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Meghan Pinedo

US Forest Service International Programs Wood Identification and Screening Center, Ashland

Deborah A. Duffield

Portland State University, duffieldd@pdx.edu

Dalin N. D'Alessandro

Portland State University

Erin R. Price

US Forest Service International Programs Wood Identification and Screening Center, Ashland

Edgard O. Espinoza

US National Fish and Wildlife Forensic Laboratory, Ashland

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Oil Identification of harp seal and other select marine mammals

Megahn H. Pinedo^a, Deborah A. Duffield^b, Dalin N. D'Alessandro^b, Erin R. Price^a, Edgard O. Espinoza^{c,*}

^a US Forest Service International Programs Wood Identification and Screening Center, Ashland, OR, USA

^b Department of Biology, Portland State University, Portland, OR, USA

^c US National Fish and Wildlife Forensic Laboratory, Ashland, OR, USA

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ABSTRACT

Because of the rich omega-3 fatty acids content, harp seal (*Pagophilus groenlandicus*) oil is a popular supplement that is packaged as pills in Canada and sold for medicinal purposes, although this practice is banned in the United States. Due to US regulations, it is important to be able to distinguish between fish oil and seal oil, but the taxonomic determination of oils provenance has been a difficult problem to solve. In this study, Direct Analysis in Real Time time-of-flight mass spectrometry (DART TOFMS) was used to analyze the chemotypes of blubber samples collected from seven species of marine mammals, including seals, sea lions, and a porpoise. Results indicated that the chemotype profiles found in negative-ion mode could be used to separate all of the species using Discriminant Analysis of Principal Components (DAPC). Consequently, this study suggests that it may be possible to identify the taxonomic source of marine mammal oils based on chemical chemotypes.

Introduction

Since the late 1900's, fish oils have been used as a dietary supplement due to their rich content of omega-3 fatty acids [25], though their purported health benefits are debatable [10]. An alternate commercial source of omega-3 fatty acids is harp seal oil (*Pagophilus groenlandicus*). Populations of harp seal are abundant in the North Atlantic, Greenland Sea, and White Sea/Barents Sea [17,18,23]. The oil is legally harvested and sold in Canada, but it is banned in the United States because harp seals are protected under the Marine Mammal Protection Act (MMPA) of 1972 [16]. In order to enforce the United States' MMPA, determination of the taxonomic source of suspected marine mammal oils is critical.

Direct Analysis in Real Time time-of-flight mass spectrometry (DART TOFMS), described by Cody & Laramée [4], has shown remarkable success in analyzing challenging types of evidence [21]. This ambient ionization approach has become a popular forensic tool in the analysis of evidence because it is minimally destructive and rapidly produces mass spectra with little to no sample preparation [21]. In 2021, the National Institute of Standards and Technology (NIST) released a DART MS database focused on the mass spectra of controlled substances [22]. Price et al. [19] reported the characterization of keratin fibers using ambient ionization, while Lancaster et al. [14], Cody et al. [2] and McClure et al. [15] used DART TOF mass spectra for determining species

source of timber.

Traditionally, the analysis of oil consists of esterifying the lipids in a sample by a process known as fatty acid methyl ester (FAME) analysis using gas chromatography (GC). This process is elegant, allows for quantitation and requires sample preparation [6]; But others have investigated approaches that do not require FAME methylation. For example, Vaclavik et al. [24] authenticated the source of pork and beef fat using DART MS. Kılıç and Koçak [13] investigated the components of pine essential oil using head space solid phase microextraction (HS-SPME) /GC-MS and chemotaxonomy to determine the oil source. Kuo et al. [11] investigated the analysis of edible oils using matrix-assisted laser desorption ionization (MALDI) mass spectrometry aided by chemometrics to identify mixtures of edible oils. Espinoza et al. [7] used DART MS in negative ionization mode to determine the taxonomic source of oils from six different species of sea turtle. Beneito-Cambra et al. [1] published a comprehensive critical review comparing multiple approaches to olive oil identification and highlights the strength and weakness of each analytical approach.

The composition of oil from marine mammal species has been investigated by various researchers. Cuq et al. [5] examined the fatty acid composition of oil from pickled seal hides to determine the usefulness of the oil in fat-liquoring. Jay et al. [12] investigated the variability of extracted walrus blubber oil from three different body sites and

* Corresponding author.

E-mail address: ed_espinoza@fws.gov (E.O. Espinoza).

Table 1

List of reference blubber samples.

Family	Common Name	Species	n:	Source
Phocidae	Harbor seal	<i>Phoca vitulina</i>	9	Portland State University
Phocidae	Northern elephant seal	<i>Mirounga angustirostris</i>	12	Portland State University
Phocidae	Harp seal	<i>Pagophilus groenlandicus</i>	20	Fisheries and Oceans Canada
Phocidae	Hooded seal	<i>Cystophora cristata</i>	20	Fisheries and Oceans Canada
Otariidae	Steller sea lion	<i>Eumetopias jubatus</i>	10	Portland State University
Otariidae	California sea lion	<i>Zalophus californianus</i>	10	Portland State University
Phocoenidae	Harbor porpoise	<i>Phocoena phocoena</i>	12	Portland State University

concluded that walrus diet could be inferred by the blubber composition.

The goal of this project was twofold: 1) to determine if taxonomic provenance for marine mammals can be determined from oil products using positive- or -negative mode ambient ionization mass spectrometry, and 2) to determine whether the same analysis could be performed on blubber samples from decomposed carcasses that washed ashore, including those representing the seven mammal species: harbor seal (*Phoca vitulina*), northern elephant seal (*Mirounga angustirostris*), harp seal (*Pagophilus groenlandicus*), hooded seal (*Cystophora cristata*), Steller sea lion (*Eumetopias jubatus*), California sea lion (*Zalophus californianus*), and harbor porpoise (*Phocoena phocoena*).

Methods

Sample sources

Blubber samples were obtained from the sources listed in Table 1. All specimens were kept frozen at 32 °F until sample preparation.

Sample preparation for DART TOFMS

To yield oil from the blubber samples, a 1" by 1" by 1" section of blubber was removed from each sample and finely diced before being transferred to a 10" non-stick skillet. The blubber was simmered over a propane stove at low temperature (estimated to be 200° F to 300° F). The oil volume yielded from this process was between 1 to 15 mL. The oil was transferred via a glass pipet to a scintillation vial and the oil remained liquid at room temperature (i.e., never solidifying). The color of the oil produced ranged from a pale yellow to a deep orange-red hue. Contamination between each specimen was prevented by thoroughly cleaning all equipment between each sample preparation.

Mass spectra collection

Mass spectrum measurements were collected on a time-of-flight mass spectrometer (AccuTOF-DART 4 G, JEOL, USA, INC., Peabody, MA, USA) fitted with a DART ion source (DART-SVP, IonSense Inc., Saugus, MA, USA). As stated by the manufacturer, the resolving power of the instrument is 10,000 full width at half maximum (FWHM). The DART was operated in positive- and negative-ion modes with a collection range of 100 to 1000 mass-to-charge ratio (m/z). For positive-ion mode, orifice 1 was set to 30 V, orifice 2 to a voltage of 5 V, and the ring lens to 5 V. In negative-ion mode, orifice 1 was set to -30 V, orifice 2 to -5 V, and the ring lens to -5 V.

To collect mass measurements using DART TOFMS, 50 μ l of oil was diluted with 0.5 mL of 2-propanol and vortexed for 10 to 15 s. For both the positive- and negative-ion analysis, the sealed end of a glass capillary tube (Pyrex #9530-4) was dipped into the diluted sample and placed in front of the DART heated helium stream at 350 °C. Positive mode spectra were calibrated with poly(ethylene glycol) 600 (Ultra Scientific, Kingstown, RI, USA) diluted with methanol (1:10 ratio), while the negative-ion mode spectra were calibrated with a fluorinated ether, Fomblin®Y (Aldrich, St. Louis, MO, USA) [3], diluted with methanol (1:10 ratio). A calibrant spectrum was collected at the beginning, middle, and end of each sample batch of 10.

After the mass measurements were obtained, the spectra were averaged, and the background was subtracted using msAxel software

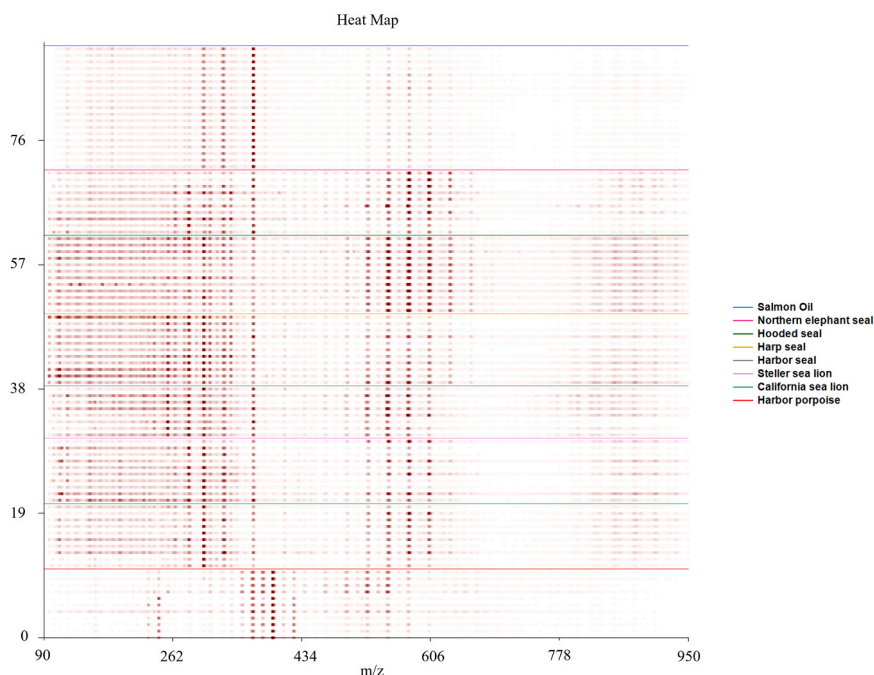


Fig. 1. Mass Mountaineer heat map results for positive mode ions. The heat map is labeled with the common names corresponding to the oil samples run. The x-axis indicates ions (m/z) and their relative intensity, the y-axis denotes individual samples.

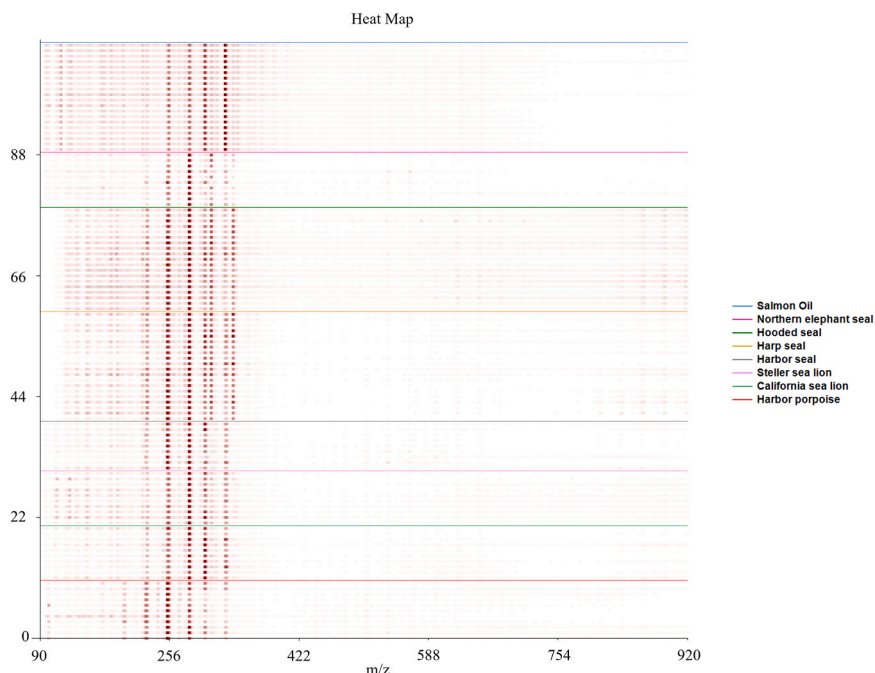


Fig. 2. Mass Mountaineer heat map results for negative mode ions. The heat map is labeled with the common names corresponding to the oil samples run. The x-axis indicates ions (m/z) and their relative intensity, the y-axis denotes individual samples.

Table 2
Comparison of analytical parameters of positive-ion and negative-ion spectra.

	Positive-ion Spectra	Negative-ion Spectra
Mass tolerance	5 mmu	5 mmu
Number of ions in model	1436	696
Number of principal components that describe 90% of variability	27	29
Variance Covered	89.81%	89.32%
Leave-One-Out Cross Validation	89.23%	92.68%

(version 1.0.5.2, JEOL Ltd.). The resulting spectra were centroided and exported as text files. All data were analyzed using the Mass Mountaineer software program (MassMountaineer.com version 7.1.10.0, Peabody, MA, USA).

Data analysis

Heat maps of positive-ion mode spectra (Fig. 1) and negative-ion mode spectra (Fig. 2) were constructed for visual analysis of similarities and differences between the species' spectra. Commercial salmon oil samples were included in both heat maps to show a visual comparison between commonly sold fish oil and marine mammals. To build statistical models, the following steps were taken for both negative and positive-ion spectra: ions were extracted from the spectra from each of the seven marine mammal species and used to construct a dataset of ions. A randomly chosen spectrum from each species was excluded from the training set and retained as blind test samples. Final selection of the ions that were chosen for multivariate analysis was done using the analysis of variance (ANOVA) algorithm in Mass Mountaineer. Ions that did not show a significant difference for the 95% confidence interval (p -value ≥ 0.05) between the two classes with the largest difference in means were excluded from further analysis.

Principal component analysis (PCA) was used to observe trends within species classes according to the ions collected from each species. Principal components (PCs) were selected to encompass the majority of the variation within the spectra. For the positive-ion spectra, a total of

27 PCs were selected to cover 89.81% of variance, and 29 PCs were selected for the negative-ion spectra to cover 89.32% of variance (Table 2).

A Discriminant Analysis of Principal Components (DAPC) algorithm was applied to the PCs collected from the PCA models in order to classify the species (Jombart 2010). Performance of each model was assessed using two criteria: 1) Leave-One-Out-Cross-Validation (LOOCV) and 2) one test spectrum from each species sample (seven total spectra) that was not included in the creation of the training set. The LOOCV algorithm sequentially treats each spectrum in the training set as an unknown, and each spectrum in the training set undergoes a k -fold= n process; a value of 100% indicates that all spectra were assigned to their appropriate class. Blind test spectra were used to assess the reliability and accuracy of the model by observing whether each test spectrum was assigned to the correct species class.

Results and discussion

Mass measurements were collected in both the positive- and negative-ion mode. Positive-ion mode analysis detected low mass fatty acids, sterols, and mono-, di-, and tri-glycerides. The negative-ion mode detected fatty acid chains and their corresponding isomers. Examples of the chemotypes produced by each mode are shown in Figs. 1 and 2.

The heat maps provide a unique opportunity to explore intraspecific variability of the chemotypes since each row corresponds to a different individual. The negative-ion heat map (Fig. 2) consists mainly of fatty acids, and it is clear that all species analyzed contained the same set of ions tentatively assigned as myristic acid, palmitoleic acid, linoleic acid, oleic acid and arachidonic acid (Table 3a and 3b). The positive-ion heat map (Fig. 1) displays a more abundant presence of ions detected, as well as chemotype patterns that appear to be characteristic to the taxa in this study. The clearest example of this is the chemotype associated with harbor porpoise. A more robust assessment of intraspecies variability necessitates a larger sample size for each of the taxa included in this study. Nevertheless, given the data, inferences can be made from the mass measurements.

Table 3a
Tentative assignments of ions from positively charged molecules.

Positive Mode Tentative Assignments			<i>Phocidae</i>				<i>Otariidae</i>		<i>Phocoenidae</i>
Assignment	Composition	Measured <i>m/z</i>	<i>P. groenlandicus</i>	<i>P. vitulina</i>	<i>C. cristata</i>	<i>M. angustirostris</i>	<i>E. jubatus</i>	<i>Z. californianus</i>	<i>P. phocoena</i>
Hypogeic Acid	C ₁₆ H ₃₀ O ₂ * (+H)	255.234	Y	Y	Y	Y	Y	Y	
18:1 Oleic Acid	C ₁₈ H ₃₄ O ₂ * (+H)	283.263	Y	Y	Y	Y	Y	Y	
20:5 Eicosapentaenoic acid	C ₂₀ H ₃₀ O ₂ * (+H)	303.232	Y	Y	Y	Y	Y	Y	
20:1 Eicosadienoic acid	C ₂₀ H ₃₈ O ₂ (+H)	311.293	Y			Y		Y	
22:6 Docosahexaenoic acid	C ₂₂ H ₃₂ O ₂ (+H)	329.248	Y	Y	Y	Y	Y	Y	
22:5 Docosapentaenoic acid	C ₂₂ H ₃₄ O ₂ * (+H)	331.263	Y	Y		Y	Y	Y	
3-Deoxyvitamin D3	C ₂₇ H ₄₄ * (+H)	369.351	Y	Y	Y	Y	Y	Y	
24-Nor-5beta-cholane- 3alpha,7alpha,12alpha,23- tetrol	C ₂₃ H ₄₀ O ₄ (+H)	381.300							Y
Methyl 9-butylperoxy-10,12- octadecadienoate	C ₂₃ H ₄₂ O ₄ * (+H)	383.315							Y
5beta-Cholane- 3alpha,7alpha,12alpha,24- tetrol	C ₂₄ H ₄₂ O ₄ * (+H)	395.316							Y
Squalene	C ₃₀ H ₅₀ (+H)	411.399	Y	Y			Y	Y	
Phenolic phthiocerol	C ₃₇ H ₆₈ O ₄ * (+H)	577.519	Y	Y	Y	Y	Y	Y	
Glycerolipid DG(19:1(9Z)/18:3 (9Z,12Z,15Z)/0:0	C ₄₁ H ₇₆ O ₅ * [M + H -H ₂ O] ⁺	631.566	Y			Y	Y	Y	
Glycerolipid DG(19:1(9Z)/20:3 (8Z,11Z,14Z)/0:0	C ₄₂ H ₇₄ O ₅ * [M + H -H ₂ O] ⁺	659.595	Y		Y	Y	Y		

* Or isotope

Table 3b
Tentative assignments of ions from negatively charged molecules.

Negative Mode Tentative Assignments			<i>Phocidae</i>				<i>Otariidae</i>		<i>Phocoenidae</i>
Assignment	Composition	Measured <i>m/z</i>	<i>P. groenlandicus</i>	<i>P. vitulina</i>	<i>C. cristata</i>	<i>M. angustirostris</i>	<i>E. jubatus</i>	<i>Z. californianus</i>	<i>P. phocoena</i>
12:0 Lauric acid	C ₁₂ H ₂₄ O ₂ * (-H)	199.169							Y
14:1 Myristoleic acid	C ₁₄ H ₂₆ O ₂ (-H)	225.185	Y	Y					Y
14:0 Myristic acid	C ₁₄ H ₂₈ O ₂ * (-H)	227.201	Y	Y	Y	Y	Y	Y	Y
16:1 Palmitoleic acid	C ₁₆ H ₃₀ O ₂ (-H)	253.218	Y	Y	Y	Y	Y	Y	Y
18:2 Linoleic acid	C ₁₈ H ₃₂ O ₂ (-H)	279.232	Y	Y	Y	Y	Y	Y	Y
18:1 Oleic acid	C ₁₈ H ₃₄ O ₂ * (-H)	281.250	Y	Y	Y	Y	Y	Y	Y
20:5 Eicosapentaenoic acid	C ₂₀ H ₃₀ O ₂ * (-H)	301.220	Y	Y	Y		Y	Y	Y
20:4 Arachidonic acid	C ₂₀ H ₃₂ O ₂ * (-H)	303.231	Y	Y	Y	Y	Y	Y	Y
20:1 Eicosadienoic acid	C ₂₀ H ₃₈ O ₂ * (-H)	309.281	Y		Y	Y	Y	Y	
22:6 Docosahexaenoic acid	C ₂₂ H ₃₂ O ₂ (-H)	327.234	Y	Y	Y	Y	Y	Y	Y
22:5 Docosapentaenoic acid	C ₂₂ H ₃₄ O ₂ * (-H)	329.250	Y	Y	Y	Y	Y	Y	Y
22:1 Cetoleic acid	C ₂₂ H ₄₀ O ₂ * (-H)	337.310	Y		Y	Y		Y	

* Or isotope

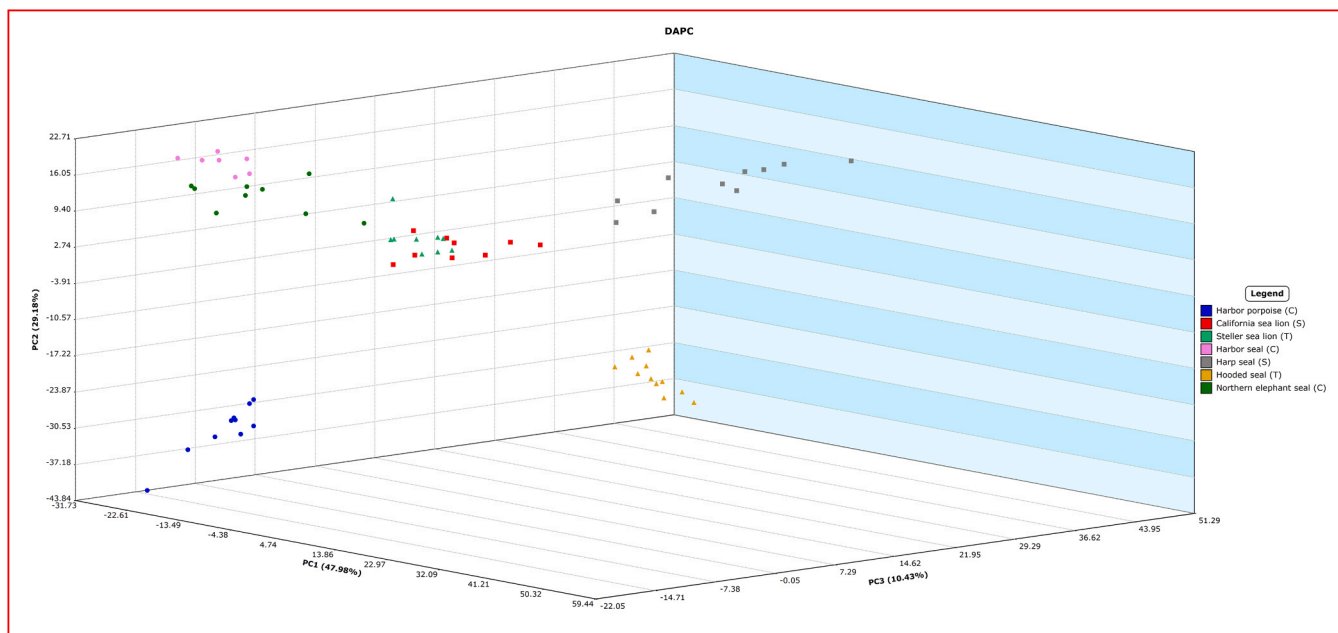


Fig. 3. Positive mode DAPC graph. LOOCV = 89.23%. PCs = 27. Each species formed individual clusters with the exception of California sea lion, Steller sea lion, northern elephant seal, and harbor seal. The legend distinguishes symbol shape where (C) = circle, (S) = square, and (T) = triangle.

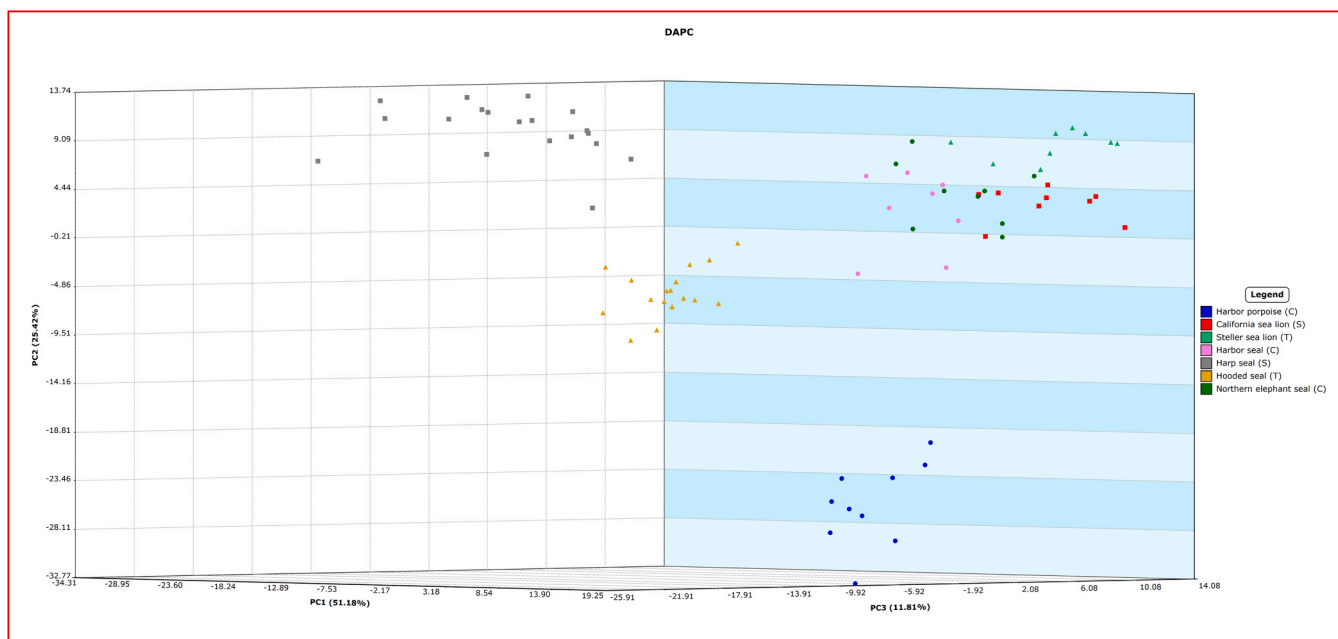


Fig. 4. Negative mode DAPC graph. LOOCV = 92.68%. PCs = 29. Each species formed individual clusters with the exception of California sea lion, Steller sea lion, northern elephant seal, and harbor seal. The legend distinguishes symbol shape where (C) = circle, (S) = square, and (T) = triangle.

Positive-ion mode

Examination of Fig. 1 revealed that the oil profile of the harbor porpoise was distinct from the other marine mammal taxa tested. This finding is not surprising since taxonomically it is the only species in the dataset that does not belong to the pinniped clade. The northern elephant seal, hooded seal, and harp seal exhibited similar chemotype profiles based on the presence of two ions at 631.566 and 659.595 m/z , while the California sea lion, Steller sea lion, and harbor seal exhibited a second chemical chemotype that was notably lacking these two ions. These ions were tentatively assigned as di-glycerolipids ($C_{41}H_{76}O_5$ and $C_{42}H_{74}O_5$ respectively) and this represents $[M + H - H_2O]^+$ because

diglycerides tend to lose one water molecule when ionized by DART in positive-ion mode. Tentative assignments of other ions are listed in Table 3a. The positive-ions uniquely characterized the harbor porpoise and separated the northern elephant seal, hooded seal, and harp seal from the California sea lion, Steller sea lion, and harbor seal.

Negative-ion mode

Examination of the ions detected in the negative-ion mode (Fig. 3b) also demonstrated that the fatty acid profile of the harbor porpoise was distinct from the other species. It is of interest to highlight that the hooded seal and the harp seal exhibited a distinct quartet of ions that

Table 4
Assignment of blind test samples from positive and negative-ion spectra.

Species	Common Name	Assignment Positive-ion Spectra	Assignment Negative-ion Spectra
<i>Phocoena phocoena</i>	Harbor Porpoise	Harbor Porpoise	Harbor Porpoise
<i>Zalophus californianus</i>	California Sea Lion	California Sea Lion	California Sea Lion
<i>Eumetopias jubatus</i>	Steller Sea Lion	Steller Sea Lion	Steller Sea Lion
<i>Phoca vitulina</i>	Harbor Seal	California Sea Lion*	California Sea Lion*
<i>Pagophilus groenlandicus</i>	Harp Seal	Harp Seal	Harp Seal
<i>Cystophora cristata</i>	Hooded Seal	Hooded Seal	Hooded Seal
<i>Mirounga angustirostris</i>	Northern Elephant Seal	Northern Elephant Seal	Northern Elephant Seal

(Positive-ion model LOOCV = 89.23%; Negative-ion model LOOCV = 92.68%)

* Indicates a misclassification.

made their chemotypes appear similar. These ions at 303.231, 309.281, 329.250, and 337.310 m/z , as well as other ions such as eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid, have been tentatively assigned and are shown in Table 3b ([8]; Iverson 2004; [20, 25]). The northern elephant seal exhibited the greatest amount of intraspecies chemotype variability.

Multivariate statistical analysis

The spectra were modeled using the DAPC algorithm. Modeling parameters for both positive and negative analysis are listed in Table 2. The DAPC graphs (Figs. 3 and 4) and the reported LOOCVs, 89.23% and 92.68% respectively, in Table 2 indicated that both models demonstrated a similar performance, but the negative-ion mode model had a slightly higher accuracy.

Results from both DAPC models indicated that the spectra of harbor porpoise, harp seal and hooded seal formed discrete clusters. Additionally, in both models there was a larger cluster formed by the northern elephant seal, California sea lion, Steller sea lion, and harbor seal.

The analysis and species assignment of blind test samples (Table 4)

not included in the training models were used to test the accuracy and reliability of the models. The blind test sample used for the California sea lion was misclassified in both the negative and positive-ion modes, supporting the possibility of intraspecific variability. This issue could potentially be remedied with an increased data set to encompass more intraspecific variability, but it was further explored by the creation of two focused DAPC models with a reduced number of species.

These models were built by removing the species that had distinctive chemotypes that clustered separately (harbor porpoise, harp seal, and hooded seal) to create a model that contained only the four species that clustered together: California sea lion, Steller sea lion, harbor seal, and northern elephant seal. These resulting DAPC models (Figs. 5 and 6) showed that the spectra of this taxon clustered separately by species. The blind test samples were evaluated, and the results are shown in Table 5. The results showed that the negative-ion mode DAPC model accurately assigned each one of the blind test samples whereas the positive-ion mode model contained two misclassifications.

Conclusions

The interpretation of the data generated in this study indicated that harp seal oil can be accurately identified and that other common sources of oil (i.e., salmon oil) can be easily and rapidly distinguished. Additionally, since the other tested marine mammal reference samples were derived from blubber, we can also infer that the taxonomic source of beach-found decomposed carcasses can be deduced by using ambient ionization mass spectrometry. Aside from the observed clustering, the results of the LOOCV indicated that the classification of these individual taxa using chemotype profiles was encouraging.

In this study, we analyzed the oils of seven species of marine animals: harbor seal (*Phoca vitulina*), northern elephant seal (*Mirounga angustirostris*), harp seal (*Pagophilus groenlandicus*), hooded seal (*Cystophora cristata*), Steller sea lion (*Eumetopias jubatus*), California sea lion (*Zalophus californianus*), and harbor porpoise (*Phocoena phocoena*) and a salmon (*Oncorhynchus* sp.) that are commonly found in commercial trade using DART TOFMS. We applied multivariate statistical analysis to spectra collected in both negative and positive-ion mode and found that the negative mode spectra consistently outperformed the positive mode spectra. These results indicated that negative-ion mode analysis is a powerful tool for species identification from oil supplements. While the

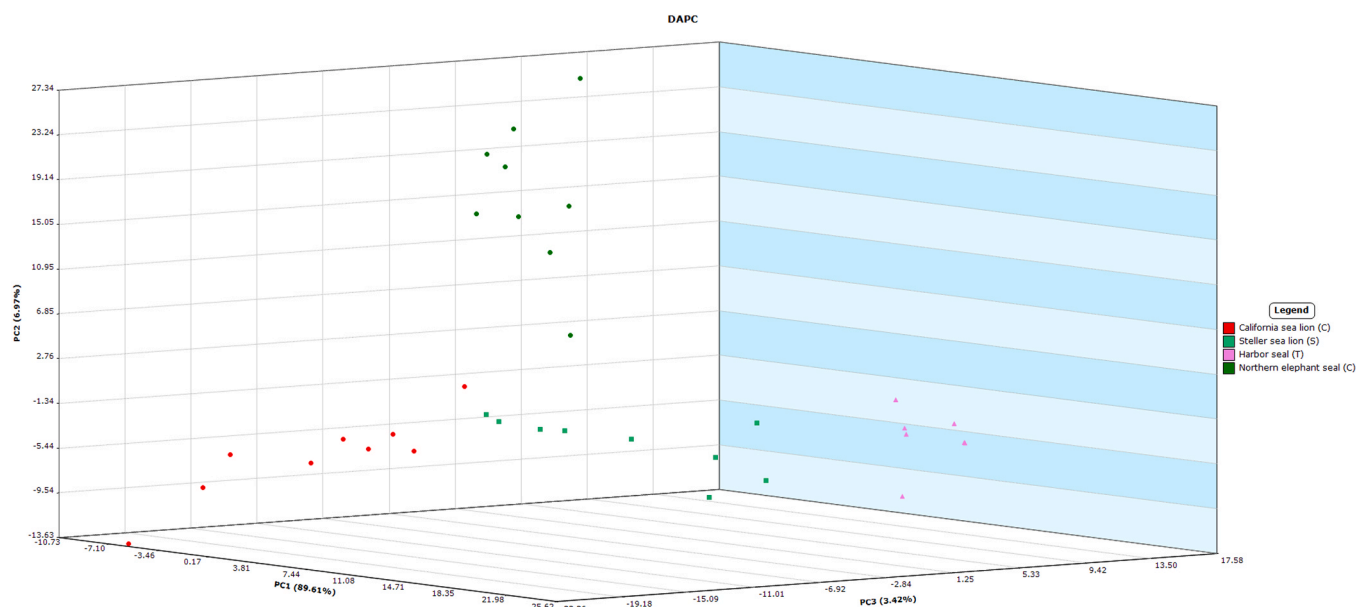


Fig. 5. Positive mode reduced DAPC graph which included California sea lion, Steller sea lion, northern elephant seal, and harbor seal. LOOCV = 85.29%. PCs = 14. The legend distinguishes symbol shape where (C) = circle, (S) = square, and (T) = triangle.

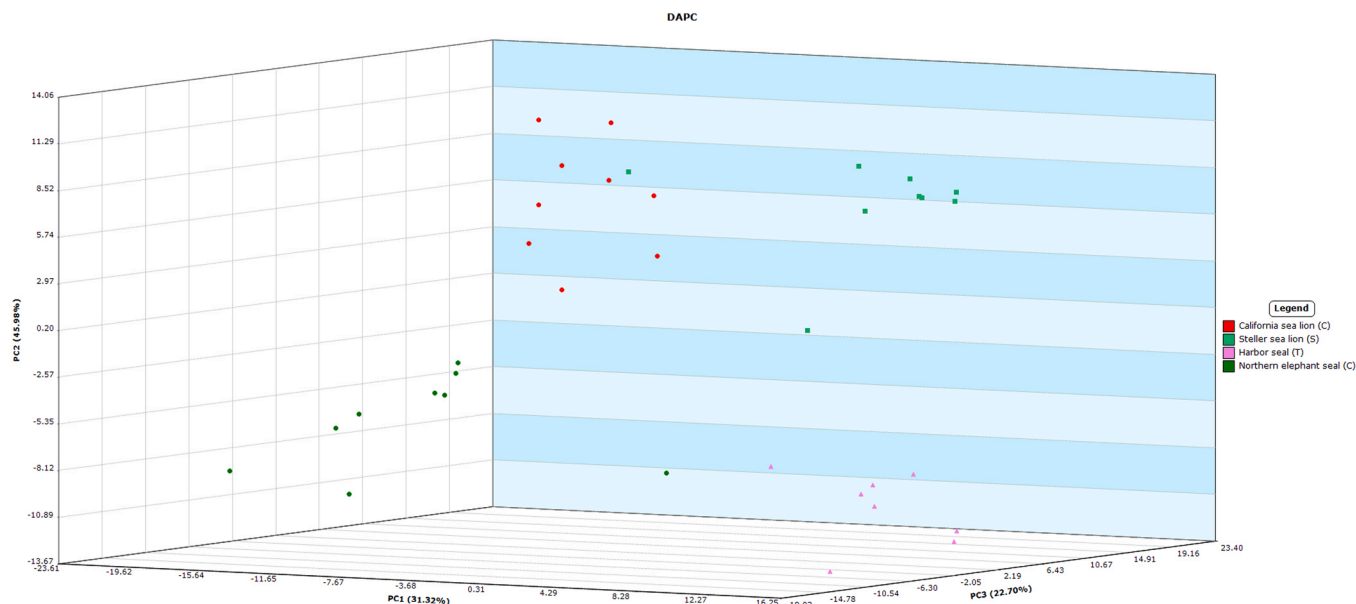


Fig. 6. Negative mode reduced DAPC graph which included California sea lion, Steller sea lion, northern elephant seal, and harbor seal. LOOCV = 91.43%. PCs = 14. The legend distinguishes symbol shape where (C) = circle, (S) = square, and (T) = triangle. These four species formed distinguishable clusters.

Table 5
Assignment of blind test samples in focused DAPC models.

Species	Common Name	Assignment Positive-ion Spectra	Assignment Negative-ion Spectra
<i>Zalophus californianus</i>	California Sea Lion	California Sea Lion	California Sea Lion
<i>Eumetopias jubatus</i>	Steller Sea Lion	California Sea Lion*	Steller Sea Lion
<i>Phoca vitulina</i>	Harbor Seal	California Sea Lion*	Harbor Seal
<i>Mirounga angustirostris</i>	Northern Elephant Seal	Northern Elephant Seal	Northern Elephant Seal

(Positive-ion model LOOCV = 85.29%; Negative-ion model LOOCV = 91.43%)
* Indicates a misclassification.

focus of this study was on pinnipeds, the addition of the harbor porpoise and salmon oil in this study were used as a means to demonstrate the difference in chemotypes between various marine taxa and to eliminate any doubt that commonly sold fish oil could be confused with harp seal oil capsules.

A limitation of this study was the small sample sizes from the available species, which may have affected the accuracy of the models, as in the case of the misclassified California sea lion (*Zalophus californianus*). Future studies should focus on including collecting samples from a wider range and increasing the number of sample species from the pinniped clade, of which there are 34 extant species [9], in addition to members of the cetacean clade. This would allow us to increase the utility of DART TOFMS for the classification of supplemental oil pills commonly found in the commercial trade and assist in the regulation of the United States' Marine Mammal Protection Act.

Ethics statement

No animals were harmed in this study and the samples analyzed were either collected from beach found carcasses or were collected for a study of physiologic health of marine mammals.

CRediT authorship contribution statement

Espinoza Edgard: Writing – review & editing, Writing – original

draft, Supervision, Methodology, Investigation, Conceptualization. **Price Erin:** Writing – review & editing, Validation, Software, Methodology. **D’Alessandro Dalin N.:** Resources, Methodology, Data curation. **Duffield Deborah A.:** Resources, Methodology, Investigation. **Pinedo Megahn H.:** Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Blubber samples from the seven comparative marine mammals came from stranded, moderately decomposed animals necropsied by the Northern Oregon/Southern Washington Marine Mammal Stranding Program. Samples were frozen prior to overnight shipment to the US Fish and Wildlife Forensic Laboratory in Ashland, OR.

Legal note

The findings and conclusions in this article are those of the authors and do not represent the views of the US Fish & Wildlife or the US Forest Service.

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