Comparing Gas Chromatographic Techniques Used in Fatty Acid Profiling of Northern Fur Seals (*Callorhinusursinus)* and Steller Sea Lions (*Eumetopiasjubatus*) from Lovushki Island Complex, Russia

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Abstract

Northern fur seal (NFS, Callorhinusursinus, n = 22) and Steller sea lion (SSL, Eumetopiasjubatus, n = 12) blubber samples were collected from adults occupying the same rookery near the Lovushki Island complex, Russia. The objective of this study was to compare identified fatty acid methyl esters (FAMEs) using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) between species for each lipid class (saturated, SFA; monounsaturated, MUFA; polyunsaturated, PUFA). GC-FID identified an average of 26 FAMEs from each species against a set of 37 FAMEs. ANOVA detected differences between detectors and species, with GC-MS recovering greater numbers of total FAMEs but with fewer SFA detected. Interestingly, the GC-MS recovered greater numbers of FAMEs for NFS when compared to SSL. The use of both GC-FID and GC-MS, rather than solely one method, seems appropriate in order to avoid drawing spurious conclusions regarding potential resource partitioning in ecological studies.

Key Words: Fatty Acids, GC-FID, GC-MS, Northern Fur Seal, Resource Partitioning, Steller Sea Lion

Introduction

There are several approaches commonly used in which to identify fatty acids or their derivatives (fatty acid methyl esters, FAME), including gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy and silver ion thin-layer chromatography (TLC, Skoog et al.1998). These methods have been used to identify the common fatty acids of animal (Budge et al. 2006) and plant (Glew et al.1997) origin, with chains typically numbering from 12 to 24 carbon atoms, including zero to six double bonds, as well as specializednon-methylene-interrupted double bonds (Budge et al. 2007). Of these techniques, gas chromatography (GC) along with any one of a number of detectors (Harris 2003), offers a simple, rapid and relatively inexpensive method for the identification or quantification of FAME in lipid research. While somewhat dependent on the detector, GC has a straightforward derivatization procedure (e.g., Budge et al. 2006), uses readily available reagents and has simple preparatory requirements. Another important asset of GC is that it is not typically necessary to isolate lipid components in pure form, as may be required by other methods (e.g., NMR spectroscopy).Currently, a considerable number of lipid researchers useGC-FIDfor FAME analysis in topics ranging from agriculture to biomedicine to ecology. However, there has been a dramatic increase in fatty acid analysis and interpretation in animals inhabiting the marine environment, specifically marine mammals. Topics of current research interest include, for example,climate change (Cooper et al. 2009), predator-prey dynamics (Iverson et al. 1997), andage-related lipidchanges(Trumble et al. 2010).

As beneficial as GC is, as with any method, there are limits associated with its use; GC-MS spectra may not always contain ions indicative of structural features (e.g.,the positions of double bonds in the aliphatic chain cannot always be definitively determined) or forGC-FID, there can be a failure to differentiate between *cis* and *trans* isomers causing misidentification of FAME.

Identification using GC-FID may also behampered by contaminants or coeluting compounds. Another limitation of using GC-FID is obtaining adequate standards, as standards are not available for many of the fatty acids found in mammalian tissue, especially for the more complicated polyunsaturated fatty acids.Therefore, there are instances when FAME analysis is best served by a combination of GC-MS andGC-FID, either for confirmatory purposes (to ensure the correct identification of a peak) or as anexploratory guide for further work (e.g., Best et al. 2003; Newland et al. 2009).

Materials and Methods

Our investigation comparesFAMEusing commonly utilized GC detectors in the blubber tissue of sympatric pinnipedspecies inhabiting remote Eastern Asia. As part of a larger study on resource partitioning, these samples were collected from the Lovushki Island complex, Russia (Figure 1), from sexually dimorphic and piscivorous adult Northern fur seals (NFS, *Callorhinusursinus*)and Steller sea lions (SSL, *Eumetopiasjubatus*).Approximately half of the total SSL breeding population in Russian waters occurs on these rookeries (Burkanov and Loughlin 2005) and after declining in numbers for 20 years, the population has remained relatively stable from 1995 through 2005 (Burkanov and Loughlin 2005). In contrast, a rapid increase in NFS population numbers on Lovushki Island began in the 1950's resulting in the current population being near their historic high (Burkanov et al. 2007).

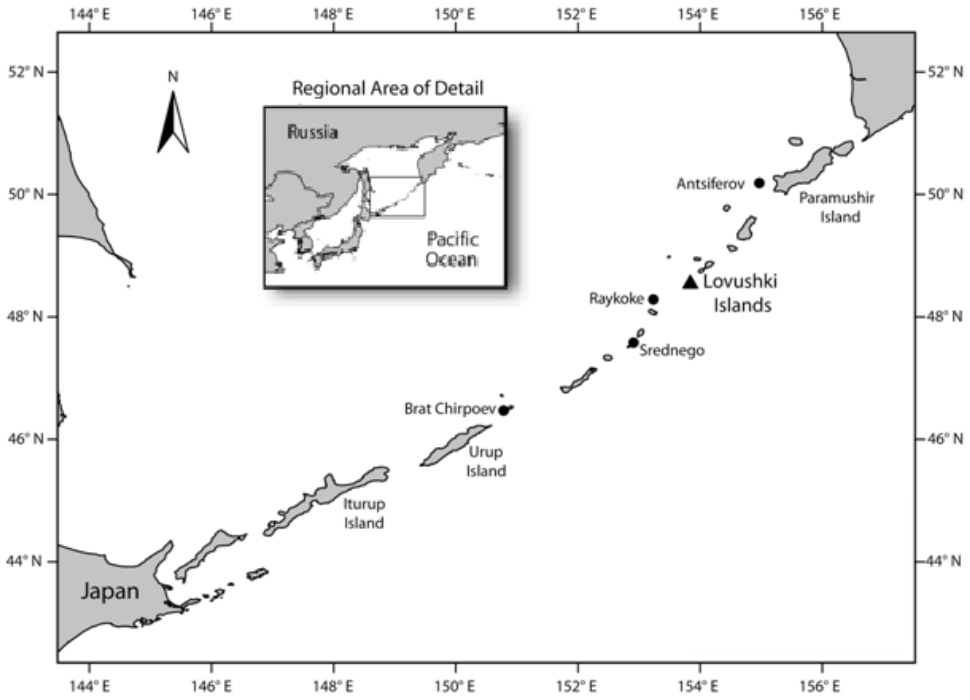

Figure 1. Lovushki Island Complex of the Kuril Island Chain, Russia (modified from Waite et al. 2012)

The goal of this study is to establishand compare FAME analysis from blubber samples from NFS and SSL using both GC-FID and GC-MS techniques, and todetermine which particular technique, if not a combination, provides the best method for characterizing blubber in these sympatrically breeding marine mammals. A secondary objective is to establish similarity indices of lipid profiles using each technique. Given the common use of GC techniques to describe FAME, we feel comparing results from samples taken from a field studywill provide information on the limits of each technique and thus allow researchers to make more informed decisions regarding methodology during ecological studies.

Blubber samples from juvenile male and non-lactating female Northern fur seal (NFS, n=22) and Steller sea lions (SSL, n=12) were collected from the same rookery near the Lovushki Island complex (Figure 1, Kuril Island chain) in Russia (48º 32.617' N, 153º 40.417' E) during the breeding season of 2008.To access the blubber sample from the ventral hip area, a 2 cm^2 area was shaved and then cleaned using a solution of 70% ethyl alcohol and betadinebefore each incision.

Biopsies were collected (30-50 mg taken under general anesthetic; 1 ml, Isoflurane) using a 4 mm biopsy cannula (Depuy, Warsaw, IN, USA). All blubber samples were frozen and stored inliquid nitrogen until later analysis. All samples were weighed after thawing to the nearest 0.001g (wwt).

Lipid extraction procedures included drying each sample with sodium sulfate (approximately 50 g, J.T. Baker, Phillipsburg, NJ) before running through an Accelerated Solvent Extractor (Dionex ASE 350, Sunnyvale, CA), using up to 100 ml dichloromethane (VWR, West Chester, PA) as the solvent.Similar laboratory conditions were maintained for all extractions.Excess solvent was evaporatedunder a steady stream of nitrogen (1.5 L/min @ 37C).The resulting lipid extract was then transesterifiedusing a method similar to Budge et al. (2006).Briefly, the samples were added to 1.5 ml dichloromethane (DCM)and 3 ml Hilditch reagent (Sigma Aldrich, St. Louis, MO), and then heated at 100°C for 1h. The lipids were then separated out by extraction by repeated centrifugation (100 X*g* for 2-5 min) with DCM (3 ml for first centrifugation, 1 ml for latter two) and deionized water (1 ml for each centrifugation). The lipid layer (bottom) was then dried with sodium sulfate (approximately 0.5 g), and heated in a water bath to evaporate solvent. Each transesterifiedsample was divided into two equal aliquots and analyzed for FAME on a GC-FID (Varian 430-GC and Varian CP-8400 AutoSampler) and subsequently on an Electron Ionization GC-MS(Varian GC 3900/MS Saturn 2100T) using, in both cases,the same CP-Select column (CP7419, Varian) 100 m x 0.25 mm ID x 0.25 um. GC-FID protocols followed Budge et al. (2006) with the following modifications: the column length was 100m;the injector temperature was 250^{o}C with a 1µl injector split ratio 100:1. Column flow was 1.0 ml min^{-1} programmed at 210^{o}C for 9.0 minutes and ramped at 15^{o}C min^{-1} to 260^{o}C for 7.7 minutes.Detector temperature was set at 300^{o}C with a hydrogen flow of 30 ml min^{-1} and airflow of 300 ml min^{-1}.The internal standard was C19:0 (Fluka 72332).

A set of 37 standard historical marine FAME (Supelco® 37 component FAME mix) were used in the GC-FID analysis (Table 1).The FAME were obtained from Sigma-Aldrich (St. Louis, MO) and Supelco® (Bellefonte, PA), chosen primarily for their physiological relevance to fatty acids commonly found in marine organisms, and were of the highest purity available.Blanks were run between each true sample. For each level of calibration, all FAME target analytes were present at equal concentrations with concentrations between 0.25 µg/µL and 10 µg/µL for each standard curve generated.

Table 1.FAME Standards with Elution Order

Elution Order	FAME Formula	Systematic Name	Common Name
1	C4:0	Butanoic acid, methyl ester	Butyric acid methyl ester
2	C6:0	Hexanoic acid, methyl ester	Caproic acid methyl ester
3	C8:0	Octanoic acid, methyl ester	Caprylic acid methyl ester
4	C10:0	Decanoic acid, methyl ester	Capric acid methyl ester
5	C11:0	Undecanoic acid, methyl ester	Undecylic acid methyl ester
6	C12:0	Dodecanoic acid, methyl ester	Lauric acid methyl ester
7	C13:0	Tridecanoic acid, methyl ester	Tridecylic acid methyl ester
8	C14:0	Tetradecanoic acid, methyl ester	Methyl myristate
9	C14:1	9-Tetradecenoic acid, methyl ester	Myristoleic acid methyl ester
10	C15:0	Pentadecanoic acid, methyl ester	Pentadecylic acid methyl ester
11	C15:1	*cis*-10-Pentadecenoic acid, methyl ester	None
12	C16:0	Hexadecanoic acid, methyl ester	Methyl palmitate
13	C16:1	9-Hexadecenoic acid, methyl ester	Palmitoleic acid methyl ester
14	C17:0	Heptadecanoic acid, methyl ester	Methyl margarate
15	C17:1	*cis*-10-Heptadecenoic acid, methyl ester	Heptadecenoic acid methyl ester
16	C18:0	Octadecanoic acid, methyl ester	Methyl stearate
17	C18:1n-9(t)	*trans*-9-Octadecenoic acid, methyl ester	*trans*-Methyl oleate
18	C18:1n-9(c)	*cis*-9-Octadecenoic acid, methyl ester	*cis*-Methyl oleate
19	C18:2n-6(t)	*trans*,*trans*-9,12-Octadecadienoic acid, methyl ester	*trans*-Methyl linoleate
20	C18:2n-6(c)	*cis*,cis-9,12-Octadecadienoic acid, methyl ester	*cis*-Methyl linoleate
21	C18:3n-6	6,9,12-Octadecatrienoic acid, methyl ester	GLA, methyl ester
22	C20:0	Eicosanoic acid, methyl ester	Methyl arachidate
23	C18:3n-3	9,12,15-Octadecatrienoic acid, methyl ester	ALA, methyl ester
24	C20:1	*cis*-11-Eicosenoic acid, methyl ester	Gondoic acid methyl ester
25	C21:0	Heneicosanoic acid, methyl ester	None
26	C20:2	*cis*-11,14-Eicosadienoic acid, methyl ester	Eicosadienoic acid methyl ester
27	C20:3n-6	*cis*-8,11,14-Eicosatrienoic acid, methyl ester	DGLA, methyl ester
28	C22:0	Docosanoic acid, methyl ester	Methyl behenate
29	C20:3n-3	11,14,17-Eicosatrienoic acid, methyl ester	ETE, methyl ester
30	C22:1n-9	13-Docosenoic acid, methyl ester	Methyl erucate
31	C23:0	Tricosanoic acid, methyl ester	Tricosylic acid methyl ester
32	C20:4n-6	5,8,11,14-Eicosatetraenoic acid, methyl ester	Methyl arachadonate
33	C22:2	*cis*-13,16-Docosadienoic acid, methyl ester	Docosadienoic acid methyl ester
34	C24:0	Tetracosanoic acid, methyl ester	Lignoceric acid methyl ester
35	C24:1	Nervonic acid methyl ester	Nervonic acid methyl ester
36	C20:5n-3	5,8,11,14,17-Eicosapentaenoic acid, methyl ester	EPA, methyl ester
37	C22:6n-3	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	DHA, methyl ester
N/A (IS)	C19:0	Nonadecanoic acid, methyl ester	None

For GC-MS analysis, the samestandards (including the C19:0 internal standard)were analyzed on an Electron Ionization (EI) mass spectrometer with the same flow rates and temperature programs as above. These standards were used to determine FA elution order, and to confirm that the same standards utilized with the GC-FID are also properly identified on the GC-MS. The NIST (National Institute of Standards and Technology) library was also utilized with GC-MS to confirm peaks matched with standards (utilizing both retention times and structural details), and additionally, to identify novel peaks in our samples using highest probability methodswith retention times, structural details, and isotopic patterns (Figure 2).

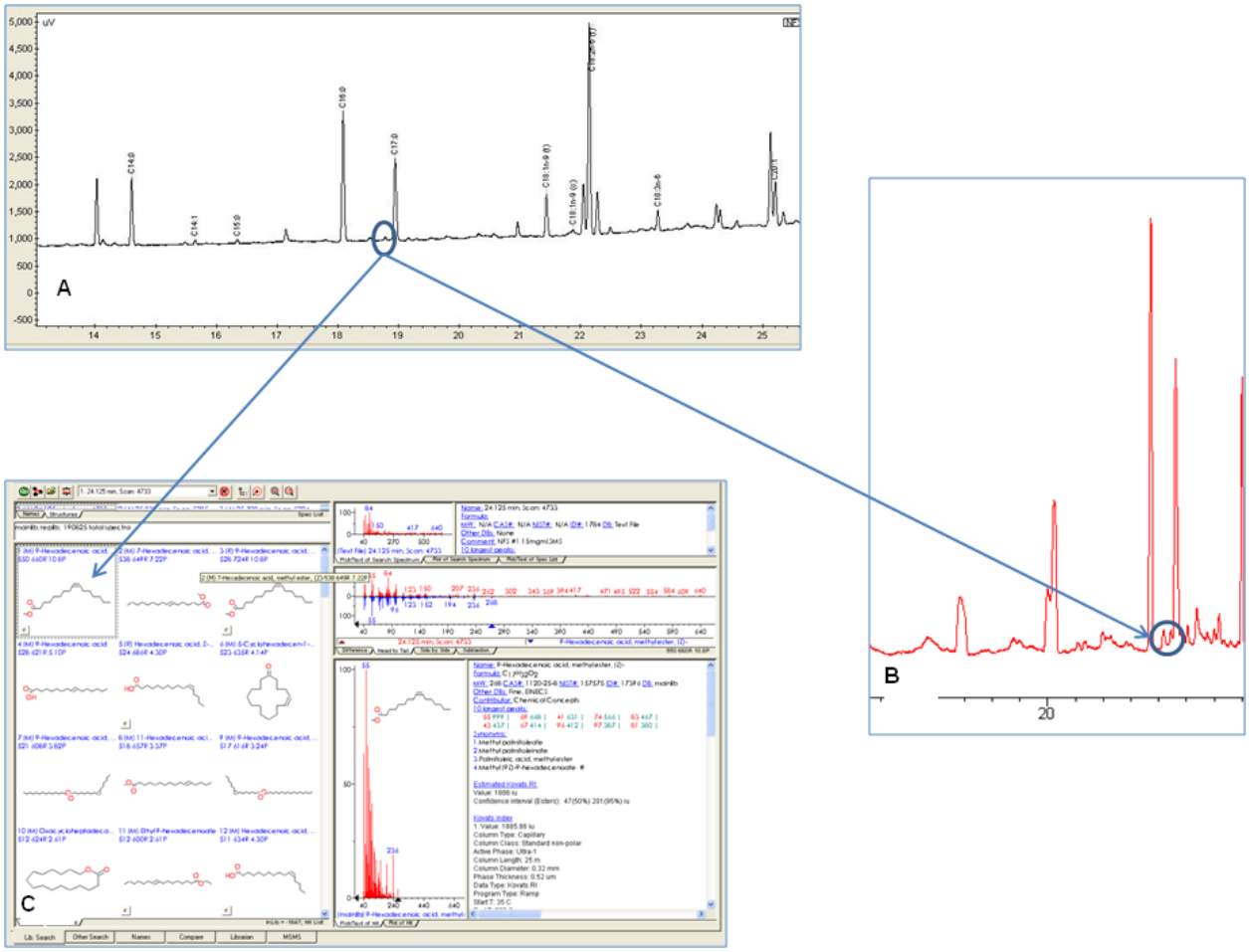

Figure 2. Comparison of same sample run through GC-FID and GC-MS; A. Magnified portion of GC-FID spectrum for a Northern fur seal sample, showing unidentified peak of interest (circled), B. Magnified portion of GC-MS spectrum of same sample, showing unidentified peak of interest (circled), now identified in (C), C. NIST library finding for the unidentified peak of interest using GC-MS

A Sørensen similarity index (SI) was used to determine differences in FAME detected between blubber samples, using GC-FID and GC-MS. Each fatty acid recovered and identified from the blubber samples was applied to the following formula: QS = 2C/A+B, where *A* and *B* are the number of fatty acid species identified for NFS (A) and SSL (B), respectively, and *C* is the number of fatty acids identified shared by the two marine mammals. SI calculations were performed for both GC-FID and the combination of GC-FID and GC-MS to establish differences between the protocols.

The commercially available standard mixture of 37 FAME (Supelco®) was used as a control in the determination of response factor for each GC detector and analyzed in triplicate before and after each series of samples run by both GC–FID and GC–MS. To test differences in the number of fatty acids between species (SSL, NFS) with detector (MS, FID) and lipid class (SFA, MUFA, PUFA), a fixed-factor ANOVA was performed (SPSS v.17). ATukey's HSD test was used to determine among group differences. Statistical significance was $P < 0.05$.Both detectors (GC-MS and GC-FID) used to identify fatty acids from blubber samples of SSLs and NFSs completely and correctly identified and recovered all FAMEs from the Supelco® 37 mixture during all controlled tests ($P > 0.05$). Therefore, we judge any differences in recovered FAMEs between the genuine field samples of NFS and SSL are, therefore, due solely to differences in the blubber composition of the sample, and not the methodology, as both detectors clearly identified all standards.

Results

GC-FID methods identified 30 FAME from NFS samples and 31 FAME from SSL samples, against a standard set of 37 FAME (Table 2, $P > 0.05$).

Table 2. Fatty Acids by Method, Class, and Species

Fatty Acids	GC-FID		GC-MS		GC-FID and GC-MS Combined	
	NFS	SSL	NFS	SSL	NFS	SSL
Saturated						
C12:0	X	X	X	X	X	X
C13:0	X	X			X	X
C14:0	X	X	X	X	X	X
C15:0	X	X	X		X	X
C16:0	X	X	X	X	X	X
C17:0	X	X	X	X	X	X
C18:0	X	X	X	X	X	X
C20:0	X	X	X	X	X	X
C21:0	X	X	X		X	X
C22:0	X	X	X		X	X
C23:0	X	X	X		X	X
C24:0	X	X	X		X	X
	X	X	X		X	X
Monounsaturated						
C14:1	X	X			X	X
C15:1	X	X	X	X	X	X
C16:1	X	X			X	
C16:1n-7(c)			X		X	X
C16:1n-9(t)			X	X	X	X
C17:1	X	X			X	X
C18:1n-7(t)			X	X	X	X
C18:1n-8(t)				X		X
C18:1n-9(t)	X	X			X	X
C18:1n-9(c)	X	X	X	X	X	X
C18:1n-12(t)			X		X	
C18:1n-13(t)				X	X	
C19:1n-9(t)				X	X	
C20:1	X	X	X		X	X
C20:1n-9(t)			X	X	X	X
C21:1n-9(t)				X		X
C22:1n-9	X	X	X		X	X
C22:1n-9(c)			X	X	X	X
C22:1n-9(t)				X		X
C23:1n-9(c)			X		X	
C24:1	X	X	X	X	X	X
C24:1n-9(t)			X		X	
Polyunsaturated						
C16:2n-4(t)				X		X
C18:2n-6(t)	X	X	X	X	X	X
C18:2n-6(c)	X	X	X	X	X	X
C18:3n-3			X	X	X	X
C18:3n-3(c)			X	X	X	X
C18:3n-6	X	X	X		X	X
C20:2	X	X	X		X	X
C20:2n-6				X		X
C20:2n-6(t)			X		X	
C20:3n-3(t)			X	X	X	X
C20:4n-3			X		X	
C20:3n-3	X	X			X	X
C20:3n-6		X			X	X
C20:4n-6	X	X			X	X
C21:4n-6(c)			X	X	X	X
C22:2	X	X			X	X
C20:5n-3	X	X			X	X
C20:5n-3(c)			X	X	X	X
C20:5n-3(t)			X		X	
C21:5n-3(t)			X		X	X
C22:4n-7(t)			X		X	
C22:5n-3(c)			X		X	
C22:6n-3	X	X		X	X	
C22:6n-3(c)			X	X	X	X
C22:6n-3(t)			X		X	X
TOTAL	29	30	43	27	53	46

Figure Legends

1. Lovushki Island Complex of the Kuril Island Chain, Russia
2. Comparison of same sample run through GC-FID and GC-MS; A. Magnified portion of GC-FID spectrum for a Northern fur seal sample, showing unidentified peak of interest (circled), B. Magnified portion of GC-MS spectrum of same sample, showing unidentified peak of interest (circled), now identified in (C), C. NIST library finding for the unidentified peak of interest using GC-MS

Comparison of FAME identification methods by lipid class, total FAMEs recovered and between species (NFS, SSL); Large and small case letters indicate statistical significance ($P < 0.05$) whereas * indicates significance between species. Error bars represent $\pm$SE.

Table Legends

1. FAME Standards with Elution Order
2. Fatty Acids by Method, Class, and Species

This equaled a 98% similarity on recovered FAMEs between both species inhabiting the same rookery.GC-MS methods identified a total of 43 FAME for NFS, including 4 not identified via GC-FID (C18:1n-13(t), C18:1n-12(t), C20:2n-6(t), C22:4n-7(t)) whereas a total of 27 FAME were identified by GC-MS for SSL ($P < 0.01$, Figure 3), including 4 not identified via GC-FID methods (C18:1n-8(t), C21:1n-9T), C16:2n-4(t), C18:3n-3(cis)). An additional 5 FAME (C18:1n-7(t), C18:3n-3, C21:4n-6(cis), C20:4n-3, C17:1(branched)) were identified by GC-MS which were common to both species. Furthermore, 7 FAME found in NFS (C16:1n-7(cis), C23:1n-9(cis), C24:1n-9(t), C20:3n-3(t), C20:5n-3(t), C22:5n-3(cis), C22:6n-3(t)) and 2 found in SSL (C22:1n-9(t), C20:2n-6) showed superior structural characterization using mass spectrometry (Figure 3). Further structural characterization of 5 FAME common to both species (C16:1n-9(t), C20:1n-9(t), C22:1n-9(cis), C20:5n-3(cis), C22:6n-3(cis)) was achieved using GC-MS. The SI calculation performed on GC-MS data determined a 51% similarity between species based on FAME composition.

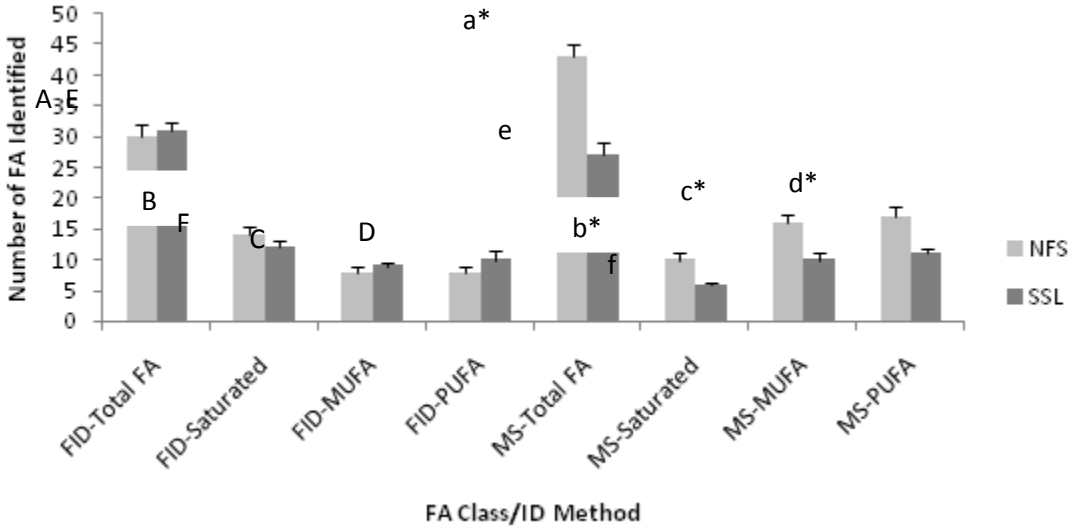

Figure 3. Comparison of FAME identification methods by lipid class, total FAMEs recovered and between species (NFS, SSL); Large and small case letters indicate statistical significance ($P < 0.05$) whereas * indicates significance between species. Error bars represent ±SE.

While a significantly greater number of saturated FAMEs were recovered using GC-FID for both species ($P < 0.05$), the GC-MS detector recovered a significantly greater number of unsaturated FAMEs for the NFS ($P < 0.05$). No difference was found in the number of unsaturated FAMEs recovered for SSL using both detectors (Figure 3, $P > 0.05$).For the GC-MS, we observed significantly greater FAMES recovered for the NFS when compared to the SSL (Figure 3, $P < 0.05$ for all lipid classes). When combining FAME profiles from both GC-FID and GC-MS, additional structural information and newly-identified fatty acids were found in both NFS and SSL samples (Table 2, Figure3). Together, the methods of GC-FID and GC-MS identified a total of 61 individual fatty acids when compiling both NFS and SSL samples. A total of 31 FA were identified by GC-FID, and 51 by GC-MS, representing 21 FA's through GC-MS which were either better characterized as to bond structure or newly identified; a 64.5% increase in FA identification was achievedusing GC-MS. Combining methods resulted in an81% agreement in fatty acids identified between SSL and NFS inhabiting the same rookery.GC-MS profiles indicate a greater number of MUFAs and PUFAs (listed above) compared to saturated FAs for both species (Table 2, Figure 3).

In our study, a greater number of saturated fatty acids in both species were found using GC-FID, while the identification of unsaturates, both MUFAs and PUFAs, was best resolved via GC-MS (Figure 3).

Therefore, we identified a greater number of fatty acids in SSL blubber using GC-FID when compared to the GC-MS, whereas GC-MS identified a greater number of FAMEs from NFS blubber samples.GC-FID analysis revealed a high degree of similarity (98%)of fatty acids between species whereasGC-MS revealed a relatively lower degree of fatty acid signature similarity (51%). When combining both methods the total number of fatty acids identified increased over using each method individually (Table 2).

Discussion

Increasednumbers of MUFAs and PUFAs were identified using GC-MS compared to GC-FID, implicating the GC-FID as aninferior method for identifying unsaturated fatty acids in these blubber samples. Typically, the GC-MS is regarded as an improvement in resolution when compared to GC-FID;the method provides greater selectivity and sensitivity over GC-FID, the ability to confirm compounds based on both retention time and additional spectral data, and an enhanced ability to separate coeluting peaks based on unique ions (Dodds et al. 2005).It has been reported that using the GC-MS as a detector leads to much greater distinguishing ability between lipids, and even among similar FAME groups (e.g. C18:Xn-x). In particular, characteristic fragmentation patterns exist for FA, with prominent fragments of m/z=43 in saturates, and m/z=41 in unsaturates (Wetzel and Reynolds 2004).However, our data indicated a decreased capabilityfor GC-MS to identify saturated fatty acidscompared to GC-FID in free-ranging marine mammal species. This result is likely due to error associated with instrument overloading. The ion trap of the MS is more sensitive to sample overload, whereas this issue is not common in GC-FID (Hilkert et al. 1999).These findings may present serious implications for studies both in the past and the future utilizing one detection method for identifying and quantifying fatty acids (see below).GC-MS identification of FAMEs can be accomplished by retention time matching, and for confirmation, by referencing the NISTlibrary for structural identification. In this study, we found GC-MS had a greater ability to resolve unsaturated fatty acids (Figure 3).

We also observed a failure by GC-FID to identify many MUFAs and PUFAs, which may lead to incomplete or incorrect conclusions pertaining to specific research involving resource use and dietary habits of marine mammal species. For example, many resource partitioning and dietary studies (Best et al. 2003, Cooper et al. 2009, Newland et al. 2009) use primarily GC-FID detection to identify FAMEs, with subsequent use of GC-MS to verify FID peaks.Using GC-FID as the sole detector limits the investigator in that several fatty acids may elute with the same retention time, and the possibility of similar non-FA lipids eluting also exists. Therefore, any results from GC-FID where peaks are tightly clustered are likely to be associated with significant error in identification (Budge et al. 2008).Results from this study show that specific FAMEs or FAs recovered from blubber and subsequently used in analysis to determine dietary habits may be best served using both GC detection methods. In other words, combining the methods of both GC-FID and GC-MS would seem to lend greater accuracy to FA blubber composition studies, and more broadly, to applications in resource partitioning and competition studies.Accurate determination of the fatty acid profile in marine mammals with the use of both GC-FID and GC-MS will add validity to ecological and physiological interpretations made with these data.Although this study was only comparing methods for identifying fatty acids, calibration curves for both detectors could be created, allowing for a complete and quantitative analysis of all fatty acids in the tissue (usually reported in relative percentage).

Surprisingly, we found that the GC-MS consistently recovered greater numbers of FAMEs for NFS when compared to SSL (Figure 3). Fatty acids are known to stratify in the blubber layers of marine mammals (Käkelä and Hyvärinen 1996; Bestet al.2003; Thiemann et al. 2004) and while these stratification patterns can be species-specific, generally, polyunsaturated fatty acids are found closer to the muscle layers (i.e. deeper), while more saturated fatty acids (particularly from de novo synthesis) are found in the outermost layers (Cooper 2004). Our species difference identified by GC-MS, then, does not seem to implicate a sampling error based on blubber depth sampled, as the number of fatty acids identified in NFS by GC-MS was higher across all FA classes. Therefore, we deem this species difference to be a factor of detector differences (discussed above) and diet (see next paragraph).

Given the importance that competition has on community structure and the patchy nature of resources, many species populating similar habitats demonstrate some degree of resource partitioning as a means to reduce inter- and intra-specific competition (Bolnick et al. 2003, Newland et al. 2009).

Traditionally, pinniped diet or dietary overlap between or among species has been determined from the identification of prey hard parts collected from stomachs, colons, spewings (regurgitations) or scat (fecal) samples (e.g., Lucas 1899, Antonelis and Perez 1984, Sinclair et al. 1994, Yonezaki et al. 2003, Gundmundson et al. 2006, Zeppelin and Ream 2006, Yonezaki et al. 2008). Our results indicated that there may be a significant level of resource partitioning between NFS and SSL, but these findings are, importantly, inherent on the use of both GC-FID and GC-MS as a means to detect FAMEs in the blubber.While food habit data on our two species is sparse in Russian waters, studies conducted in the 1950's and 1960's revealed some dietary overlap, with walleye pollock (*Theragrachalcogramma*) being a primary prey item (Belkin 1966). Additionally, while some overlap was detected, Kuzinet al. (1997) used stomach content analysis to show a clear partitioning of foraging resources between these pinniped species.Their data indicated that the NFS diet was composed of 60.1% pollock, 21.7% salmon (*Oncorhynchus* sp.), 14.1% anchovy (*Engraulis* sp.), and 4.1% squid, while the diet of SSL consisted of 37.5% squid, 35.6% rockfish (*Sebastes* sp.), and 26.8% pollock. Our findings are, therefore, substantiated by previous dietary studies.The fatty acid profiles of these prey species, in large part, explain the species differences we see in GC-MS detection.NFS fatty acid detection by GC-MS, while higher across all FA classes, shows particularly large recoveries for MUFAs and PUFAs. NFS prey are composed largely of high-PUFA and MUFA fish (pollock, salmon, squid), with only a small percentage of diet determined by anchovy (with a composition more saturated).

SSL diet, on the other hand, is composed in larger proportions of species comparatively lower in unsaturated fatty acids (rockfish), and a smaller proportion of species higher in PUFAs (squid and Pollock) (Iverson et al. 2002; Kaya and Turan 2010). Therefore, the difference we see in GC-MS detection between NFS and SSL can be explained by the relative proportion of their diets composed of unsaturated fatty acids as composed to SFA.Had we used solely GC-FID to investigate potential resource partitioning between these animals, our results would be drastically different (98% SI for GC-FID versus 81% SI in combined methods), failing to identify many of the unsaturated fatty acids present in the samples. Additionally, had we used solely GC-MS, our analysis would have failed to identify many of the common saturated fatty acids that were clearly present in the samples. Thus, using a combination of both GC-FID and GC-MS provided, we believe, a more complete analysis of fatty acids.

FA signature analysis is considered to be a robust method in determining dietary preferences (Iverson et al. 2004). However, a few caveats in GC work do exist, limiting the scope of our results.Although the differences seen between NFS and SSL can likely be attributed to differences in prey composition, this assumes ideal, representative samples from both species have been obtained. In other words, some differences in blubber FA composition between the species may simply stand as an artifact of sampling technique. However, because of the substantially different FA composition between the two species, a reasonable assumption can be made that SSL and NFS in our study location are largely feeding on different prey. As SSL and NFS are both otariids with a similar life histories, the FA differences we have identified in depot lipids are almost certainly representative of arecent diet, rather than any difference resulting from the way in which prey are digested (Falk-Petersen et al. 2009).Despite these caveats, gas chromatographic fatty acid analysis remains one of the most rigorous methods to study dietary habits of marine organisms in remote locations, and combining detectors in marine mammal studies provides a more complete and reliable fatty acid profile.

GC-FID/MS analysis of marine mammal blubber revealed many individual methyl esters ranging in total carbon number from C12 to C24. However, FAME detection using FID provided greater resolution for saturates, whereas GC-MS demonstrated higher sensitivity for unsaturates. This information could prove highly beneficial with broad implications in studies involving dietary overlap and FA stratification in blubber.

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