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Characterization of lipids in adipose depots associated with minke and fin whale ears: Comparison with "acoustic fats" of toothed whales

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In an underwater environment where light attenuates much faster than in air, cetaceans have evolved to rely on sound and their sense of hearing for vital functions. Odontocetes (toothed whales) have developed a sophisticated biosonar system called echolocation, allowing them to perceive their environment using their sense of hearing (Schevill and McBride 1956, Kellogg 1958, Norris *et al.* 1961). Echolocation has not been demonstrated in mysticetes (baleen whales). However, mysticetes rely on low frequency sounds, which can propagate very long distances under water, to communicate with potential mates and other conspecifics (Cummings and Thompson 1971).

The mechanism of sound reception in cetaceans has been debated for centuries. Cetaceans have lost the external pinna and the ear canal has also been reduced to a narrow, sometimes discontinuous channel (Lillie 1915, Yamada 1953). The bones containing the middle and inner ears have migrated out of the skull in what is called the tympano-periotic complex (Hunter 1787, Eschricht and Reinhardt 1866, Kernan 1919, Mead and Fordyce 2009). The increased separation between the skull and ears is thought to reduce bone conduction, aiding directional hearing under water (Claudius 1858 in Yamada 1953, van Heel 1962).

In the 1960s the "jaw hearing" hypothesis was proposed for odontocete cetaceans (Norris 1964). Odontocetes possess unusual mandibles, which have enlarged mandibular hiatuses filled with discrete fat bodies that are in direct contact with the tympano-periotic complex. These fats also cover the outer parts of the mandible in most

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species. It had been noted earlier that physical properties of sound in water are similar to those in most body tissues (Reysenbach de Haan 1957), so the ear canal is not well-suited for underwater sound reception. However, Norris suggested that the fat bodies associated with the mandibles act as a preferential pathway for sound to get from the aquatic environment to the ears because "fat especially is closely impedance-matched to sea water" (Norris 1968). While the detailed mechanisms are still unclear, Norris's theory has been subsequently validated by behavioral, physiological, and anatomical studies (*e.g.*, Bullock *et al*. 1968, Brill *et al*. 1988, Ketten 2000).

The "acoustic fats" involved with odontocete sound reception are an example of a structural fatty tissue, as opposed to a storage tissue. Whereas the volume and lipid composition of storage fat, such as human abdominal fat and marine mammal blubber, generally change with body condition and diet, structural fats, such as those found in the feet, joints, and eye sockets, are metabolically inert and do not expand during obesity or shrink during fasting (Pond 1998). These structural fats contain fewer dietary components than storage tissues. The fatty melon in the odontocete forehead, which is part of the high frequency sound transmission pathway during echolocation, is another structural "acoustic fat" in odontocetes. Cranford *et al*. (1996) noted that the melon remains intact even in emaciated animals, and Koopman *et al*. (2003) showed that the lipid content and fatty acid (FA) composition of the melon is stable across body conditions, while the blubber lipids show significant differences between robust and emaciated individuals.

Odontocete acoustic fats are unique in that they are comprised of endogenously synthesized lipids that are not typically found in mammalian adipose tissues. While typical mammalian fat is primarily composed of triacylglycerols (TAG), with individual FA having chain lengths of 14–22 carbon atoms (Pond 1998), odontocete acoustic fats contain high levels of short, branched chain FA with 5–16 carbons. The acoustic fats also contain wax esters, a class of lipids synthesized by several groups of marine organisms but not synthesized in other mammals (Nevenzel 1970; Varanasi and Malins 1970*a*, *b*, 1971; Litchfield and Greenberg 1974; Litchfield *et al*. 1975; Morris 1986; Koopman *et al*. 2006). Wax esters and short, branched FA have not been found in mysticete tissues examined to date (Ackman *et al*. 1965; Tsuyuki and Itoh 1970; Lockyer *et al*. 1984, 1985; Ackman and Lamothe 1989; Olsen and Grahl-Nielsen 2003; Reynolds *et al*. 2006; Ruchonnet *et al*. 2006; Budge *et al*. 2008).

The lipids within the acoustic fats are arranged in a specific pattern, with wax esters and shorter, branched FA found in the highest quantities in the inner core of the acoustic fat depots (Litchfield *et al*. 1973, Wedmid *et al*. 1973, Morris 1975, Varanasi *et al*. 1975, Blomberg and Lindholm 1976, Koopman *et al*. 2006). Because sound travels more slowly through wax esters and shorter, branched FA than through TAG and longer, straight chain FA (Guow and Vlugter 1967, Hustad *et al*. 1971, Flewellen and Morris 1978), it has been hypothesized that the topographical arrangement of these lipids serve to focus sound in the outgoing echolocation beam and the incoming sounds to the ear (Litchfield *et al*. 1973, Norris and Harvey 1974, Blomberg and Lindholm 1976, Koopman *et al*. 2006). Measurements of sound speed through different regions of the melon have supported this notion (Norris and Harvey 1974, Blomberg and Lindholm 1976, Goold and Clarke 2000). However, recent studies indicate that there may be subtle variations in these patterns (Goold and Clarke 2000, Zahorodny *et al*. 2009) and some studies have concluded that the majority of the sound refraction and collimation occurs at the tissue/seawater interface rather than within the acoustic fat, owing to factors such as the curvature of the head and the higher sound speed of seawater compared to acoustic fats (Litchfield *et al*.

1979). The acoustic fats are also well-vascularized, which may provide an additional mechanism for altering sound speed profiles through temperature regulation (Houser *et al*. 2004, Costidis and Rommel 2012).

Although it is widely accepted that specialized fat bodies are involved in odontocete sound reception, sound reception pathways in mysticete cetaceans remain unknown. However, a recent study by Yamato *et al*. (2012) identified a well-formed fat body referred to as "ear fats" in the minke whale (*Balaenoptera acutorostrata*), one of the smallest and most abundant mysticete species. These fats attach to the tympano-periotic complex and contact the malleus, which raises the question of whether mysticetes also use fatty tissues for sound reception.

The purpose of this study was to identify the biochemical composition of these newly described fat bodies in mysticetes and compare them with the "acoustic" fats of odontocete cetaceans. We used samples from two Balaenopteridae: minke and fin whales (*Balaenoptera physalus*). The three main questions we addressed in this study are: (1) Do the fats associated with the ears of baleen whales contain wax esters, short, branch-chained FA, or other unusual lipids? (2) Do the lipids within the fat bodies display any topographical distribution patterns similar to some odontocete acoustic fats? (3) Are there any systematic differences in the lipids of the ear fats and blubber? In addition, we examined the effect of tissue decomposition on FA profiles, the effect of body site and body condition on blubber lipid content and FA composition, and compared tissues from the two species of baleen whales.

Tissues were available from the ear fats of three minke whales (B-acu18, B-acu19, B-acu22) and one fin whale (B-phy11). All specimens stranded on or were found floating off of Cape Cod, Massachusetts. B-acu18 was a female that stranded in August 2007, B-acu19 was a female that stranded in June 2008, B-acu22 was a male that stranded in May 2011, and B-phy11 was a male that stranded in February 2009. All individuals were subadults. Body condition was categorized as "robust," "thin," or "emaciated" by experienced stranding network personnel based on standard observations. The area of the epaxial muscle in robust individuals is convex, while it is hollowed in emaciated individuals. Thin animals have an intermediate appearance, with a slight sunken aspect to the dorsal-lateral body. Prominent indentations of the nape are another indicator of emaciation (Pugliares *et al*. 2007). Ear fats were extracted from the right side of each animal. For B-acu18 and B-acu22, fat bodies were also extracted on the left side. The extracted fat bodies were sectioned transversely and then subsampled in a grid to provide three-dimensional representation of all regions, with approximately 25 subsamples per ear fat. Subsamples were approximately 2 cm × 2 cm × 2 cm.

In addition to the ear fat samples, blubber was sampled above the pectoral fin on the mid-dorsal side for each animal, which is a standard collection location for the stranding network. Several previous studies found that there are no significant differences in FA composition between blubber from different body sites in mysticetes (Tsuyuki and Itoh 1970, Ruchonnet *et al*. 2006, Budge *et al.* 2008). However, Reynolds *et al*. (2006) reported that in one bowhead whale, the blubber from sites along the umbilical girth contained slightly more omega-3 FA compared to blubber from sites along the axillary girth (10.14%, SD 2.16 *vs*. 7.41%, SD 4.58). Furthermore, Lockyer *et al*. (1984, 1985), and Ruchonnet *et al.* (2006) reported that the lipid content was lower in ventral blubber compared to dorsal blubber in fin whales and sei whales (*Balaenoptera borealis*), although Ackman *et al.* (1975) found variable results across six individuals of fin whales and four individuals of sei whales, perhaps due to body condition of the individual. Therefore, we also sampled blubber from

three additional body sites in one individual (B-acu22): in the region of the external auditory meatus, mid-lateral blubber, and mid-ventral blubber over the ventral grooves.

It is well known that lipid content and composition of blubber vary with depth from the epidermis: the lipid content is generally highest in the external blubber except in pregnant females, and internal blubber is more reflective of the diet compared to external blubber (Ackman *et al*. 1965, Lockyer *et al*. 1984, Aguilar and Borrell 1990, Koopman *et al.* 1996, Olsen and Grahl-Nielsen 2003, Ruchonnet *et al*. 2006, Koopman 2007). Therefore, each piece of blubber was subsampled from the surface, the middle, and the deepest layer except for B-acu19, in which the sampled piece of blubber was too small to subsample. The blubber sample location was labeled "unknown" for B-acu19.

Total lipids were extracted from each sample (~0.5 g) following a modified Folch procedure (Folch *et al*. 1957, Koopman 2007) to obtain lipid content (% wet weight). Lipid classes were quantified and analyzed *via* Thin Layer Chromatography with Flame Ionization Detection (TLC/FID) with an Iatroscan Mark VI using 94/6/1 hexane/ethyl acetate/formic acid as the solvent. For FA analysis, total lipids were converted to FA butyl esters using BF_3 in butanol (10% Supelco), and analyzed using gas chromatography (GC) with FID on a Varian 3800 GC fitted with a Zebron ZB-FFAP nitroterephthalic acid modified polyethylene glycol 30 m × 0.25 mm column (Phenomenex, Torrance, CA). Butyl esters were used instead of the more commonly used methyl esters because short chain FA are more volatile and likely to be underestimated as lighter methyl esters (Shantha and Napolitano 1992, Koopman *et al*. 1996, Budge *et al*. 2006). Sixty-six FA were identified from known standard mixtures (Nu-Chek Prep, Inc., Elysian, MN; Koopman *et al*. 2003). Sixteen of the FA were consistently found above 0.5% and were included in our analyses (see Table 1). Quantities were expressed as the percentage of the total weight (wt%). Further details of the methods are described in Koopman *et al*. (2006) and Koopman (2007).

FA profiles for all samples were examined using the statistical software program Primer 6 (Plymouth Routines In Multivariate Ecological Research, Primer-E, Ltd., Ivybridge, U.K.). In Primer, resemblance matrices were created using the Bray-Curtis method (Clarke and Gorley 2006). This was followed by a nonmetric, multidimensional scaling analysis (NMDS), which represents each sample on a two-dimensional map according to the resemblance matrix. The algorithm is an iterative process and the confidence level of the output is represented by the "stress value." A low stress value (<0.1) indicates that the model is confident in placement of the samples relative to each other, while a high stress value (>0.2) indicates that the relationships between the samples may not be represented faithfully (Clarke and Warwick 2001, Clarke and Gorley 2006).

Analyses of Similarity (ANOSIM) tests were conducted to determine the effect of species and sample type. ANOSIM is the approximate analogue of the standard univariate 1- and 2-way Analysis of Variance (ANOVA) tests to assess whether differences in FA profiles exist between groups of samples specified by a particular factor. Under the null hypothesis that there are no differences between groups of samples, the histogram of the permutation distribution of the test statistic R is centered on 0. The global R value is the observed test statistic, which ranges from approximately 0–1, with higher values indicating more deviation from the null hypothesis (Clarke and Gorley 2006). When differences between sample groups were found in ANOSIM, one-way similarity percentages analyses (SIMPER) were conducted to determine which FA were driving the differences.

Table 1. The major fatty acids present in minke and fin whale ear fats and blubber. The numbers indicate quantities in wt%, averaged from all samples of the tissue (SD in parentheses). The second column ("type") is taken from Iverson *et al*. (2004)'s data from detailed studies on marine carnivores (pinnipeds and minks), with B = all or primarily from biosynthesis or biotransformation, D = all or primarily from direct dietary intake, and b = relatively large contributions from both biosynthesis and diet.

		B-acu18		B-acu19		B-acu22		B-phy11	
Fatty acid	Type	Ear fat	Blubber	Ear fat	Blubber	Ear fat	Blubber	Ear fat	Blubber
14:0	b	4.7 (0.2)	3.9 (0.6)	4.9 (0.4)	4.7	5.6 (0.6)	5.5 (1.1)	5.0 (0.2)	4.9 (0.3)
16:0	b	11.6 (1.5)	6.7 (1.9)	12.1 (1.3)	9.5	13.0 (3.1)	10.1 (1.5)	18.0 (0.9)	16.0 (1.5)
16:1n-7	b	9.6 (1.1)	5.1 (2.6)	13.7 (0.6)	13.3	12.0 (1.0)	8.0 (2.8)	8.6 (0.5)	7.5 (2.5)
18:0	b	3.4 (1.2)	4.1 (0.7)	2.4 (0.3)	2.1	2.6 (0.6)	2.7 (0.7)	4.0 (0.4)	4.2 (0.9)
18:1n-11	B	4.7 (0.9)	5.5 (0.7)	4.0 (0.2)	4.1	4.0 (0.4)	3.0 (1.2)	1.4 (0.1)	1.4 (0.1)
18:1n-9	b	20.2 (1.0)	18.9 (0.2)	17.7 (0.4)	16.2	19.1 (1.0)	14.6 (1.2)	26.8 (0.8)	24.7 (1.0)
18:1n-7	b	4.6 (0.1)	3.7 (0.5)	5.0 (0.2)	4.5	4.1 (0.3)	3.2 (0.4)	6.3 (0.2)	5.6 (0.1)
18:2n-6	D	1.7 (0.0)	1.6 (0.1)	1.1 (0.0)	1.0	1.4 (0.1)	1.4 (0.2)	2.2 (0.1)	2.0 (0.0)
18:3n-3	D	0.8 (0.1)	0.4 (0.2)	0.5 (0.0)	0.4	0.7 (0.1)	0.6 (0.1)	1.1 (0.0)	1.0 (0.1)
20:1n-11	D	4.5 (0.4)	7.4 (2.1)	3.6 (0.1)	3.7	3.8 (0.3)	3.9 (1.1)	1.4 (0.1)	1.7 (0.3)
20:1n-9	D	12.4 (0.7)	16.8 (3.1)	11.2 (0.6)	12.0	10.1 (0.7)	12.3 (1.9)	3.9 (0.1)	4.5 (0.8)
20:4n-3	D	0.6 (0.1)	0.5 (0.2)	0.5 (0.0)	0.6	0.5 (0.2)	0.9 (0.2)	1.0 (0.1)	1.0 (0.1)
20:5n-3	D	1.4 (0.3)	1.1 (0.6)	2.4 (0.5)	3.5	2.2 (1.0)	3.9 (0.8)	2.4 (0.4)	2.6 (0.7)
22:1n-11	D	8.5 (0.5)	10.2 (1.1)	7.1 (0.7)	7.7	7.4 (0.7)	9.9 (0.3)	2.0 (0.1)	2.2 (0.3)
22:5n-3	b	1.2 (0.3)	1.9 (0.1)	1.3 (0.4)	2.2	0.9 (0.5)	2.7 (0.6)	1.8 (0.3)	2.9 (0.4)
22:6n-3	D	1.6 (0.6)	1.8 (0.6)	1.9 (1.0)	3.5	2.7 (1.8)	6.3 (2.1)	3.4 (1.2)	6.8 (0.7)
Total B+b		60.0	49.8	61.1	56.6	61.3	49.8	71.9	67.2
Total D		31.5	39.8	28.3	32.4	28.8	39.2	17.4	21.8

We adopted the commonly used notation A:Bn-X, where A indicates the number of carbon atoms in the chain, B is the number of double bonds, and X is the position of the first double bond relative to the terminal methyl (CH_3) group. An italicized *i* before the A:Bn-X notation indicates a branched FA with a methyl branch at the second carbon (see Budge *et al*. 2006). Individual FA were identified as originating from biosynthesis (endogenous) or from direct dietary intake following the classification of Iverson *et al*. (2004; Table 1). Endogenous lipids include FA with chain lengths of less than 14 carbons, which are oxidized immediately following ingestion (Pond 1998). Dietary lipids originate either entirely or primarily from direct dietary intake, and include lipids such as 20:1n-9 and 22:1n-11, which have a specific source in calanoid copepods and organisms feeding on calanoid copepods (Falk-Petersen *et al*. 2000). FA that may originate from the diet but also have a large contribution from biosynthesis and biotransformation were classified in a separate category. An example of the latter case is 16:0, the primary product of *de novo* synthesis in marine predators according to Budge *et al*. (2006).

Fresh mysticete tissue samples are rare, and all specimens in our study were classified as Code 3 (moderate decomposition). The effect of tissue decomposition on FA profiles was examined by comparing the right and left ear fat samples of B-acu18. The ear fat samples from the right side were extracted and frozen 2 d after the animal was first seen, floating in Vineyard Sound, Massachusetts. The animal was classified as "Early Code 3" and was estimated to have died the day before the sighting. The rest of the head was frozen for one year. After being completely thawed, the specimen was placed at room temperature for 5–8 h a day for 4 d, with refrigeration at 4°C in between each session. The specimen was then left in a chiller at 4°C for 7 d before the ear fat from the left side was extracted and subsampled for FA analysis. At this time, the tissue was quite decomposed and would be classified as "Late Code 3."

In answering our first main question, we did not find any wax esters, short, branch-chained FA, or other unusual lipids in our samples. We found that the majority of lipids within both ear fats and blubber consisted of TAG, which is typical for mammalian adipose tissues. For all ear fat samples combined, the average TAG content was 95.4 ± 8.7 wt%. For all blubber samples combined, the average TAG content was 99.1 ± 0.75 wt%. Lipid class composition was individual- and tissue-specific. All blubber and ear fat samples of B-acu18, B-acu19, and B-phy11 were composed of >98% TAG. The blubber of B-acu22 contained 99% TAG, while the ear fat samples of B-acu22 contained on average approximately 84% TAG, 1% sterol ester, 6% free FA, 2% cholesterol, and 6% phospholipid.

Previous studies have found these non-TAG lipids in fin and sei whale blubber (Bottino 1978, Lockyer *et al.* 1984, Ruchonnet *et al*. 2006). Lockyer *et al*. (1984) attributed the presence of these other lipid classes to decomposition artifacts. Therefore, the high levels of non-TAG lipids in B-acu22 may be due to decomposition. However, the ear fat samples of B-acu18L, which were more decomposed than B-acu22, still contained over 95% TAG. Another possibility is that the lipid class composition may change through development, as B-acu22 was the largest of the minke whales in our study. A greater sample size of fresh tissues as well as tissues from different age classes is necessary for further exploration of this issue. For reference, odontocete mandibular acoustic fats have wax ester at levels ranging from 1.2% in the outer mandibular fat body of the harbor porpoise (*Phocoena phocoena*) to more than 60% in the inner mandibular fat body in Sowerby's beaked whale (*Mesoplodon bidens*; Koopman *et al*. 2006). Blubber of delphinid, monodontid, and phocoenid odontocetes contains very little (0%–10%) wax esters, whereas the blubber of kogiid,

physterid, and ziphiid odontocetes contains high levels of wax esters (60%–100%; Koopman 2007).

Both ear fats and blubber of minke and fin whales were primarily composed of medium to long-chain FA ranging from 14 to 22 carbons in length, in agreement with previous studies on minke and fin whales (Ackman and Lamothe 1989, Moller *et al.* 2003, Olsen and Grahl-Nielsen 2003). Shorter or branched chain FA such as 12:0, 13:0, *i*-14:0, *i*-15:0, and 15:0 were present in the ear fats at quantities <1 wt %. There was a significant difference between the FA composition of the fin whale compared to the three minke whales (Fig. 1 ANOSIM global $R = 0.958$, $P < 0.01$, stress = 0.09). The average dissimilarity of 25.42% between the two groups was primarily driven by the FA 18:1n-9, 20:1n-9, 16:0, and 22:1n-11 (SIMPER, 17.83%, 16.63%, 13.54%, and 13.20% contributions to overall dissimilarity). All of the minke whale samples contained lower levels of 16:0 and18:1n-9 and higher levels of 20:1n-9 and 22:1n-11 compared to the fin whale samples (see Table 1).

For our second main question, we did not find any topographical distribution patterns that are found in some odontocete acoustic fats. There was no significant difference in FA composition of the anterior sections *vs*. posterior sections of the ear fats (ANOSIM global $R = 0.049$, $P > 0.01$) or the dorsal *vs*. ventral sections of the ear fats (ANOSIM global $R = 0.092$, $P > 0.01$). We did find that there was a large spatial variability in the lipid content of ear fats, ranging from less than 10% lipid by wet weight to greater than 90% lipid. The mean lipid content value for all samples was 61 ± 24 wt %. All samples with lipid content values of less than 10% were from ventral locations, consistent with a transition to a fibrous joint with the mandible.

For our third main question, we found systematic differences in the lipids of the ear fats and blubber. The ear fat samples from all three minke whales were more similar to each other compared to blubber (Fig. 1). The diversity in blubber FA composition between individuals is consistent with previous studies. For example, Budge *et al.* (2008) found that the blubber FA of bowhead whales (*Balaena mysticetus*) vary

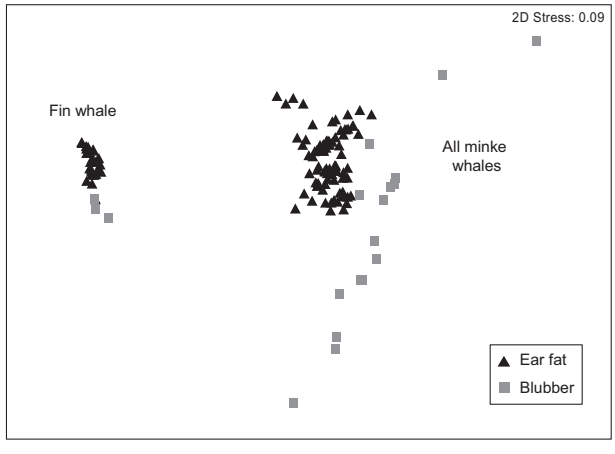

Figure 1. NMDS plot of all fatty acid data of ear fat and blubber samples for minke and fin whales based on a Bray-Curtis resemblance matrix. Data points represent subsamples from a total of four different individuals. Subsamples that are more similar to each other are placed closer together on the two-dimensional map than subsamples that are dissimilar from each other.

with age, season, and year, but not with sex. The minke whales used in this study were all subadults that stranded between the months of May and August, but were from different years (2007, 2008, and 2011).

There was a significant difference in the FA composition of blubber and ear fat samples for the minke whales (ANOSIM global $R = 0.777$, $P < 0.01$, stress = 0.11). The average dissimilarity of 16.43% was primarily driven by 16:1n-7, 18:1n-9, 22:6n-3, and 16:0 (15.22%, 12.56%, 12.00%, and 10.61% contributions to dissimilarity; see Table 1). There was also a significant difference in the FA composition of blubber and ear fat samples in the fin whale (ANOSIM global $R = 0.958$, $P < 0.01$, stress = 0.06). The average dissimilarity of 8.49% was primarily driven by 22:6n-3, 18:1n-9, 16:1n-7, and 16:0 (22.77%, 14.45%, 14.35%, and 14.09% contributions to dissimilarity). Blubber contained higher levels of dietary FA compared to ear fat samples (Table 1), indicating that the ear fat may be less metabolically active than blubber. Studies comparing the lipids of blubber and acoustic fats of odontocetes have shown that the FA found in the blubber have higher dietary components, with consistently higher average chain lengths than those found in the acoustic fats (Ackman *et al*. 1971; Varanasi and Malins 1971; Litchfield *et al*. 1975, 1976; Koopman *et al*. 2003).

There was a slight but significant difference in the FA composition of samples from the right ear fat of B-acu18 (relatively fresh) and left ear fat of B-acu18 (more decomposed) (ANOSIM global $R = 0.325$, $P < 0.01$; Fig. 2). The average dissimilarity of 6.45% was driven by the FA 18:0, 16:0, 18:1n-11, and 16:1n-7 (15.91%, 15.35%, 12.77%, and 11.85% contributions to overall dissimilarity). These difference between the right and left ear fat of B-acu18 were much smaller than the difference between the ear fat of B-acu18 compared to the blubber of B-acu18 (ANOSIM global $R = 0.782$, $P < 0.01$; see Table 1), which had an average dissimilarity of 14.44%, driven by the FA 16:0, 16:1n-7, 20:1n-9, and 20:1n-11 (18.45%, 17.26%, 16.89%, and 11.10% contributions to overall dissimilarity).

The lipid composition and content of blubber was strongly stratified through its depth. In all individuals, lipid content increased from the inner layer of blubber to

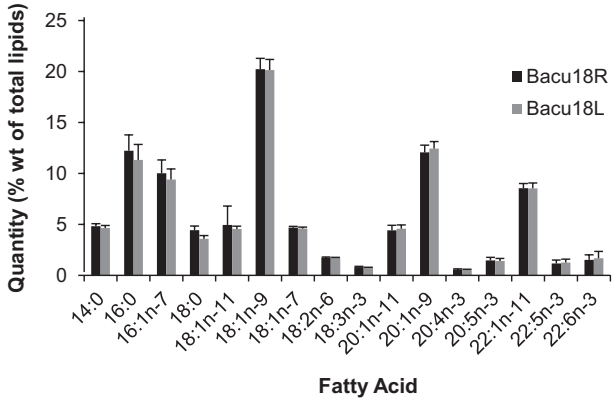

Figure 2. Fatty acid profiles for the ear fat samples from minke whale B-acu18. The right side (B-acu18R) was sampled in a relatively fresh state (Early Code 3) and the left side (B-acu18L) was sampled 11 d later with intermittent refrigeration (Late Code 3). Error bars indicate one standard deviation. The quantities are expressed in % weight of the total lipids.

the outer layer of blubber, closest to skin (Table 2), which is consistent with previous studies on fin whales (Ackman *et al*. 1965, Lockyer *et al*. 1984, Aguilar and Borrell 1990). However, it should be noted that Koopman (2007) found that the inner layer of blubber contained more lipid than the outer layer in some odontocete species. Blubber from animals described as "robust" had the highest lipid content values (55.5%–86.4%), while thin or emaciated animals had blubber with low lipid content (8.9%–46.2%). These values are within previously reported ranges for fin whales of comparable body conditions (Ackman *et al*. 1975).

In contrast to several previous studies on fin, sei, and bowhead whales (Lockyer *et al.* 1984, 1985; Reynolds *et al.* 2006; Ruchonnet *et al*. 2006), we did not find striking differences in the FA composition or lipid content of blubber from various body sites for our minke whale specimen B-acu22. We did not find any significant differences in the FA composition of dorsal blubber, ventral blubber, lateral blubber, and the blubber in the region of the external auditory meatus (global $R = -0.281$, $P > 0.1$). On average, dorsal blubber contained 79.6% lipid (SD = 14.4), ventral blubber contained 77.0% lipid (SD = 16.6), lateral blubber contained 72.0% lipid (SD = 11.9), and blubber from the external auditory meatus contained 67.7% lipid (SD = 20.1).

While the blubber lipid content was strongly influenced by body condition, the lipid content values of the ear fats were much more stable across individuals. For example, the blubber of the emaciated individual (B-acu18) was depleted in lipid, but the average lipid content of its ear fat was still comparable to that of robust individuals and consisted of >50% lipid (Fig. 3). While the conservation of lipid in the tissue does not necessarily point to an acoustic function it is consistent with the ear fat being a structural fatty tissue and more than just an additional site for lipid storage, in agreement with previous studies on the acoustic fats of the melon (Cranford *et al*. 1996, Koopman *et al*. 2003).

In summary, we described for the first time the lipid composition of the fatty tissues associated with minke and fin whale ears. Unlike odontocete acoustic fats, the mysticete ear fats in our study did not contain wax esters or short, branched chain FA and are instead composed of lipids typically found in mammalian adipose tissues. In the ear fat of one minke whale (B-acu22), we did find low levels of non-TAG lipids, which have been previously reported for fin and sei whale blubber (Bottino 1978, Lockyer *et al.* 1984, Ruchonnet *et al*. 2006). However, a greater

Table 2. Lipid content (% wet weight) of minke and fin whale specimens used in this study.

Individual	Body length	Body condition	Blubber sample depth	% lipid
B-acu18	430 cm	emaciated	inner	8.9
			middle	11.2
			outer	27.6
B-acu19	465 cm	robust	unknown	73.6
B-acu22	530 cm	robust	inner	55.5
			middle	77.3
			outer	86.4
B-phy11	1,221 cm	thin	inner	24.3
			middle	40.2
			outer	46.2

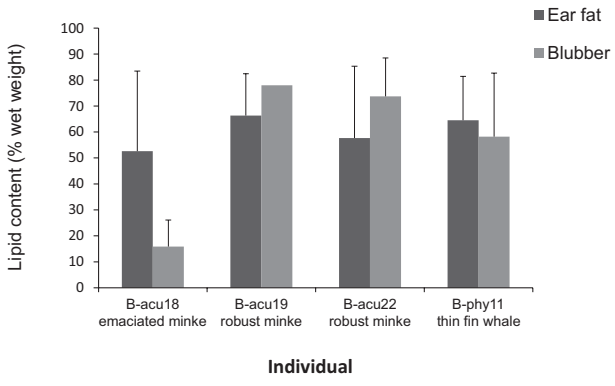

Figure 3. Lipid content of ear fats compared to blubber in three minke whales and one fin whale. B-acu18 was an emaciated individual and B-phy11 (the fin whale) was a thin individual. Lipid content data was only available from the left side of B-acu18. For B-acu22, data from both right and left ear fat samples were pooled. Error bars indicate one standard deviation.

sample size of fresh, mature individuals is necessary to understand the presence of these lipids.

We did not see any fine scale topographical distribution patterns similar to odontocete acoustic fats. However, we found systematic differences in the lipids of the ear fats compared to blubber. All ear fat samples from the minke whales converged to a similar FA profile, while the blubber FA profiles were more variable. Like odontocete acoustic fats, the ear fat lipids are conserved under starvation and have fewer dietary components compared to blubber, indicating that the tissue is more than just an additional site for lipid storage.

It has been recognized that there is a large variability in the identity of the lipids found in various odontocete taxa and that no single lipid turns a fat body into "acoustic" fat (Litchfield *et al*. 1975, Morris 1986). Although the precise reason for having wax esters and short, branched FA in acoustic fats is unknown, they all reduce sound speed through the acoustic fat compared to normal fats and surrounding tissues (Gouw and Vlugter 1967, Hustad *et al*. 1971, Bamber and Hill 1979, Duck 1990). Because sound bends towards regions of slower sound speed, using a particularly low sound speed tissue in their hearing pathway may help to focus sound towards the ears of odontocetes. The mysticete ear fats, having a lower sound speed than the surrounding, nonfatty tissues (Bamber and Hill 1979, Duck 1990), may also help to channel sound towards the ears.

Validating the idea that fat bodies composed of typically mammalian lipids may also be acting as "acoustic" fats in some mysticete cetaceans will require additional experiments that are beyond the scope of this study. Although collection of adequate samples from mysticete specimens is logistically challenging, future investigations should include additional species as well as individuals of different age classes because ontogeny plays a role in the composition of odontocete acoustic fats (Gardner and Varanasi 2003, Koopman *et al*. 2006, Koopman and Zahorodny 2008). Furthermore, other potentially important functions of the fatty tissue must be investigated. The location of the fatty tissue coincides with the temporo-mandibular joint region of the

head (Lambertsen *et al*. 1995), leading to speculations that it may also be involved in other functions besides sound reception in a way that is similar to the multi-purpose odontocete mandible, which is involved in both feeding and sound reception (Yamato *et al*. 2012).

Odontocetes and mysticetes both face the challenge of listening entirely under water, where external pinnae and air-filled ear canals are ineffective for collecting and amplifying sound. It is proposed that both suborders of cetaceans have evolved to incorporate fatty tissues into their auditory systems for aquatic sound reception. The different lineages of odontocetes may have subsequently acquired the ability to synthesize and deposit wax esters and short, branched FA as they specialized in echolocation and ultrasonic hearing.

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