair (bp) sequence with the 13 genes adjusted to the same reading frame. All edits were made using Geneious (Kearse, Moir, Wilson, Stones-Havas, Cheung, Sturrock, Buxton, Cooper, Markowitz, Duran, Thierer, Ashton, Meintjes & Drummond, 2012). The phylogenetic analyses and divergence time estimates were performed in BEAST ver. 2.3.0 (Bouckaert, Heled, Kuhnert, Vaughan, Wu, Xie, Suchard, Rambaut & Drummond, 2014). The concatenated and aligned mitogenome data were partitioned into 1st, 2nd and 3rd coding positions and analysed using the HKY model with estimated frequencies, fixed mean mutation rates, a birth-death model with gamma shaped rates, and a random local clock to account for branch heterogeneity. We defined monophyletic taxa sets with three exponential distributed calibration points and minimum age set as offset. We used the bowhead whale as an outgroup, and the age of the
*Ferecetherium kelloggi* (Mchedlidze, 1970) specimen at 23 Ma for calibration of the odontocete lineage (Mchedlidze, 1970), *Globicetus hiberus* (Bianucci et al., 2013) at 13.7 Ma for the Ziphiidae lineage (Bianucci, Miján, Lambert, Post & Mateus, 2013), and *Salumiphocaena stocktoni* (Wilson, 1973) at 7.3 Ma for the Phocoenidae lineage (Wilson, 1973). Trees were generated across 50,000,000 runs, logging every 5000th tree. Further processing was conducted in TRACER and TREEANNOTATOR, while ensuring that acceptance rates for each operator were between 0.12 and 0.25 (Gelman, Meng & Stern, 1996), and all ESS values above 200.

**Hearing in extinct and extant odontocetes**

Three studies (Churchill et al., 2016; Mourlam & Orliac, 2017; Racicot, Darroch & Kohno, 2018) have shown that nine measurements of the inner ear labyrinth in a variety of terrestrial, semi-aquatic, and fully aquatic artiodactyl groups correspond to hearing sensitivity, such that ultrasonic and infrasonic hearers show clear separation in a principal components analysis. To illustrate the hearing ranges of our selected NBHF, BB, and possible predator species alongside other artiodactyls, we built on two of these previous analyses by obtaining the same measurements, and adding them to the PCA performed by Mourlam and Orliac (Mourlam & Orliac, 2017). The measurements were: cochlear length (Cl), secondary bony lamina length (SBL), cochlear width (Cw), cochlear width perpendicular to Cw (W2), inter-turn distance (ITD), spiral ganglion canal width at the first quarter-turn (GAN), surface area of the fenestra cochleae (FC), and number of turns (T). The PCA was performed using the FactoMineR package (Le, Josse & Husson, 2008) in the R programming language (Team, 2015). Following Mourlam and Orliac (2017), any measurements that could not be taken from the CT scans were imputed using the missMDA package (Josse & Husson, 2016), although these were few in number (see Data S1). Cochlear spiral length (Cl), a proxy sometimes used to infer hearing sensitivity, was measured from the start of the laminar gap (just interior to the fenestra cochleae) to the cochlear apex within the cochlea. Secondary bony labyrinth lengths were measured externally just interior to the fenestrae cochleae at
the start of the laminar gap. The radial (outer) base of the secondary bony lamina is almost always better preserved than the axial edge, making the external measurement potentially more appropriate for hearing range estimates. The number of cochlear turns was determined by counting the number of times the cochlea crossed a line going through the start of the laminar gap through the axis of rotation and is reported as number of turns (total degrees divided by 360°) following previous methods (Ekdale, 2010; Racicot, Gearty, Kohno & Flynn, 2016). We added measurements to previous data from the inner ear labyrinths of 11 extant and extinct odontocete species using CT scans of 13 individual petrosals (Supplementary Table S2). Digital endocasts of the inner ear labyrinths were extracted using VGStudioMax 2.2. Anterior views of the inner ear labyrinths were oriented with the anterior semicircular canal positioned vertically along the dorsoventral axis (Figure 1, Supplementary Figures S1 and S2).

*Palaeoclimate modelling and ambient noise levels*

To explore the noise regimes during NBHF evolution we used the coupled atmosphere-ocean-vegetation general circulation model (GCM) HadCM3BL-M2.1aD (Valdes, Armstrong, Badger, Bradshaw, Bragg, Crucifix, Davies-Barnard, Day, Farnsworth, Gordon, Hopcroft, Kennedy, Lord, Lunt, Marzocchi, Parry, Pope, Roberts, Stone, Tourte & Williams, 2017) to simulate modern climate and project patterns back in time.

The Beaufort scale (sea states (SS)) correlates with wind velocity, which determines wave heights and ultimately ocean noise (Wenz, 1962). Wind velocities simulated by the GCM are determined by the atmospheric circulation, which is determined by the spatial gradients in atmospheric moisture content. The simulated annual mean wind speeds compare favourably to observations at the global scale ((Dee, Uppala, Simmons, Berrisford, Poli & others, 2011); Figure S3). Therefore, we have confidence that the model can be used to simulate wind speed patterns from which SS across oceans back to the middle Miocene can be estimated. There are large uncertainties when attempting to estimate wind patterns millions of years ago. We tested the
sensitivity of mean wind speeds to changes in ocean gateways, the Greenland ice sheet and atmospheric CO$_2$ concentrations by forcing the GCM with boundary conditions for the Pliocene, and the late and middle Miocene (Figure S3). The Pliocene simulation is documented in Bragg, Lunt and Haywood (2012), Late Miocene simulations include Bradshaw, Lunt, Flecker, Salzmann, Pound, Haywood and Eronen (2012) and others (Bradshaw, Lunt, Flecker & Davies-Barnard, 2015) while middle Miocene simulations are all previously unpublished. The Miocene simulations use the palaeogeography of Markwick (Markwick, 2007) and between them include uncertainties in CO$_2$ concentration, land-sea mask and continental ice sheet extent. A full list of simulations and boundary condition assumptions is provided in Table S1. Uncertainties in simulated annual mean wind speeds over Miocene oceans due to boundary condition uncertainties differ only in extent, not in magnitude.

**Ecological and behavioural characteristics of NBHF species**

To identify ecological and behavioural characteristics of NBHF species relative to BB species we mapped species information on body length, weight, sociality and habitat to a phylogenetic tree. Body weights, lengths, sociality and habitat were obtained primarily from Culik (2011); Perrin, Wursig and Thewissen (2009), and the tree was based on our mitogenomic analysis with missing species added according to previous studies (McGowen, 2011; McGowen, Spaulding & Gatesy, 2009).

**RESULTS AND DISCUSSION**

**Timing of NBHF evolution**

Analysis of odontocete mitogenomes provided a phylogenetic tree with strong (>0.90) support for most branch nodes, confirming that NBHF biosonar evolved independently at least four times (Figure 1). These instances may have occurred over a considerable time span, with estimates of 20 Ma and 22 Ma for the origins of Kogiidae and Pontoporiidae respectively, 16-7 Ma for
Phocoenidae, and 2.5-2 Ma for the Delphinid NBHF-using clade containing the genus *Cephalorhynchus* and the species *Lagenorhynchus cruciger* (Quoy & Gaimard, 1824) and *Lagenorhynchus australis* (Peale, 1848). These estimates fit well with previous estimates based on a smaller subset of mitochondrial and nuclear loci (McGowen et al., 2009), however, the estimated period for NBHF evolution in Kogiidae, Phocoenidae and Pontoporiidae can be significantly narrowed down by fossil evidence.

Measurements of the inner ear labyrinth from CT scans of a sampling of extant NBHF species, extinct relatives, and BB species supplement the phylogenetic analysis to indicate that anatomical adaptations to high frequency hearing had evolved by the time each species was recognizably a member of its respective clade, except the kogiid fossil (5.9-4.6 Ma; Figure 2, Table S2, Figures S1, S2, Data Table S1). Extinct relatives of modern NBHF species, with the exception of the kogiid fossil, lie within or close to the morphospace of extant relatives. The variables factor map (inset in Fig 2) indicates that most of the variation shown among NBHF species and other odontocetes can be described by aspects of cochlear width (Cw and W2). In NBHF species, Cw ranges from 8.38–9.93 and W2 ranges from 6.205–7.49 mm. NBHF species are differentiated from terrestrial artiodactyls mostly by number of turns, and a combination of FC, Ch, CI, and CW and W2 distinguishes them from mysticetes. The PCA illustrates that that adaptations for NBHF hearing were present in the most basal investigated species of the NBHF clades, in the phocoenid lineage 7.5–9.5 Ma (*Salumiphocaena*) and in pontoporiid lineages 9.9-8.8 Ma (*Brachydelphis*), based on dating of strata from which the taxa were found and confirmed by the phylogenetic analysis outlined above (Figure 1). The kogiid fossil that occupies a position in the PCA among non-NBHF odontocetes is referred to as a “large” morphotype kogiid (Velez-Juarbe, Wood & Pimiento, 2016). Smaller morphotypes are described from the same formation (Velez-Juarbe et al., 2016), suggesting the presence of two sympatric kogiid species and that other kogiid species may have evolved NBHF earlier (the smaller morphotype may have had NBHF, but has not been CT scanned). Intriguingly, *Kentriodon pernix* (Kellogg, 1927), a small odontocete from the Calvert Formation (18 Ma)
(Mourlam & Orliac, 2017) recently hypothesized to be an early-diverging delphinidan (Lambert, Bianucci, Urbina & Geisler, 2017), is nested within the morphospace of NBHF species (Figure 2, labelled ‘Ke pe’). If this species used NBHF signals, the earliest occurrence of NBHF evolution can be pushed back to a minimum of 18 Ma.

Investigations of general skull shape of extant Delphinoidea members did not reveal any consistent patterns related to NBHF use. Previously, low levels of skull asymmetry have been used to imply NBHF use in odontocete fossils (Morisaka & Connor, 2007). However, there is no straightforward relationship between skull asymmetry and BB/NBHF use among extant Lissodelphininae (Galatius & Goodall, 2016). Thus, we have not used skull asymmetry to assess the use of NBHF sounds.

**Predation as a driver of NBHF evolution**

It has been hypothesized that predation by killer whales exerted selection pressure for acoustic signals with bandwidths exclusively above 100 kHz (Andersen & Amundin, 1976; Kyhn et al., 2009; Kyhn et al., 2010; Madsen et al., 2005; Miller & Wahlberg, 2013; Morisaka & Connor, 2007), although younger killer whales may have lower thresholds for high frequencies (Branstetter et al., 2017). Our phylogenetic analysis indicates that the killer whale lineage originated about 6 Ma, more recent than previous estimates of 10 Ma (McGowen et al., 2009; Steeman et al., 2009) and postdating the origin of three of the four NBHF lineages. The earliest known killer whale fossils are only 3.1 My old (Bianucci, 2005), and there is currently no evidence that earlier members of the lineage occupied the same ecological niche as extant killer whales. Thus, killer whales may have provided the selection pressure for evolution of NBHF properties in the recent *Cephalorhynchus-Lagenorhynchus* clade about 2.5 Ma, and perhaps for the kogiids if NBHF evolved after 4.8-3.1 Ma. However, if the predator hypothesis is correct, species other than killer whales must have contributed to the much earlier NBHF adaptation in Phocoenidae and Pontoporiidae.
There were indeed odontocetes potentially preying on smaller odontocetes during the three earlier instances of NBHF evolution. The fossil record of the raptorial sperm whales (stem physeteroids) shows a wide diversity and global distribution. These include relatively complete fossils from the middle to late Miocene like *Acrophyseter* (ca. 13.0-6.7 Ma), *Brygmophyseter* (ca. 16.0-13.7 Ma, Japan) and *Zygophyseter* (ca. 10.4-8.1 Ma, Europe,) all of which were of similar sizes as extant killer whales (Bianucci & Landini, 2006; Lambert, Bianucci & de Muizon, 2016). More fragmentary material referred to *Hoplocetus* stretches the temporal range of this type of predators into the Pliocene (Hampe, 2006).

*Acrophyseter deinodon* (Lambert et al. 2008) had cochlear morphology similar to that of the modern killer whale (Figure S1), indicating similar low sensitivity to frequencies above 100 kHz. The raptorial sperm whales had large teeth, strong mandibular musculature, and were adapted for feeding on large prey (Lambert et al., 2016). In contrast to killer whales, whose fossils are only from the Pliocene, the occurrence of raptorial sperm whales coincides in space and time with fossils of three taxa of current NBHF species (Kogiidae, Pontoporiidae, Phocoenidae). Some formations, like the Pisco Formation, have yielded several fossils of predators like *A. deinodon* and *A. robustus* and potential prey like *Brachydelphis* (Pontoporiidae) and *Piscolithax* (Phocoenidae), indicating co-existence (Lambert et al., 2016).

We conclude, therefore, that predation by ancient predators like *A. deinodon* provided selection pressure for the use of NBHF signals in three older families of small odontocetes (Figure 2, NBHF morphospace) while predation by the modern killer whale, *O. orca*, is responsible for NBHF signals in a more recent fourth taxon, the NBHF using delphinids. We also conclude that the hearing of an ancient predator like *Acrophyseter* was similar to that of *O. orca* based on inner ear anatomy and their close proximity in the odontocete morphospace (Figure 2).

**NBHF signals and ambient ocean noise**

Wind generated wave action is the major contributor to ambient sea noise at depths of less than 200 m and especially at lower frequencies (Hildebrand, 2009; Wenz, 1962). Our palaeoclimate
simulations indicate that mean annual surface wind speeds were stable at 4-9 m/s from the mid Miocene (about 16 Ma) in oceanic regions with high occurrence of fossils from NBHF lineages (Figure 3A-C; see Table S1 for conditions of palaeoclimate modelling and Figure S3 for evaluating mean annual wind velocities). Beaufort sea states (3-5) corresponding to observed annual mean wind speeds (4-9 m/s) meet thermal noise at frequencies about 100-140 kHz (Figure 3D). These conditions form an ambient noise minimum at the frequencies used for echolocation and communication by NBHF species that have centre frequencies at about 125-140 kHz in a narrow frequency bandwidth of about 11-20 kHz emitted at about 20 dB lower intensities than BB species (Kyhn et al., 2009; Kyhn, 2010; Kyhn et al., 2010; Madsen et al., 2005; Melcon et al., 2012; Miller & Wahlberg, 2013; Morisaka & Connor, 2007). Heavy acoustic absorption, which increases 10 times from 100 kHz to 200 kHz, and the doubling of thermal noise with each doubling of frequency, were probably selection pressures limiting the upper frequencies of NBHF biosonar. Thus, the ambient noise minimum provides an acoustic “window”, which has existed for about 16 M years, giving a selective advantage for odontocetes using NBHF signals. A favourable ambient noise environment is probably not in itself a selection pressure for evolution of these signals, but has been conducive to NBHF evolution in the presence of predation pressure.

Ecology, behaviour and species in transition?

NBHF species are smaller in length and weigh less than most odontocetes, particularly their closest BB relatives, and most NBHF species occur in small groups (Figure 4). This pattern is particularly striking when comparing closely related NBHF and BB species in the subfamily Lissodelphininae (Lissodelphis-Lagenorhynchus-Cephalorhynchus complex). The small size of NBHF species lends further support to the hypothesis of predation as the main selection pressure for NBHF evolution, as smaller species are more likely targets of predation. We do not find patterns related to habitat in that NBHF species occur in coastal waters, over the continental shelf and in the open ocean. The only
river dolphin using NBHF biosonar (Pontoporiidae) occurs coastally and, therefore, in a habitat with potential extinct or current odontocete predators.

If NBHF signals help protect against predation, why are there not more species using these signals? A NBHF signal is not adaptive for all odontocetes. First, obligate NBHF species have a reduced vocal repertoire, which may limit social interactions, contributing to the decreased sociality observed in these species (Figure 4), although more complex communication calls than previously known have been identified in *P. phocoena* (Sørensen, Wisniewska, Jensen, Johnson, Teilmann & Madsen, 2018). Second, when comparing BB and NBHF clicks of equal energy, the narrow bandwidth and the low noise window jointly provide the NBHF signal with the best echo-to-noise ratios for ranges shorter than some 100 meters (Kyhn, 2010), thus limiting the effective range of NBHF biosonar. Third, the higher frequencies in BB clicks can penetrate further, since source levels of a dolphin’s (e.g., *Tursiops truncatus* Montagu, 1821) BB clicks can be 30 to 80 times greater than the clicks of NBHF species (Villadsgaard et al., 2007). This may be particularly useful for detecting prey at longer ranges. Fourth, the much wider bandwidth of BB biosonar can provide more information in the echo than can narrow band biosonar (Simmons, Houser & Kloepper, 2014). All BB dolphins studied use lower frequency whistles for communication over longer distances (Rasmussen, Lammers, Beedholm & Miller, 2006) and BB clicks for communication at close ranges (Blomkvist & Amundin, 2004) as well as for orientation and hunting (Jensen, Bejder, Wahlberg & Madsen, 2009). Killer whales can hear dolphin whistles, as well as the lower frequencies in their BB clicks, but many dolphin species travel in pods, sometimes up to thousands (Culik, 2011), potentially gaining security in numbers (Morisaka & Connor, 2007).

Intriguingly, a recent paper documents BB clicks and whistles, as well as NBHF signals, from Commerson’s dolphin (*Cephalorhynchus commersonii* Lacépède, 1804) in Argentina (Reyes, Tosenberger, Ilíñiguez, Hildebrand & Melcón, 2016), although Commerson’s dolphins have been kept in captivity for decades without BB sounds ever being recorded. BB clicks have also been reported from another NBHF species, Haviside’s dolphins (*Cephalorhynchus heavisidii*) (Martin,
Gridley, Elwen & Jensen, 2018). The four *Cephalorhynchus* species have only evolved NBHF signals within the last two million years. In contrast, despite numerous recordings of phocoenid species in the wild and in captivity, neither BB signals nor whistles have been recorded in this, much older, lineage. Thus, the older lineages may be obligate NBHF species, while at least two species of *Cephalorhynchus* apparently also use BB clicks and whistles, indicating either adaptive value in conserving BB capabilities, or that these species are in a transition phase to becoming obligate NBHF species.

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**AUTHOR CONTRIBUTIONS**

LAM conceived of the study and designed it with AG, MTO, MES and RAR. LAM and LAK dealt with the acoustic aspects, MTO conducted the phylogenomic analyses, MES and RAR organized acquisition of CT scans, RAR generated and analysed the data on inner ear labyrinths, MES compiled fossil data, CDB performed the palaeoclimate modelling, and AG, LAK and LAM gathered and analysed the behavioural and ecological data. AG, MTO, LAK and LAM drafted the manuscript with input from the other authors. All authors approved of the final version of the manuscript.
FIGURE LEGENDS

Figure 1  Phylogeny of 41 extant odontocetes, illustrating the separate origin and convergent evolution of NBHF in four odontocete lineages. The phylogeny is based on an 11,388bp fragment of the mitogenome and divergence time estimates obtained by calibration with the fossil record (see text), Cenozoic epochs are indicated under the cladogram. The bowhead whale (*Balaena mysticetus*) was used as out-group. The blue node bars are 95% probability density estimates and node labels are the posterior branch support values (all above 0.500). The 10 species in bold are known to use NBHF biosonar. Five other species not shown here are known or presumed to use NBHF biosonar: *Kogia sima*, *Phocoenoides dalli*, *Phocoena spinipinnis*, *Phocoena sinus* and *Phocoena dioptrica*. The skulls of two predators potentially exerting selection pressure for evolution of NBHF signals, *Orcinus orca* and *Acrophyseter deinodon* are shown in Box I. Periods with fossil evidence for macroraptorial odontocete predators are indicated with grey-tinted boxes, in the early period (Box II) period killer sperm whales were present (timespans provided for three likely predators of small odontocetes), in the later period (Box III) members of the genus *Orcinus*. Photos courtesy of Mikkel Høegh Post (*Orcinus orca*) and Olivier Lambert (*Acrophyseter deinodon*), with permissions.

Figure 2  
PCA of nine cochlear measurements including extinct and extant NBHF odontocete species and other artiodactyls with variables factor map inset. The dataset includes measurements from novel scans as well as previous work to include six representative taxa from known extant NBHF species, five extinct relatives of extant NBHF species, the killer whale, and one proposed extinct predator (*Acrophyseter*). Squares indicate extant species and thick crosses indicate fossils from the present study. Circles and thin crosses represent extant and fossil taxa respectively from the previous studies (Churchill et al., 2016; Mourlam & Orliac, 2017). Representative digital endocasts of the
inner ear labyrinths are shown near their associated morphospaces. See Supplement Data S1 for taxa associated with the abbreviations used in the plot and comprehensive list of measurements.

**Figure 3** Palaeoclimatic modelling of mean annual wind speeds (A-C), as well as the relationship between sea state and thermal noise (D), showing that ambient ocean noise has remained constant (Beaufort 3-5, wind speed 3.4-10.7 m/s) at the current NBHF hearing optimum for at least 16 million years (A-C). Simulated wind speeds (the colour code bar at the right) are shown during Pliocene (ca. 3 Ma), Late Miocene (ca. 8 Ma) and Middle Miocene (ca. 14 Ma), respectively; and D) shows the relationship between thermal noise and sea states re-drawn and modified from Wenz (Wenz, 1962) with the NBHF hearing optimum marked in grey. (Resolution of the maps is 2.5-3.75 degrees. The maps were created in ArcGIS version 10.2.2 with the model output (Bradshaw et al., 2015; Bragg et al., 2012) and the late Miocene and middle Miocene palaeogeography of Markwick (Markwick, 2007). Simulated wind speeds have been corrected for systematic model bias using the modern (ERA-Interim (Dee et al., 2011)) mean annual wind speeds for 1979-2012 (courtesy of Dr. Jean Bidlot, The European Centre for Medium Range Weather Forecasts, Reading, UK (see Fig. S3)).

**Figure 4.** Odontocete size, behaviour and ecology imposed on the phylogenetic relationships. There is a clear tendency towards reduced size and low sociality in NBHF species (in **bold**), while they are found in all habitats. The four grey scale shades for each category attempt to cover the properties of the species. Information is taken from species entries in Perrin et al. (2009) and Culik (2011). Branch lengths are arbitrary and dashed branch lines indicate species appended following McGowen (2011); McGowen et al. (2009).
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