

Topographical Distribution of Lipids Inside the Mandibular Fat Bodies of Odontocetes: Remarkable Complexity and Consistency

Heather N. Koopman, Suzanne M. Budge, Darlene R. Ketten, and Sara J. Iverson

Abstract—Odontocetes possess unusual and specialized mandibular fat bodies in and around their lower jaws. These tissues have been proposed to facilitate sound reception and are composed of unusual endogenously synthesized lipids. Little is known about how the topographical arrangement of the lipid molecules in these tissues influences sound reception. We examined the lipid composition of the mandibular fat bodies, using a fine-scale approach, on six specimens (representing four odontocete families). We show that odontocete jaw lipids exhibit a complex structural three-dimensional topography. Different odontocetes synthesize and deposit slightly different molecules, but the relative arrangement of the lipids within each head showed marked consistency. Mandibular fats of beaked whales were uniquely dominated by isolauric acid (*i*-12:0). In contrast, the dolphin and porpoise biosynthesized isovaleric acid (*i*-5:0), while the pygmy sperm whale deposited medium-length (10–14 carbons) straight-chain lipids. In all heads examined, the shortest and branched-chain (“*z*”) fatty acids were concentrated in the center of the jaw fats, which connect intimately with the ears. We hypothesize that in odontocete jaws, this arrangement may serve to channel an incoming sound to the ears because sound travels slower through shorter branched-chain fatty acids than through longer straight-chain fatty acids.

Index Terms—Branched-chain fatty acid, delphinid, hearing, isolauric acid, isovaleric acid, kogiid, mandibular fat, odontocete, phocoenid, ziphiid.

I. INTRODUCTION

AMONG mammals, the toothed whales (*Suborder Odontoceti*) possess highly adapted ears, with distinct specializations for high-frequency underwater sound [1]. For odontocetes, sound is used in foraging, socializing, and navigation [2]. Although the mechanisms for the generation and transmission of high-frequency sound have received considerable attention in recent years [2]–[4], and recordings of characteristic acoustic

signals from many species have been made [5], there is little understanding of how sound is received by the odontocete head and transferred to the inner ear. Understanding the mechanisms of odontocete hearing has important conservation implications. Concern over possible negative impacts of sound in the ocean has recently received considerable attention for two important reasons: 1) There is a general increase in ocean noise, potentially affecting the marine-animal behavior and ecology, and 2) several recent mass strandings of beaked whales (Family *Ziphiidae*) have been linked to anthropogenic noise, particularly to certain types of sonar used in major military exercises [6]–[8].

It has been widely suggested that lipids play a unique and important role in the acoustics of toothed whales [2], [9]–[12]. Early work on the biochemical structure of the melon (the fat body located in the “forehead” region) of odontocetes led to the suggestion that the melon is a specialized lipid depot, adapted for echolocation (e.g., [13], [14]). The presence of unusual endogenously synthesized fatty acids (FA) and fatty alcohols (FAlc) in the triacylglycerols (TAG), and wax esters (WE) comprising the melon, and their arrangement in a complex three-dimensional manner suggest that the melon plays an important role in collimating outgoing high-frequency sound [9]–[11], [15]–[17].

Norris [18] was the first to propose that the large fat bodies found in and around the mandibular fossae of dolphins (Family *Delphinidae*) serve as part of the acoustic pathway, since these fats had different physical properties than the surrounding cranial tissue. Limited and early studies of the mandibular fat bodies suggested that they also contain high concentrations of unusual endogenous lipids, similar to those found in the melon (e.g., [15], [16], [17], [19]). However, little attention has been paid to the lipids in the mandibular fats at either gross or fine scales, or to how these properties might affect acoustic reception and functionality.

Here, we compare the composition and organization of the major lipid constituents in the mandibular fat bodies in five species of odontocetes (including representatives from families *Delphinidae*, *Phocoenidae*, *Ziphiidae*, and *Kogiidae*): Gervais’ beaked whale (*Mesoplodon europaeus*), Sowerby’s beaked whale (*M. bidens*), Atlantic spotted dolphin (*Stenella attenuata*), harbour porpoise (*Phocoena phocoena*), and the pygmy sperm whale (*Kogia breviceps*). This is the first study to map the concentrations of FA and FAlc components of mandibular TAG and WE in a three-dimensional framework. Our work permitted: 1) Examination of topographical patterns

Manuscript received April 7, 2005; revised June 24, 2005; accepted July 11, 2005. This work was supported by the Effects of Sound in the Marine Environment Program of the United States Office of Naval Research, Woods Hole Oceanographic Institution, the Seaver Foundation, and the Natural Sciences and Engineering Research Council (NSERC), Canada. **Guest Editor: D. S. Houser.**

H. N. Koopman is with the Biological Sciences, University of North Carolina at Wilmington, Wilmington, NC 28403 USA (e-mail: koopmanh@uncw.edu).

S. M. Budge was with the Department of Biology, Dalhousie University, Halifax, NS B3K 1M4, Canada. She is now with the Department of Food Science and Technology, Dalhousie University, Halifax, NS B3J 2X4, Canada.

D. R. Ketten is with Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA.

S. J. Iverson is with the Department of Biology, Dalhousie University, Halifax, NS B3K 1M4, Canada.

Digital Object Identifier 10.1109/JOE.2006.872205

TABLE I
ODONTOCETE SPECIMENS SAMPLED FOR ANALYSIS OF MANDIBULAR FAT COMPOSITION

Species and Common Name	Sex and Maturity status	# jaw fat subsamples (left/right)
<i>Mesoplodon europaeus</i>	Female	65/65
Gervais' beaked whale	Adult (<i>mother of specimen below</i>)	
<i>M. europaeus</i>	Male	37/37
Gervais' beaked whale	Calf (<i>of specimen above</i>)	
<i>M. bidens</i>	Male	20/21
Sowerby's beaked whale	Adult	
<i>Stenella attenuata</i>	Male	33/38
Atlantic spotted dolphin	Adult	
<i>Phocoena phocoena</i>	Male	20/20
Harbour porpoise	Adult	
<i>Kogia breviceps</i>	Male	0/33
Pygmy sperm whale	Adult	

of distribution of these lipid constituents within individuals; 2) comparison of these distribution patterns across species; 3) consideration of the potential functional significance of such organizational patterns for the transmission and channeling of sound from the environment to the ears; and 4) we also examined potential ontogenetic influences on mandibular fat composition in ziphiids using the data collected from an adult female beaked whale and her month-old calf.

II. MATERIALS AND METHODS

A. Sample Collection

We were able to collect fresh heads from one representative adult specimen from each of the five odontocete species, as well as from one young beaked whale calf (Table I). Because the tissues we were examining represent structural materials, comprised of lipids that are uniquely biosynthesized within the odontocetes according to phylogeny [10], [15], we used one head from each species to perform an extremely detailed mapping and characterization of its components. Specimens sampled in this study were all obtained from the Atlantic coast of North America during 2001–2002, and all had either stranded or died as the result of interaction with the fishing gear. All specimens were considered to be fresh (SI code 2) and all sexually mature [20], [21] with the exception of the *M. europaeus* (Gervais' beaked whale) calf, estimated to be one

month old [52]. Heads were removed during standard necropsy procedures, and either sampled immediately or frozen and subsequently thawed for later dissection. Preliminary sampling trials (data not shown) indicated that mandibular fat bodies are well protected by external tissues, and composition does not vary significantly with freezing and thawing over short periods. Fat bodies [see Fig. 1(b) and (d)] located inside the mandibular fossa ("inner") and those lying superficial to the mandible but subdermal to the blubber ("outer") were carefully removed from the skull and mandibles. Because these lipids are liquid at room temperature, the whole fat bodies were wrapped with a plastic and chilled until firm enough to subsample. Each fat body was divided into a series of transverse sections [Fig. 1(c)], and, from each section, a number of subsamples (each ca. 1 cm³) were collected [Fig. 1(d); Table I]. Every effort was made to analyze homologous samples from each specimen in order to facilitate comparisons across the species using digital images of sample collection. Bilateral sampling of four of the heads was also standardized with the use of digital photography. Samples were immediately placed in 2:1 chloroform:methanol with 0.01% butylated hydroxytoluene (BHT) and stored frozen until further processing.

B. Lipid Analysis

Total lipids were extracted from each sample following a modified Folch procedure [22], as previously described [23],

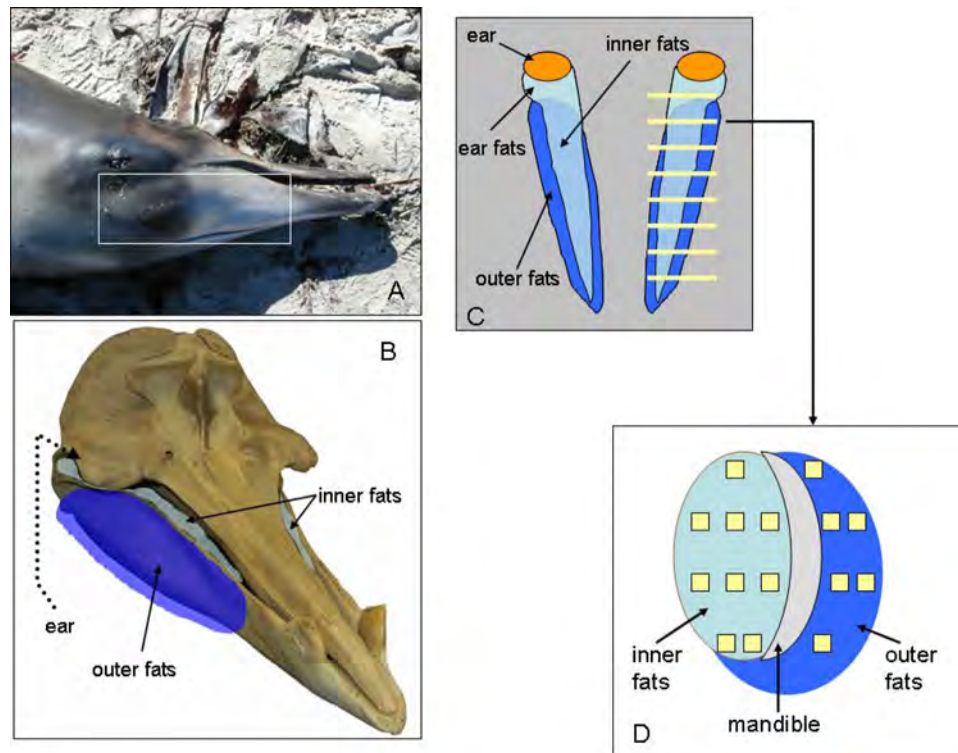


Fig. 1. Location and sampling regime for odontocete mandibular fats. (A) Image of intact *Mesoplodon bidens* head, with white box indicating positions of mandibular fats. (B) Skull of *Mesoplodon europaeus*, with schematic overlay of inner mandibular fats (lying inside the mandibular fossa; pale blue) and outer mandibular fats (external to the mandible but beneath the blubber; darker blue). Position of ear in skull is indicated by arrow. (C) Schematic of dorsal view of mandibular fats showing positions of transverse sections (represented by the yellow bars) collected from a representative head. Orange ovals represent the ears. (D) Schematic of frontal (cross sectional) view of representative transverse section oriented so that dorsal is at the top of the box; subsampling locations are indicated by small yellow squares. The numbers of sections and subsamples obtained from each head are given in Table I. *M. bidens* photograph taken by HNK *M. europaeus* skull photograph appears, courtesy of the Smithsonian Marine Mammal Program. (Color version available online at <http://ieeexplore.ieee.org>.)

[24], to yield extracted lipid and total lipid content (wet weight) and to ensure no losses of very short-chain FA. The lipid classes (TAG, three FA bound to a glycerol backbone, and WE, one FA bound to an FAlc) were separated by thin-layer chromatography (TLC) using 94/6/1 hexane/ethyl acetate/formic acid, visualized with dichlorofluorescein under UV light, and quantified by subsequent extraction of the silica-gel bands. The three main lipid components [FA from TAG (TAG-FA) and from WE (WE-FA), and FAlc from WE (WE-Alc)] were then analyzed separately by gas chromatography (GC). Isolated TAG were converted to FA butyl esters (FABE) using boron trifluoride in butanol, and analyzed on a Perkin-Elmer Autosystem GC fitted with a 30 m \times 0.25 mm i.d. column, coated with 50% cyanopropyl polysiloxane (J&W Scientific DB-23 column) and equipped with a flame ionization detector. Relatively, temperature-stable FABE were used to again ensure no loss of short-chain FA during both butylation and subsequent GC analysis. Temperature programs were set [23] to permit identification and quantification of both short- and long-chain FA components. WE-FA and WE-Alc in odontocete acoustic samples could not be quantified in a single GC run due to significant coelution problems, and, thus, had to first be separated for GC analysis. WE were esterified to produce FABE and free FAlc, which were then separated by TLC using 70/30/1 hexane/ethyl ether/acetic acid. FABE were then quantified by GC as above. FAlc were quantified on a 30 m \times 0.25 mm i.d. column coated with nitroterephthalic acid modified polyethylene glycol (Zebtron ZB-FFAP column) with the following

temperature program: Initial temperature 100°C, hold 5 min; increase temperature at 10.0°C/min to 250°C, hold 15 min. Internal standards were used to account for any losses during the WE processing. Identification of straight-chain FA was made from known standard mixtures [24]. Branched-chain FA were isolated using urea adducts [25] and their identities were confirmed with GC analysis. FAlc were identified from known standard mixtures, as well as conversion of a suite of FAlc from a representative sample into FA [26], for the conventional GC identification to confirm identity. FA and FAlc are named according to a short-hand notation of $X:Y_n-z$, where X is the number of carbons, Y is the number of methylene-interrupted double bonds, and z denotes the position of the last double bond relative to the methyl terminus. The three main classes of components (TAG-FA, WE-FA, and WE-Alc) were each converted to weight percentage (wt.%) of the total array of each class present. In addition, the use of standards and knowledge of lipid-class composition permitted the integration of all three components into a reconstruction of the complete lipid composition of each sample, incorporating TAG-FA, WE-FA, and WE-Alc. Results are presented as mean \pm standard error (SE), unless otherwise indicated.

III. RESULTS

Lipid content, major lipid class (TAG versus WE) composition, and the constituents (FA and FAlc) of the major lipid classes were determined in a total of 389 samples from the

TABLE II
CONTRIBUTIONS OF DOMINANT FA AND FALCs TO THE OVERALL TOTAL LIPID COMPOSITION OF THE MANDIBULAR FATS OF FIVE ODONTOCETE SPECIES

Species/site	% lipid (wet wt)	%WE (wet wt)	<i>i</i> -5:0 FA	<i>i</i> -10:0 FA	<i>i</i> -12:0 FA	12:0 FA	<i>i</i> -15:0 FA	<i>i</i> -16:0 FALc	16:0 FALc
<i>m.europaeus</i>									
(adult) inner	90.5	15.1	0.3	8.1	32.8	11.9	0.2	5.9	1.3
<i>M.europaeus</i>									
(adult) outer	86.9	21.0	0.2	0.7	5.1	11.7	0.5	2.8	9.3
<i>M.europaeus</i> (calf)									
inner	85.3	55.4	<0.1	0.4	1.8	10.0	0.3	2.7	16.9
<i>M.europaeus</i> (calf)									
outer	65.7	27.5	<0.1	<0.1	0.3	2.2	0.1	0.6	6.6
<i>M. bidens</i> inner	83.4	64.7	<0.1	6.9	28.5	4.7	<0.1	33.2	7.1
<i>M. bidens</i> outer	90.7	39.3	<0.1	1.4	9.7	13.4	<0.1	15.1	4.1
<i>Stenella</i> inner	84.7	57.9	26.1	<0.1	0.2	0.1	20.7	4.1	2.8
<i>Stenella</i> outer	70.1	10.0	22.0	<0.1	0.4	0.3	9.2	1.0	1.1
<i>Phocoena</i> inner	77.7	6.9	34.8	<0.1	0.5	1.7	7.1	-	-
<i>Phocoena</i> outer	77.2	1.2	20.9	0.2	1.2	2.8	3.0	-	-
<i>Kogia</i> inner	81.9	50.2	<0.1	0.8	3.1	20.8	0.3	3.4	14.1
<i>Kogia</i> outer	84.9	29.6	<0.1	0.2	1.0	10.8	0.4	1.5	14.9

For each specimen, data are provided for two representative sampling locations: where these specialized lipids occur in the highest concentrations (in the centre of the inner mandibular fat body – “inner”), and where they are present in lower concentrations (dorsal portion of the outer mandibular fat body – “outer”). For reference, lipid content and proportion of wax esters (WE) are reported; the balance of lipid content is almost entirely TAG. The five fatty acids (FA) and two fatty alcohols (FALc) were selected from a total of 118 components quantified in each sample. Values presented are wt% proportions of the total lipid composition (rather than wt% of lipid classes), thus FA components represent the sum of both WE-FA and TAG-FA. WE-FA and WE-FALc were not quantified in *Phocoena* due to extremely low yields of WE (see results); FA values for this specimen represent TAG-FA wt% adjusted for the relative concentrations of TAG vs. WE in each sample.

mandibular fat bodies of six individuals (Table I; Fig. 1). The lipid content was generally very high (> 75% wet weight) in most samples. The calf’s mandibular fats contained only slightly lower amounts of lipid, on average (mean inner lipid content $80.6 \pm 2.0\%$; outer $73.2 \pm 1.5\%$), than that of its mother (mean inner lipid content $83.9 \pm 1.9\%$; outer $80.7 \pm 1.7\%$). The mean lipid content in the inner mandibular fat

bodies of the other four specimens ranged from $66.7 \pm 6.0\%$ in *Kogia* to $82.3 \pm 1.6\%$ in *M. bidens*; mean outer mandibular lipid content ranged from $59.0 \pm 5.6\%$ in *Kogia* to $84.9 \pm 1.7\%$ in *M. bidens*. Unlike the heterogeneous distribution of individual FA and FALc components (see below), there were no consistent regions of high lipid content in either the inner or the outer mandibular fat bodies.

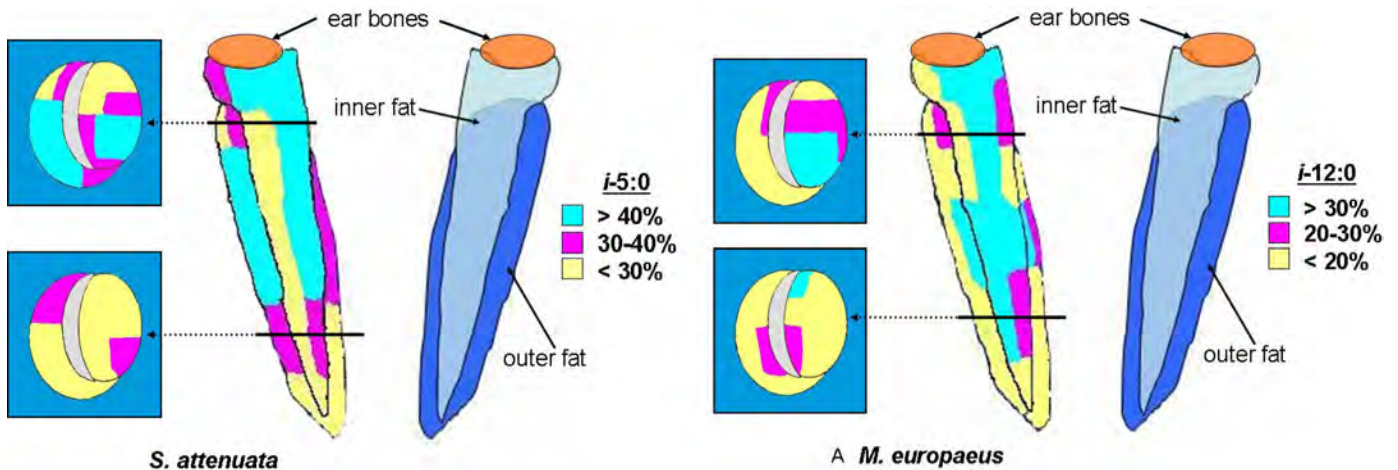


Fig. 2. Concentration gradient of the dominant fatty acid, *i*-5:0 (wt.% total TAG-FA), in the right mandibular fats [see Fig. 1(c)] of *Stenella attenuata*. Concentrations of *i*-5:0 are by color such that teal = highest, pink = intermediate, and yellow = lowest. Note that the highest concentrations of this fatty acid occur in the center of the inner mandibular fat body and around the earbones (orange ovals). Two cross sections (indicated by black bars) show frontal view [see Fig. 1(d)] of the concentration map; grey crescent in cross sections represents mandible. (Color version available online at <http://ieeexplore.ieee.org>.)

A. Lipid-Class Composition

All samples comprised of a mixture of TAG and WE; these two lipid classes were so dominant that all others (cholesterol esters, mono and diacylglycerols, phospholipids) were undetectable. Thus, only the WE levels are reported, and TAG can be assumed to comprise the balance of lipids in all samples.

The *Kogia* and adult beaked whale (*Mesoplodon*) samples contained the highest levels of WE as a proportion of the total lipids, at $36.1 \pm 2.4\%$ in *M. europaeus*, $44.9 \pm 4.7\%$ in *Kogia*, and $55.0 \pm 4.1\%$ in *M. bidens* samples. The *Stenella* samples contained less WE on average ($21.8 \pm 2.1\%$). In *Phocoena*, WE concentrations were extremely low, at only $5.0 \pm 0.6\%$; in many of the samples, WE were present only in trace (< 1 wt%) quantities. The low yield of WE from the porpoise samples precluded us from a comprehensive analysis of WE-FA and WE-FALC in *Phocoena* and, consequently, we report only the TAG-FA here.

Regardless of the overall WE concentrations, there was a considerable spatial variation in WE content within each head, with highest relative WE concentrations occurring in the caudal-most portions of the inner mandibular fat bodies, which are also the points connecting most intimately with the earbones.

B. Dominant FA and FALC in Each Species

We identified and quantified all FA and FALC in the TAG and WE lipid classes from each sample to provide a complete reconstruction of the mandibular lipid composition, and because FA and FALC differ both structurally and functionally. A total of 86 individual FA and 32 FALC were identified in most samples. In reporting the lipid composition below, we place an emphasis on the branched-chain (*i*-) FAs and FALCs, as these were generally present in high concentrations, and this is unusual among mammals. We first present FAs and FALCs as weight percentage of the lipid-class fraction from which they were extracted (i.e., as a proportion of all FA in TAG or all FALC in WE). We then present a reconstruction of the total lipid composition (including all FA and FALC, and accounting for relative proportions of WE and TAG in the total lipid, which will influence the overall FA

and FALC contributions to the sample) of the representative samples for each specimen (Table II).

Despite the large number of FA present, most samples were dominated by only a few compounds, the identity of which varied with family. Generally, concentrations of long-chain polyunsaturated and monounsaturated FA were negligible ($< 5\%$) across the species, with the exception of 16:1n-7, 16:1n-9, and 18:1n-9 in some samples/specimens.

In both *Stenella* and *Phocoena*, *i*-5:0 was the dominant FA, accounting for > 30 wt.% of TAG-FA (and of WE-FA for *Stenella*) in most samples (although amidst a considerable spatial variation; see below). *I*-15:0 was the second most prevalent FA in *Stenella*, representing 10–25 wt.% of all TAG-FA and WE-FA. In *Phocoena*, *i*-15:0 was also present in considerable concentrations, at 5–13 wt.% of all TAG-FA. In contrast, *i*-5:0 and *i*-15:0 levels in both *Kogia* and the two beaked whale species (*Mesoplodon*) were < 1 wt.% in TAG-FA and WE-FA. Other major FAs in the *Stenella* and *Phocoena* samples were 16:1n-7 and 18:1n-9. However, the levels of these two FAs in any given sample varied inversely to those of *i*-5:0 and *i*-15:0.

In adults of both *Mesoplodon* species, *i*-12:0 was the major TAG-FA and WE-FA, generally comprising > 30 wt.%, whereas this FA accounted for ≤ 5 wt.% in *Stenella*, *Phocoena*, and *Kogia* TAG and WE. Other principal FAs in the *Mesoplodon* TAG-FA and WE-FA fractions were *i*-10:0 (6–10 wt.%), 10:0 (4–11 wt.%), *i*-11:0 (3–17 wt.%), and 12:0 (10–26 wt.%).

Kogia samples exhibited greater concentrations of medium-length straight-chain FA, with 10:0 and 12:0 together accounting for approximately 25–32 wt.% of all TAG-FA and 35 wt.% of WE-FA. In contrast to all of the other specimens, branched-chain FA were negligible ($< 1\%$) in most of the *Kogia* samples, except for a few samples containing small quantities (2–3 wt.%) of *i*-12:0.

Interestingly, FALC were far less diverse, both in number and intraspecific variation. Only four components routinely represented > 5 wt.% of all FALC present: *i*-15:0 FALC, *i*-16:0 FALC, 16:0 FALC, and 18:1n-9 FALC. In all specimens (except *Phocoena*, in which FALC could not be quantified), 16:0 FALC

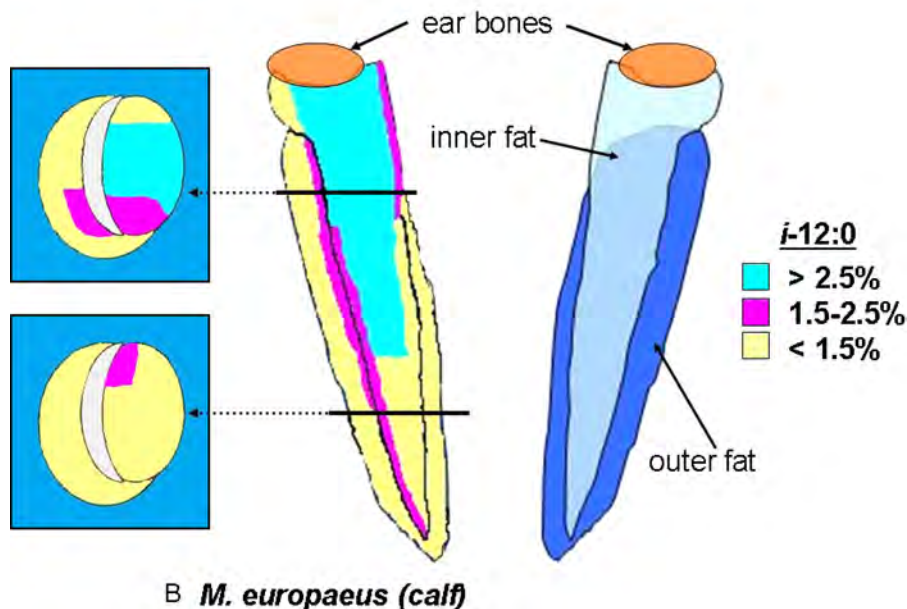


Fig. 3. Concentration gradient of the dominant fatty acid, *i*-12:0 (wt.% total TAG-FA), in the right mandibular fats [see Fig. 1(c)] of *Mesoplodon europaeus*. (A) Mature female and (B) her month-old calf. Concentrations of *i*-12:0 are given as weight percentage in the colored boxes, so that teal = highest, pink = intermediate, and yellow = lowest. Two cross sections (indicated by black bars) show frontal view [see Fig. 1(d)] of concentration map; grey crescent in cross sections represents mandible. Note the similarity in the distribution of *i*-12:0 in the mother and calf, despite the 10-fold concentration difference. (Color version available online at <http://ieeexplore.ieee.org>.)

and *i*-16:0 FALc were the dominant components, together representing >60 wt.% of all FALc. Only in *Stenella* was *i*-15:0 alc also a consistently significant fraction (12–34 wt.%).

C. Patterns of Spatial Distribution of FA and FALc

One of the most striking findings was the systematic distribution of individual lipids throughout the mandibular fat bodies. Regardless of identities of dominant lipid components or species, mandibular lipids exhibited a pattern of spatial distribution common to all heads, with the shortest and branched-chain compounds concentrated in the middle of the inner fat body and around the earbones. Usually, there was also a region of high concentrations of short-chain lipids overlying a portion of the mandible, in the outer mandibular fat. This was most clearly demonstrated in *Stenella* by the TAG-FA, where the concentrations of *i*-5:0 in the center of the inner fat body and around the ears were 44–51 wt.% (Fig. 2); the distribution of *i*-5:0 in *Phocoena* was comparable to that of *Stenella*. In both *Stenella* and *Phocoena*, the samples containing the highest concentrations of *i*-5:0 also contained the largest amounts of *i*-15:0. A similar pattern, but with different compounds (*i*-10:0 and *i*-12:0), was observed in adults of both *Mesoplodon* species (see Fig. 3). Unlike the other species, *Kogia* lacked high concentrations of branched-chain FA. However, the center of *Kogia*'s inner fat body was again dominated by the shortest chain FA present (10:0 and 12:0). In all heads, the regions with low concentrations of short-chain branched molecules contained mixtures of other medium- and longer chain FA, with no single component comprising more than 20%. There was also a predictable pattern to the distribution of FALc in the mandibular fat bodies, with *i*-16:0 FALc occurring in highest concentrations (ranging from 26 wt.% of FALc in *Kogia* to 75

wt.% in *M. bidens*) around the earbones and in the center of the inner fat body of all heads. In *Stenella*, the highest levels of *i*-15:0 FALc (up to 34 wt.%) were also found in the center of the inner mandibular fat bodies and around the ears.

In the calf of *M. europaeus*, the same branched-chain components (*i*-10:0 and *i*-12:0) were present in the mandibular lipids as were found in the adults, but at much lower concentrations (<2.5% of TAG-FA). Nevertheless, despite these low concentrations in the calf, there was a discernible pattern, remarkably similar to that of its mother, in the distribution of *i*-12:0 [Fig. 3(b)]. In the calf, as in the adult, the *i*-12:0 concentrations were highest around the ears and in the center of the inner fat body [Fig. 3(a) and (b)]. Finally, for all specimens in which both left and right mandibular fat bodies were examined (all but *Kogia*, Table I), a bilateral symmetry in composition and spatial arrangement of lipids was pronounced, as illustrated in both *Stenella* and *M. europaeus* (Fig. 4).

The significant contribution made by branched- and short-chain compounds to the center of the inner mandibular fat body is more evident when these constituents are expressed as proportions of the total lipid composition (Table II), rather than as weight percentage of each fraction. In *M. bidens*, for example, branched-chain lipids accounted for >68% of all lipids present in the inner jaw fats, but <30% of lipids in the outer jaw fat lipids (Table II). In *Stenella*, branched-chain lipids also occurred in higher concentrations in the inner mandibular fats (>50%) than in the outer samples (<35%), but in these species, this differential distribution was due to a different series of branched-chain lipids (Table II). This view of the data permits reconstruction of the total-lipid profile of the mandibular fats, and because it takes into account the different contributions made by the TAG and WE components, it permits examination of the data from

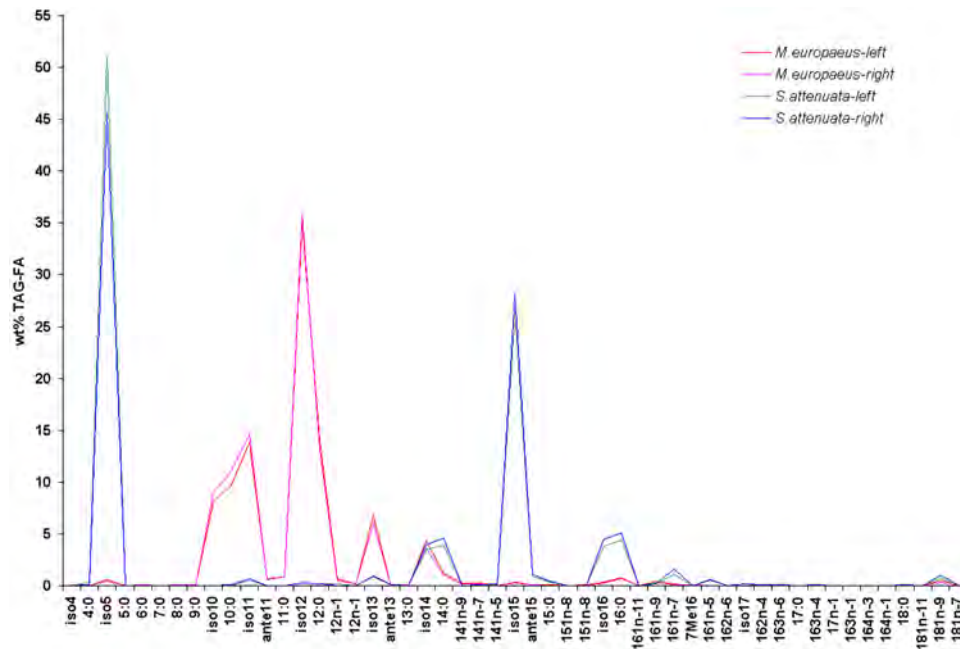


Fig. 4. Fatty-acid concentrations from GC analysis of samples collected from the middle of the inner fat channels on each side of the adult *Stenella attenuata* and *Mesoplodon europaeus* heads show the high degree of bilateral symmetry present. Data presented are weight percentages of total TAG-FA at the same site (left and right sides for each head). Although 86 individual FA were quantified in each sample, only 5–7 major peaks (>5 wt.%) were detected. All 34 components beyond 18:1n-7 were <0.2 wt.% and are not shown. (Color version available online at <http://ieeexplore.ieee.org>.)

another perspective. This presentation of the data also emphasizes the high degree of variability in identity of the dominant components of the mandibular fats for the different odontocete species (beaked whales = $i - 12 : 0$; dolphin, and porpoise = $i - 5 : 0$ and $i - 15 : 0$; pygmy sperm whale = straight chain 12:0 and 14:0).

IV. DISCUSSION

Our results demonstrate several significant new findings about odontocete mandibular lipids. The distribution of the mandibular lipids is far more complex than originally thought, in effect forming an internal channel of branched- and/or short-chain lipids inside the mandibular fat bodies. This pattern was remarkably consistent across a series of animals in which the synthesis of the specific lipid molecules appears to be phylogenetically constrained [10], [15], [16], [25]. In addition, our observations suggest that mirror-image channels exist on either side of the head. Our data also imply that the foundation for the complex arrangement of mandibular lipids is established at a very young age, but that acquisition of the full “adult” concentrations of individual FA and FALc may require additional development.

We mapped the complete lipid structure of the mandibular fats of the six individuals from the five odontocete species (Table I; Fig. 1). All heads were collected from fresh-dead specimens with no signs of decomposition or putrefaction. Because we were examining a structural material, which comprised of components that are uniquely biosynthesized within different families of odontocetes [10], [15], this can be expected to exhibit a relatively little variability [27]. In contrast to classic and metabolically active interabdominal or subcutaneous mammalian adipose depots, diet has no bearing

on the composition of odontocete acoustic lipids, as virtually all components are of endogenous origin [3], [24], [28]. For instance, the previous work on odontocete melons [10], [16], [17], [28] suggests a strong phylogenetic influence on acoustic lipid synthesis, and there is considerable evidence from at least two odontocete species that, like known structural adipose depots in other mammals (such as the eye socket fat pad in humans), acoustic lipids undergo no change in lipid content or composition during fasting and starvation [24], [27] and are, therefore, conserved and relatively metabolically stable. We consider the mandibular fats to be primarily structural elements of the odontocete body, and data from an individual can thus be used to generally characterize species trends.

A. Lipid Content and Lipid-Class Composition

The high lipid content of the mandibular fat bodies we examined is not surprising, as similar values have previously been reported for these tissues and for melons [9], [11], [28], [29]. We assume the balance of the tissue content (i.e., all nonlipid materials) to consist of vasculature, nervous tissue, and connective tissue, as sparsely distributed blood vessels and nerves are apparent upon gross examination of these tissues. However, a thorough examination of the microanatomical structure of the mandibular fats should be conducted to confirm this assumption.

Immature adipose tissue generally contains less lipid than that of adults, as the adipocytes require time to hypertrophy [30]. Therefore, we might have expected lower lipid-content values in samples collected from the month-old calf. However, the calf’s mandibular fat bodies contained only slightly less lipid than that of its mother; these values fell well within the range of lipid-content values observed in the other specimens. Even at one

month of age, the calf's mandibular fat bodies contained lipid at levels comparable to those of all adult heads; an observation that could be interpreted as an indication of the importance of investing in this tissue.

Although waxes are not commonly found in adipose tissue, the high WE content of the mandibular fat bodies we examined is not surprising. The WE content of our specimens parallels the previous observations of odontocete melons, and the WE in mandibular fat bodies have also been reported by other researchers [15], [28]. Yet, mammalian physiology has potentially undergone some major adaptations in the toothed whales to permit such a high degree of synthesis of waxes. WE are unusual because they are not found in the adipose depots of any other mammals [31], [32]. WE have different physical properties, as well as unique synthetic pathways that make them metabolically and physiologically distinct from TAG [31], [33]. Although some (but certainly not all) of the FA and FAlc constituents of WE can be traced to dietary sources, the formation of WE rather than TAG by adipose tissue is completely independent of diet. That is, eating WE does not lead to their deposition in adipose tissue [31], [32], as the metabolic processes of their digestion and synthesis are decoupled. Interestingly, WE are also known to occur in the blubber of some groups of odontocetes, which might suggest that WE synthesis represents a generalized physiological adaptation of toothed whales. Yet, the presence of WE in acoustic fats and blubber is not entirely parallel. Most species of toothed whales have some amount of WE in their acoustic tissues [15], [28], [29], but blubber does not appear to follow this pattern. Although WE have been observed in the blubber of a small number of individuals from a few delphinid species, a recent survey of a larger number of odontocete species and specimens indicates that the presence of WE as the dominant lipid class in blubber is entirely restricted to the ziphiids, kogiids, and physeterids [15], [29], [34], [35]. The potential functional significance of WE in blubber remains unknown. However, it is possible that these unusual lipids might play a role in acoustic pathways (see below) when they occur in the cranial adipose depots.

B. Topographical Distribution of FA and FAlc in the Mandibular Fat Bodies

Although our analysis was limited to only one adult specimen per species, the consistency of the patterns of distribution of FA and FAlc within each head was remarkable. It is important to stress here that the spatial arrangements of individual FA and FAlc are all relative: It is the positioning of the shortest chain (within each head) molecules in the middle of the inner mandibular fat bodies, and immediately adjacent to the ears, that displayed such uniformity across specimens. *I-5:0* was the dominant FA in the center of the inner mandibular fat body and around the ears of the dolphin and porpoise, and was also the shortest (five carbons) lipid constituent present in those heads. In contrast, the adult beaked whale's inner mandibular central "channels" were filled with *i-12:0* rather than *i-5:0*; these tissues contained only negligible (<1%) amounts of *i-5:0*, and no FA shorter than 12 carbons or FAlc shorter than 16 carbons at concentrations over 15%. Curiously, and unlike the other species we examined, the *Kogia* inner mandibular fat bodies were not

dominated by branched-chain FA. In the *Kogia* head, it was 12:0 and 14:0 (rather than branched-chain *i*-FA and *i*-FAlc) that formed the inner mandibular channel, but once again these were the shortest lipid constituents found in that head. Thus, it is the relative, rather than absolute, chain length within a head that is important in terms of the consistent heterogeneous distribution pattern that we describe here.

Although the FAlc showed less diversity both in number of components and among species, there was still a clear pattern to their distribution throughout the mandibular fat bodies, particularly the branched-chain alcohols. In all heads, (including the *M. europaeus* calf) *i-16:0* Alc occurred in higher concentrations in the inner fat bodies compared to the outer (Table II). All specimens (except *Phocoena*, in which FAlc could not be quantified) had the highest within-head concentrations of this branched-chain FAlc in the middle of the inner mandibular fat body and around the earbones, and, in *Stenella*, *i-15:0* Alc occurred at its highest levels in these same regions. Robische *et al.* [36] also reported high concentrations of *i-15:0* in the mandibular fat bodies of the narwhal, directly adjacent to the earbones.

Norris [18] originally proposed the mandibular acoustic waveguide as a simple homogeneous channel of fat leading from the environment to the ear. We have provided biochemical evidence suggesting that the system is actually far more complex, with the intricate arrangement of lipid compounds forming a well-defined channel inside the larger mandibular fat body. The fact that these patterns of distribution also appear to be bilaterally symmetrical (Fig. 4) confirms our contention of a highly organized arrangement of individual lipids within the mandibular fat bodies. Regardless of the physiological processes underlying the topographical distribution of these molecules (as yet unknown), or the functional significance of this arrangement (see hypotheses below), the mandibular fat bodies of odontocetes clearly exhibit a complicated characteristic directed molecular architecture that is consistent across species.

C. Sources of Mandibular Lipids

Previous studies on melon lipids have emphasized the unusual accumulation of *i-5:0* by dolphins, porpoises, and belugas, and the apparent lack of branched-chain FA in river dolphins and sperm whales [15], [24], [37], [38], but until now, little mention [17] has been made of the seemingly unique presence of *i-12:0* in beaked whales. Even more remarkable than the phylogenetic variation in the presence of various FA in cranial adipose depots is the fact that this diverse suite of lipids is biosynthesized by the animals. Although branched-chain FA can be products of bacterial decomposition [39], this is not the case in our study, as samples obtained and analyzed were extremely fresh, and both the magnitude of occurrence of these molecules and their extremely organized spatial patterns preclude such a possibility. Further, the diet cannot be the source of these molecules dominating the acoustic fats. Unlike longer (>14C) FA, which are transported to adipose tissues for deposition via chylomicrometers in the blood, ingested short- and medium-chain FA are oxidized immediately after intestinal absorption [40]–[42]. Short-chain FA with carbon lengths ≤ 6 can be synthesized by the mammary glands of some ungulates [43], but these are only straight-chain

forms; *i*-5:0, for example, does not occur in milk (see [44] and [53]). The branched-chain FA dominating the mandibular fats of the *Mesoplodons*, the *Stenella*, and the *Phocoena*, and the 10:0 and 12:0 of the *Kogia*, must therefore arise entirely from biosynthesis.

Our understanding of the biosynthetic pathways of branched-chain lipids in odontocetes is limited, but it is clear that like WE, the *i*-FA represent another departure from typical mammalian physiology. Unlike typical FAs, branched-chain FAs are formed through modification of amino acids [10], [45]. Additionally, branched-chain acids of different chain lengths have different amino acid precursors [45] and there is a strong phylogenetic influence on prevailing branched-chain acid type. *In vitro* labeling experiments [45] with fresh odontocete acoustic tissues have shown that *i*-5:0 and *i*-15:0, the dominant FAs in the dolphin and porpoise acoustic fats are derived from the breakdown of leucine. The *i*-12:0 dominating ziphiid tissues is a product of the valine catabolic pathway, as are *i*-14:0 and *i*-16:0. Why various odontocetes appear to utilize generally similar (modifications of amino-acid metabolism) yet slightly different (leucine versus valine precursors) pathways for the synthesis of branched-chain FAs, apparently according to some kind of phylogenetic pattern, will be a difficult question to answer. An added complication to this problem is apparent “replacement” of *i*-FA in the mandibular lipids of pygmy sperm whales with 10:0 and 12:0. What is clear is that these compounds, whether they be short- or medium-length straight-chain FAs, or branched-chain FAs, exhibit consistency in their distribution, strongly suggesting that there is some functional aspect to their positions in the mandibular fats.

D. Functional Implications for Observed Patterns of Lipid Distribution in the Mandibular Fat Bodies

It is clear that the distribution of lipids in the jaw fats is far more complex than originally thought, in effect, forming a channel of branched- and/or short-chain lipids inside the large fat body originally imagined as the acoustic pathway through the head [18]. This revelation permits the first detailed consideration of these specialized fat depots from a functional perspective. Empirical inverse relationships between carbon chain length and sound speed [46], [47] mean that acoustic waves will move faster through longer lipids than through shorter ones. In the mandibular fat bodies of all odontocetes we examined, the shortest FA present in each head were concentrated in the center of the inner fat body and immediately adjacent to the earbones. Theoretically, this arrangement would cause sound entering an odontocete head to bend inwards toward the center of the mandibular fat body and be directed to the ears. The high concentrations of short-chain FA in certain regions of the outer mandibular fat bodies overlying the thin part of the bone (Norris’ “acoustic window” [18]) are most obvious in *Stenella* and *M. europaeus* [Figs. 2 and 3(a)] and may also serve to direct sound. Similar patterns for *i*-5:0 have also been observed in a *Tursiops* (bottlenose dolphin) specimen [48], leading those authors to suggest that *i*-5:0 is preferentially deposited in that area in order to channel sound waves “to the interior mandibular waveguide.” Unfortunately, there are no empirical data in the literature on sound velocity through branched-chain FA or FAlc;

thus, these ideas can be based only on the acoustic properties of straight-chain FA.

It has also been suggested [49] that WE may exhibit lower sound velocities than many forms of TAG, as WE are, by nature, smaller molecules. In fact, Varanasi *et al.* [19] showed this to be true, using lipids extracted from the melon of a *Stenella attenuata graffmani* (coastal spotted dolphin) to demonstrate that even small increases in the proportions of WE, relative to TAG, led to considerable reductions in ultrasonic velocity. Altering the WE content also produced corresponding changes in density and compressibility, leading the authors [19] to conclude that small changes in lipid composition can achieve a “major acoustic accommodation” in dolphin melons. Interestingly, in all of the specimens we examined, the highest within-head WE concentrations were found in samples collected from the middle of the mandibular fat bodies and immediately adjacent to the earbones—exactly where the shortest FA were located. If sound does travel more slowly through WE than TAG in the jaw fats, as it does in the melon [19], then WE could be expected to have the same effect on sound pathways, as would high concentrations of short chain FA, of bending sound toward the earbones. This remains a tentative suggestion, as the only published data on the acoustic properties of WE [19] involved lipids dominated by *i*-5:0, not *i*-12:0 or those lacking *i* acids entirely. The role of the high concentrations of branched-chain FAlc in the middle of the inner mandibular fats and adjacent to the earbones also remains a mystery. Regardless of the dominant FA type, however, *i*-16:0 Alc was present in considerable quantities in all specimens, once again suggesting some kind of functional significance for this molecule.

Yet the concept of an acoustic channel formed by specific arrangements and concentrations of short-chain lipids does have support from studies on the other specialized cranial adipose depot: the melon. The melons of bottlenose dolphins (*Tursiops truncatus*) and pilot whales (*Globicephala melas*) both exhibit highly organized patterns of distribution of lipids, with an inner core containing high concentrations of *i*-5:0, and an outer shell with a much lower *i*-5:0 content [9], [11], [50]. Measurements of acoustic velocity through the lipid extracted from these two melon regions indicate that sound speed varies inversely with *i*-5:0 content, so that the regions with higher *i*-5:0 levels exhibit lower acoustic velocities [11], [49], [51]. Sound traveling through the delphinid melon, therefore, has a greater velocity toward the outside than through the central core, exactly as would be predicted based upon the lipid composition. Given what is accepted about the role that the arrangement of melon FA plays in the collimation of the outgoing sound [11], [49], it is reasonable to suggest that a corresponding series of adaptations in the lipid synthesis and deposition has evolved for sound reception. We, therefore, put forth the hypothesis that the arrangement of the lipid molecules inside the odontocete mandibular fat body functions as an acoustic waveguide to channel sound from the environment to the ear.

Our suggestion that the spatial arrangement of specific lipids in the mandibular fat bodies is fundamentally important for odontocetes garners additional support from the youngest specimen in our study. Despite the low concentrations of *i*-12:0 in the *M. europaeus* calf [Table II and Fig. 3(b)], its distribution

was remarkably similar to that of its mother [Fig. 3(a)] and to the distribution of other branched- or short-chain FA in other species (e.g., Fig. 2). This suggests that the complex organization of mandibular lipids may be a feature established fairly early in life. Yet, it also suggests that the formation and concentration of these lipids takes time to synthesize and develop, which may have implications for the ontogeny of hearing and echolocation in juveniles. Parallel physiological development hypotheses have been generated for the melon lipids of porpoises and dolphins [24], [37], but this is the first time the proposed sound reception lipids have been given such attention. If young animals do not possess the same acoustic apparatus as adult conspecifics, there may be ontogenetic differences in hearing.

We recognize that validating these ideas requires an entire suite of additional experiments, including: confirmation of the uniformity of this lipid distribution pattern in additional species and specimens and in complete ontogenetic series; determination of physical properties (melting point, phase-change properties) of key lipid constituents; empirical data on acoustic velocity through branched-chain lipids and WE; a better understanding of the metabolism of these unusual endogenous lipids; and studies on the development of echolocation and sound perception. A further challenge in this paper is presented by the relatively rare opportunity to obtain fresh tissues from standings of beaked whales. Nonetheless, the impressive complexity of the organization of such unusual FA and FALc in the mandibular fat bodies, combined with the remarkable consistency of this arrangement even when different molecular “building blocks” are used, is clearly a nonrandom physiological phenomenon with likely functional significance.

E. Concluding Remarks

The specific physical implications for sound transmission and reception, based on the differences in branched-chain acids in the jaw fats of toothed whales, remain unknown. Although our understanding of odontocete sound reception is limited, we know that the acoustic properties of organic compounds vary with chemical structure [46], [47], and that there will be an inherent variation in sound transmission through the fat bodies constructed from different materials. Thus, by nature of their mandibular lipids, odontocetes from certain families may receive and perceive sounds differently than those from others. How these biochemical differences are affected, or perhaps influenced, by other factors including peak echolocation frequency, habitat (coastal versus pelagic), diving behavior (shallow surface divers versus deep divers), and associated physiological effects (e.g., pressure and temperature differences) are as yet unknown and require investigation. Experiments aimed at tracing the specific path that artificially applied sound that follows through the mandibular fats and the rest of the head, using stranded or bycaught specimens from a variety of taxonomic groups, are needed to test our hypotheses.

ACKNOWLEDGMENT

The authors would like to thank the following organizations for access to samples and assisting with logistics: the Marine

Mammal Network of the Smithsonian Institution’s National Museum of Natural History; the Grand Manan Whale and Seabird Research Station, NB, Canada; The Marine Mammal Stranding Program at the University of North Carolina at Wilmington; the Virginia Marine Science Museum; and the National Marine Fisheries Service—Southeast. The authors also would like to thank A. J. Westgate for comments on an earlier version of the manuscript, and J. G. Mead, T. Cranford, and C. W. Potter for helpful suggestions during the early stages of the project. This manuscript was also improved by the comments of two anonymous reviewers. Samples were collected and all work were performed under appropriate permits issued by the Canadian and United States officials to the stranding networks and institutions listed above.

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Heather N. Koopman was born in Burlington, ON, Canada. She received the B.Sc. degree in marine biology, in 1992, and the M.Sc. degree in zoology, in 1994, from the University of Guelph, ON, Canada, while working with Dr. David Gaskin during both degrees. She received the Ph.D. degree in environment from Duke University, Durham, NC, in 2001, under advisor Dr. Andy Read.

She was a Postdoctoral Researcher in Woods Hole Oceanographic Institution until the end of 2003 while working with Dr. Darlene Ketten. She joined the University of North Carolina, Wilmington, in 2004, as an Assistant Professor in animal physiology. She is also a Research Associate at the National Museum of Natural History, Smithsonian Institution, and a Senior Scientist at the Grand Manan Whale and Seabird Research Station. Her research interests include biochemistry, functionality, and evolution of specialized lipids in marine vertebrates.

Dr. Koopman was awarded a Postdoctoral Fellowship from Woods Hole Oceanographic Institution 2001–2003.



Suzanne M. Budge received the B.Sc. degree in chemistry from Acadia University, NS, Canada, in 1993, and the Ph.D. degree in marine chemistry from Memorial University of Newfoundland, NF, Canada, in 1999.

She spent several years as a Postdoctoral Fellow in the Biology Department of Dalhousie University, NS, Canada. She is presently an Assistant Professor in the Food Science Program at Dalhousie. Her research interest is on the analysis of marine lipids, specifically their trophic cycling, and subsequent oxidation.



Darlene R. Ketten received the B.A. degree in biology and french from Washington University, Seattle, in 1971, the M.S. degree in biological oceanography from Massachusetts Institute of Technology, Cambridge, in 1979, and the Ph.D. degree from the Johns Hopkins University, Baltimore, MD, (jointly awarded neuroanatomy, behavioral ecology, and experimental radiology), in 1984.

She is a Marine Biologist and a Neuroanatomist specializing in functional and biomedical imaging of sensory systems. She currently holds joint appointments as a Senior Scientist in Biology at Woods Hole Oceanographic Institution, Woods Hole, MA, and as an Assistant Professor in Otolaryngology and Laryngology at Harvard Medical School, Boston, MA. Her speciality training includes otopathology at Harvard Medical School, in 1987, neuroradiology at Armed Forces Institute of Pathology (AFIP), in 2003, veterinary pathology at AFIP, in 2005, and forensic pathology at AFIP, in 1995 and 2005. She serves as a Specialty Lecturer on inner ear imaging, anatomy, and diagnostic pathology for American Medical Association—head and neck surgery courses. Her research focuses on two areas: how structural differences in marine versus terrestrial-vertebrate ears relate to physical differences habitats and feeding behaviors and how electrode placement and inner-ear pathologies impact the effectiveness of cochlear implants.



Sara J. Iverson received the Ph.D. degree in nutritional sciences from the University of Maryland, College Park, in 1988.

She is currently a Full Professor in the Biology Department at Dalhousie University, Halifax, NS, Canada, and an Affiliate Professor at the Institute of Marine Science, School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks. Her work focuses on the physiology and metabolism of lipids in vertebrates and their implications for understanding ecology and life histories of free-ranging animals.