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MMS 2010-016

Trophic Aspects of Sperm Whales (*Physeter macrocephalus*) in the Northern Gulf of Mexico Using Stable Isotopes of Carbon and Nitrogen

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Prepared under MMS Contract
1435-01-06-CT-39790 (M06PC00011)
by
New Mexico State University
Arts & Science Research Center
Box 3000, MSC RC
Las Cruces, NM 88003

Published by

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

New Orleans
April 2010

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CITATION

Suggested citation:

Ruiz-Cooley, R.I. and D.T. Engelhaupt. 2010. Trophic aspects of sperm whales (*Physeter macrocephalus*) in the northern Gulf of Mexico using stable isotopes of carbon and nitrogen. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2010-016. 28 pp.

ABOUT THE COVER

Cover shows mother and calf sperm whales surfacing (upper photo) and a sperm whale fluking (lower photo) in the northwest Gulf of Mexico on the 2005 S-tag cruise (photos by Craig Hayslip, Oregon State University).

ACKNOWLEDGMENTS

This work was made possible thanks to the significant support and collaborative work of many scientists from the Sperm Whale Seismic Study (SWSS). For their extensive support during field work, we thank Ann Jochens and Doug Biggs (Texas A&M University), Bruce Mate and Joel Ortega-Ortiz (Oregon State University), Deborah Epperson and Carol Roden (Minerals Management Service) and the scientific team and crew of the RV-Gyre (SWSS project).

We are grateful to John Wormouth (TAMU), Kim Johnson, Andre Dubose, and Alonzo Hamilton (Department of Commerce/National Oceanic and Atmospheric Administration/ National Marine Fisheries Service) for the collection of squid specimens.

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1. Summary

In the northern Gulf of Mexico (GoM), seismic operations and oil exploration are increasing in deepwater areas which are inhabited by sperm whales (*Physeter macrocephalus*). Yet, little is known about the feeding ecology of sperm whales. This report provides initial information on the variability in the relative trophic position of sperm whales in the northern GoM. Stable isotope ratios of carbon (C) and nitrogen (N) were measured from skin samples of free-ranging whales. The isotopic signatures were different between females and immature males. However, no differences were detected between samples from the Mississippi Canyon and samples from the northwestern GoM, perhaps due to similar consumption of average diet. We compared the isotopic values between individuals from the GoM and Gulf of California (GC), and between squid from inshore and offshore areas of northern GoM. The isotopic signatures of sperm whales from the GoM were contrastingly lower than whales from the GC. This isotopic difference may be associated with a variation in diet and different trophic level. However, the variability in the amount of C and N and biochemical cycling seems to influence this isotopic difference. Although no annual isotopic variation was detected during the years of study, this work recommends continued evaluation of how the relative trophic position (indicated by $\delta^{13}C$ and $\delta^{15}N$) in sperm whales' skin may change through time. We documented significant spatial isotopic variability in squid between inshore and offshore sites of the northern Gulf; this variation has important implication for evaluating trophic relationships using stable isotope analysis. We recommend continued investigation of the feeding ecology of free-ranging whales by using stable isotope ratios in association with mesopelagic cephalopods.

The objectives of the project were

I. Analyze sperm whale skin samples collected in previous SWSS cruises (2002-2005)

II. Conduct stable isotope analysis of different cephalopod species collected in 2003 from SWSS cruises where sperm whales were sighted

III. Conduct analysis of cephalopods collected by NOAA/NMFS scientists in the summer and fall of 2005 in the northern Gulf of Mexico.

Accomplished objectives include

I. Completed stable isotope analysis of sperm whale tissue samples from 2002, 2003, 2004 and 2005.

II. Completed stable isotope analysis from squid tissue samples collected in 2003 in areas where sperm whales were distributed

III. Completed stable isotope analysis from squid collected in 2005 by NOAA/NMFS scientists.

Deliverables

I. Final Report

II. Presentation at the 2007 Information Transfer Meeting in Kenner, Louisiana

III. Presentation at the 2007 17th Biennial Conference on the biology of Marine Mammals in South Africa

IV. Reference values of isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) in sperm whale skin samples collected from 2002 to 2005

Trophic Aspects of Sperm Whales (*Physeter macrocephalus*) in the Northern Gulf of Mexico Using Stable Isotopes of Carbon and Nitrogen

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2. Introduction

Optimal foraging theory predicts that animals select diets that maximize energy intake and minimize the cost to obtain nourishment (Schoener 1971). Optimal diet is crucial for endangered species as it may affect reproductive success. This is particularly important for those populations inhabiting areas where anthropogenic activities are increasing. Sperm whales inhabit the northern Gulf of Mexico (GoM) in water depths between 200 to 2000 m, near cyclone and confluence zones (Hansen et al. 1995, Davis and Fargion 1996, Davis et al. 2000). The distribution of sperm whales might be related to the occurrence of cephalopod species (Davis et al. 2000), but there is not much information about their diet composition and attributes of potential prey (e.g. abundance and diversity of cephalopods) in the GoM.

The main used method to investigate the diet of sperm whales is analysis of stomach contents from carcasses obtained during whaling or from strandings (e.g. Okutani and Nemoto, 1964, Clarke et al. 1988). Measuring stable isotopes of carbon (C) and nitrogen (N) from animal tissues offers an alternative and non-invasive method to traditional analysis to investigate the trophic ecology of sperm whales (Ruiz-Cooley et al. 2004, Marcoux et al. 2007). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios from skin samples of sperm whales and muscle samples of cephalopods indicate a trophic position that depends on their assimilated diet. Isotopic ratios can be used to evaluate changes in trophic position at temporal and spatial scales, and also to infer trophic relationships between sperm whales and potential prey (Ruiz-Cooley et al. 2004, Marcoux et al. 2007).

The goals of this study were: (1) to quantify $\delta^{13}C$ and $\delta^{15}N$ in skin samples of free-ranging sperm whales from the northern Gulf of Mexico, (2) to investigate temporal (interannual) variation in relative trophic position, and (3) to compare the relative trophic position between whales from two locations: Mississippi Canyon and the Northwestern Gulf of Mexico. We tested the null hypothesis of no differences in sperm whale trophic position between years and areas due to low changes in the average assimilated diet. Squid were collected in sites where sperm whales were observed and muscle tissue samples used to determine isotopic ratios. Potential trophic

relationships between sperm whales and squid were inferred comparing their isotopic ratios. Since we lacked a representative sample size of mesopelagic squids (e.g.> 50 individuals by site), we also collected squid from inshore waters from the upper northern Gulf to understand spatial isotopic variation between inshore and offshore sites. This allowed us to recognize how $\delta^{13}C$ and $\delta^{15}N$ in squid change as a function of location within the northern Gulf of Mexico. Efforts are needed to investigate mesopelagic squid and evaluate their relationship with sperm whales as potential prey simultaneously and at different temporal scales.

3. Methods

3.1 Sample Collection, Preservation and DNA Extraction

Skin tissue from free-ranging sperm whales was collected under Marine Mammal Protection Act/Endangered Species Act permit #909-1465 and #909-1726. Samples included in this study were from adult female and immature whales. No samples were collected from physically mature adult males or calves. Tissue samples were collected with a biopsy dart or from sloughed skin (Lambertsen 1987, Palsbøll et al. 1991, Amos et al. 1992), including sloughed skin opportunistically collected from suction cups that had been attached to sperm whales (Miller et al. 2004a and b). Biopsy samples were collected with a sterilized corer tip attached to a dart (with float) fired from a crossbow or PAXARMS biopsy system. A total of 53 skin samples of sperm whales were collected from 2002 to 2004 and stored in an aqueous solution of 20 % (v/v) dimethyl sulfoxide (DMSO) saturated with sodium chloride (Amos and Hoelzel 1991). In 2005, each collected skin sample (n = 18) was divided into two pieces: one half was kept frozen at -20 C for isotopic analysis and the other half in DMSO for gender determination. Whole-cell DNA was extracted for use in subsequent polymerase chain reactions (PCR) by a standard phenol/chloroform extraction technique (Hoelzel 1998).

3.2 Squid Muscle Samples

C and N isotopic ratios in some squid species vary by size and location (Ruiz-Cooley et al. 2006). Therefore, it is highly recommended to collect animal tissues from both predator and prey from the same area to evaluate trophic relationships properly. We obtained muscle tissue samples from mesopelagic cephalopods collected at night (Table 1) using 14.8 m^2 Isaacs-Kidd midwater trawl at different depths (0-400 m, 0-400-600 m and 0-600-800 m; Wormuth 2006). These specimens were obtained in areas of the GoM where sperm whales were observed (Figure 1) and presumed to be engaged in foraging activity (Miller et al. 2004a; Wormuth 2006).
During the summer and autumn of 2005, we collected muscle tissues from inshore neritic species in areas where sperm whales do not occur. Our goal was to evaluate the spatial isotopic variation from the northern Gulf of Mexico and distinguish isotopic values from potential prey (mesopelagic squid) of sperm whales from those of a non-prey (inshore shallow squid). These inshore squid species were collected along the inshore northern Gulf of Mexico by personnel from the National Marine Fisheries Service, Pascagoula, Mississippi, aboard National Oceanic

and Atmospheric Administration research vessels (Figure 1). For these specimens, we measured mantle lengths to the nearest mm using a digital caliper. We analyzed muscle samples from the arrow squid *Loligo plei* (Blainville 1823, n = 61), the brief squid *Loligo pealei* (Lesueur 1821, n = 29) and the long-finned inshore squid *Lolliguncula brevis* (Blainville 1823, n = 54). For these inshore species, *L. brevis* (mean and SD: 75.7 and 74.8 mm ML) had the smallest mantle length, while *L. plei* (mean and SD: 93.2 and 38 mm ML) and *L. pealei* (116 and 40 mm ML) were comparable in size.

We analyzed muscle tissue samples from the mesopelagic squid *Ommastrephes bartrami (*n = 1; 10 cm mantle length (ML)), and from unidentified species from the family Ommastrephidae (n = 1) and Histioteuthidae (n = 2) collected in 2003 in the same area where sperm whales were observed.

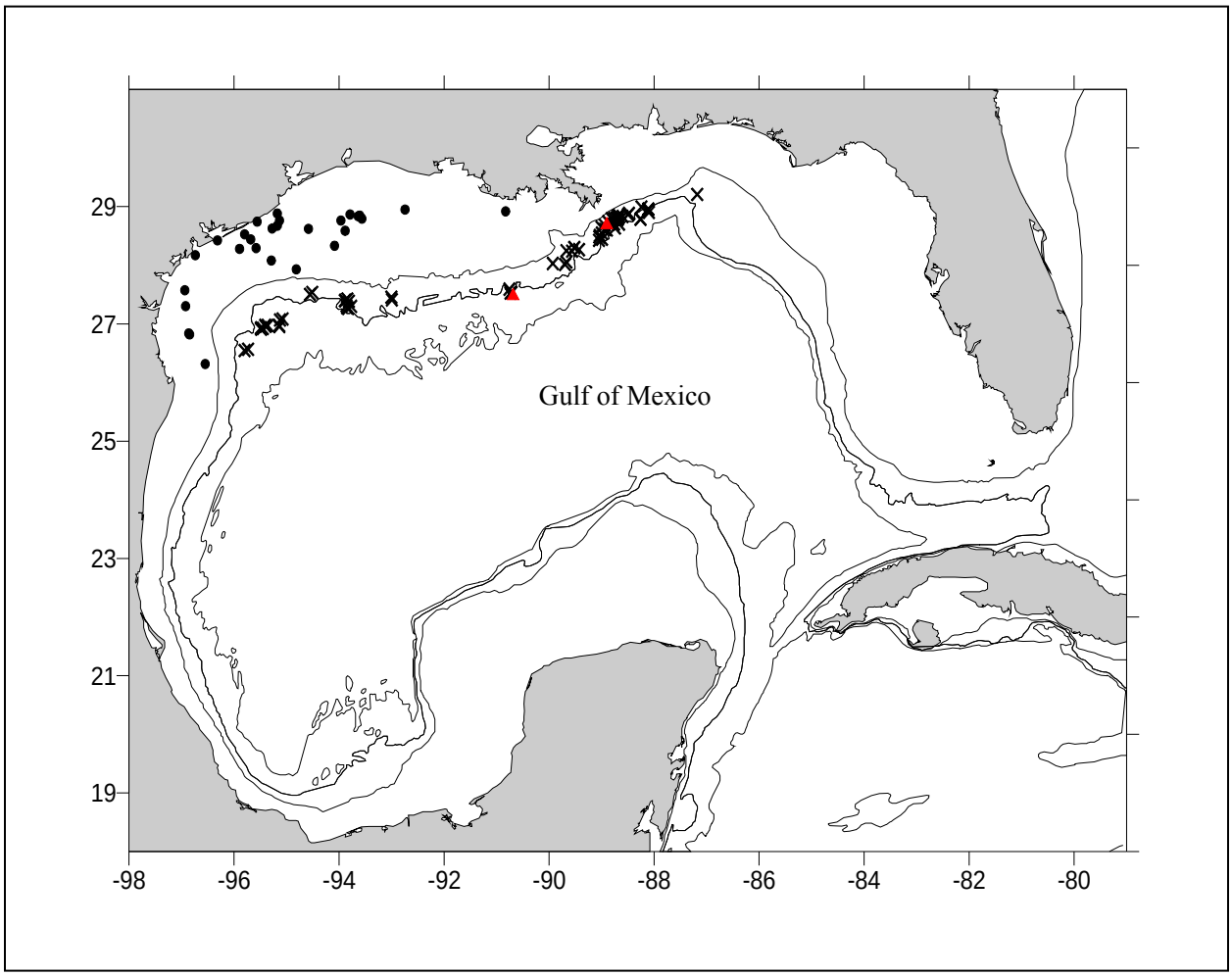

Figure 1. Sampling location of skin of sperm whales (X) and pelagic squid (▲, in red), and inshore shallow water squid (•) from the northern Gulf of Mexico. Contours indicate the 200 m, 1,000 m, and 2,000 m isobaths.

3.3 Genetic Analysis

Sex was determined using the ZFX/ZFY technique described by Bérubé and Palsbøll (1996) and used to evaluate differences between females and males with respect to prey. Male and female strandings of known gender from the Gulf of Mexico and Northern Sea were included as a means of positive confirmation for PCR amplifications. To avoid biases associated with the unintentional inclusion of multiple samples from the same individual, genotype screening at a minimum of ten polymorphic microsatellite loci was performed on all samples, and any duplicates were eliminated as a result (Engelhaupt 2004).

3.4 Stable Isotope Analysis

Most of the tissue samples originated from biopsy darts rather than from sloughed skin. Therefore, we followed a lipid extraction of chloroform and methanol (2:1) modified from Bligh and Dyer (1959) to maximize removal of lipids. Ruiz-Cooley et al. (2004) analyzed mostly sloughed skin samples of sperm whales from the Gulf of California and followed a lipid extraction using chloroform and methanol with a volume ratio 1:1. To compare our result with the previous study, we obtained two subsamples of a total of five skin samples. For each, we extracted lipids following a mixture of chlorophorm: methanol with a volume 1:1 and the other one with a volume 2:1. Regarding potential effect from preservatives (i.e. DMSO) on the isotopic signature of skin samples, no effects of dimethyl sulfoxide (DMSO) were observed on lipid-extracted skin samples of cetaceans in short-term studies (Todd et al. 1997, Marcoux et al. 2007). Thus, we assumed that presence of DMSO in our samples did not affect the results.

Skin samples were analyzed by using an isotope ratio mass spectrometer (IRMS, Thermo Finnigan Delta Plus) at the New Mexico State University. The $\delta^{13}C$ and $\delta^{15}N$ were obtained for each sample. Isotope ratios are expressed in standard notation: $\delta^{15}N$ or $\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 1000$, where R_{sample} and $R_{standard}$ are the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample and standards (PDB for carbon and atmospheric nitrogen, respectively). IAEA-N-1 and ammonium sulfate (IAEA-N-2) provided by NIST were used as a primary standard for nitrogen. For carbon, we used LSVEC (Li_2CO_3) and USGS24 (graphite). The typical long-term external precision obtained by repeated analyses of internal laboratory standards was lower than 0.2 ‰.

3.5 Statistical Analysis

The resultant isotopic ratios of C and N from the northern Gulf of Mexico were categorized by location: Mississippi Canyon and northwest Gulf of Mexico. We tested the effect of location (using only isotopic data of females) and sex on $\delta^{13}C$ and $\delta^{15}N$ using a student t-test and assuming equal variances. Samples from females and males were pooled by year; we tested the effect of year on $\delta^{13}C$ and $\delta^{15}N$ values using ANOVA at $\alpha = 0.05$. Ruiz-Castro (2002) performed a lipid extraction with chloroform and methanol with a volume 1:1, whereas we extracted lipids using the same mixture but with a volume 2:1. Therefore, we tested the effect of lipid extraction with these two methods using a paired t-test. Then, we used published isotopic ratios from females and immature sperm whales reported in Ruiz-Castro (2002) from the central Gulf of California to compare this area with our two sampling sites using 1-way ANOVA.

4 Results

4.1 Relative Trophic Position of Sperm Whales

A total of 71 sloughed skin samples and skin biopsies from free-ranging sperm whales were analyzed from 2002 to 2005 for stable isotope analysis. Based on molecular sexing analysis, we identified a total of 57 females and 14 immature males.

Differences between genders were observed in $\delta^{15}N$ (t = 2.1, P = 0.04, DF = 69). N-isotopic values from females (mean, standard deviation (SD) = 12.31 (0.4) for $\delta^{15}N$) were higher than males (12.05 (0.45) for $\delta^{15}N$) by 0.26 ‰. However, variation in $\delta^{13}C$ (t = -0.47, p = 0.63, DF = 69) was minimal between sexes (mean (SD) for females was -16.55 (0.43) and for males was -16.5 (0.27)). No significant differences were found between years for either $\delta^{15}N$ (ANOVA, $F_{3,67}$ = 1.04, P = 0.38) or $\delta^{13}C$ ($F_{3,67}$ = 0.86, P = 0.46) (Figure 2, Table 1). Female isotopic signatures (mean (SD)) from the Mississippi Canyon (n = 41, -16.53 (0.47) for $\delta^{13}C$ and 12.36 (0.40) for $\delta^{15}N$) and Northwest Gulf (n = 16, -16.58 (0.29) for $\delta^{13}C$ and 12.17 (0.38) for $\delta^{15}N$) were not detectably different between sites for either carbon (t = 0.41, P = 0.68, DF = 55) or nitrogen (t = 1.64, P = 0.107, DF = 55) stable isotope ratios.

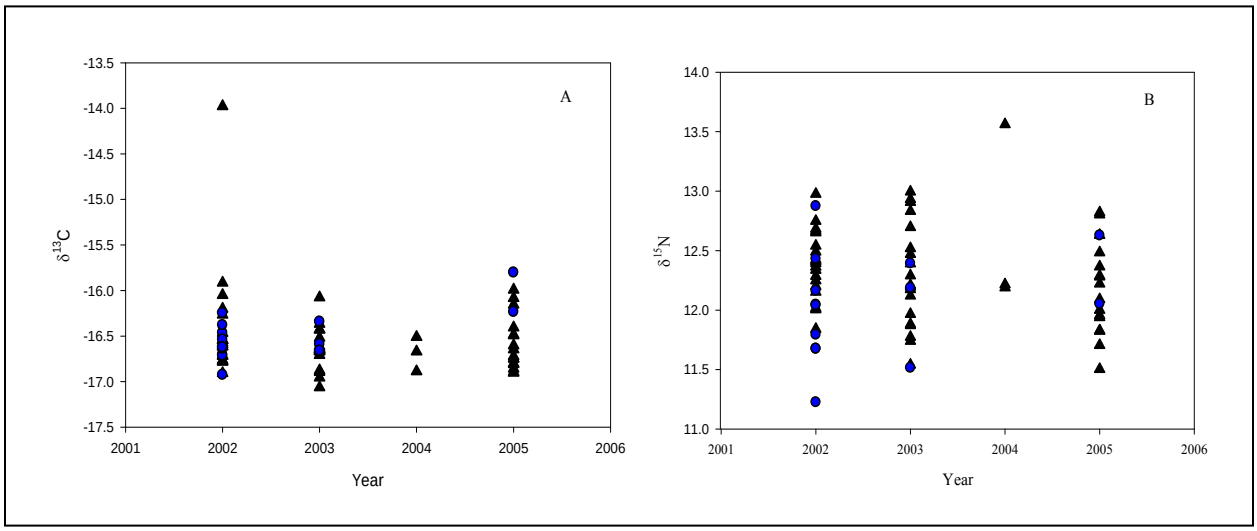

Figure 2. Temporal variation in carbon (A) and nitrogen (B) isotopic ratios (‰) of skin samples from immature male (○ in blue) and female (▲) sperm whales from the northern Gulf of Mexico.

Table 1

Stable isotope signatures (‰) from sperm whale (*Physeter macrocephalus*) skin tissues from the Mississippi Canyon (MC) and Rio Grande Slope (North West (NW)) Gulf of Mexico.

			Gender	$\delta^{15}N$			$\delta^{13}C$		
Year	n	Area	F:M	Mean (SD)	Min	Max	Mean (SD)	Min	Max
2002	28	MC	19:09	12.26 (0.39)	11.22	12.97	-16.46 (0.53)	-16.92	-13.97
2003	22	MC	19:03	12.25 (0.43)	11.51	12.99	-16.62 (0.21)	-17.06	-16.07
2004	3	MC	3:00	12.65 (0.64)	12.19	13.56	-16.69 (0.15)	-16.89	-16.51
2005	18	NW	16:02	12.19 (0.37)	11.50	12.82	-16.52 (0.33)	-16.9	-15.80

4.2 Comparison between Sperm Whales from the Gulf of Mexico and Gulf of California

The $\delta^{13}C$ ($t = 1.56$, $p = 0.19$, DF = 4) and $\delta^{15}N$ ($t = 2.69$, $p = 0.054$, DF = 4) values of lipid extracted skin samples with 1:1 and 2:1 chloroform: methanol ratios were not statistically different. The $\delta^{13}C$ (ANOVA, $F_{1, 105} = 1097.45$, $P < 0.0001$) and $\delta^{15}N$ (ANOVA, $F_{1, 105} = 5489$, $P < 0.0001$) from skin tissue samples from the Gulf of Mexico and Gulf of California were significantly different. Females and immature males from the GoM showed mean isotopic signatures that were lower by 7.42 ‰ for $\delta^{15}N$ and by 2.74 ‰ for $\delta^{13}C$ in comparison with mean values from the Gulf of California (Table 1).

4.3 Isotopic Signatures between Sperm Whales and Squid from the Gulf of Mexico

Stable isotope differences between sperm whales and muscle samples from the family Histiotheutidae and Ommastrephidae were 1.6 ± 0.36 ‰ for $\delta^{13}C$ and 4.3 ± 0.58 ‰ for $\delta^{15}N$ (Figure 3). In contrast, the $\delta^{15}N$ between sperm whales from the Gulf of Mexico and small shallow water squid, *L. plei* and *L. pealei,* were similar; they varied by 0.45 ‰ for and 0.2 ‰, respectively. However, the $\delta^{13}C$ values between sperm whales and *L. plei* were distinct; they varied by 1.27 ‰, while the difference between whales and *L. pealei* was 0.9 ‰ (Figure 3). Remarkably, the $\delta^{15}N$ and $\delta^{13}C$ values from *L. brevis* were higher than those from sperm whales by 2.81 ‰ and 0.7 ‰, respectively (Figure 3).

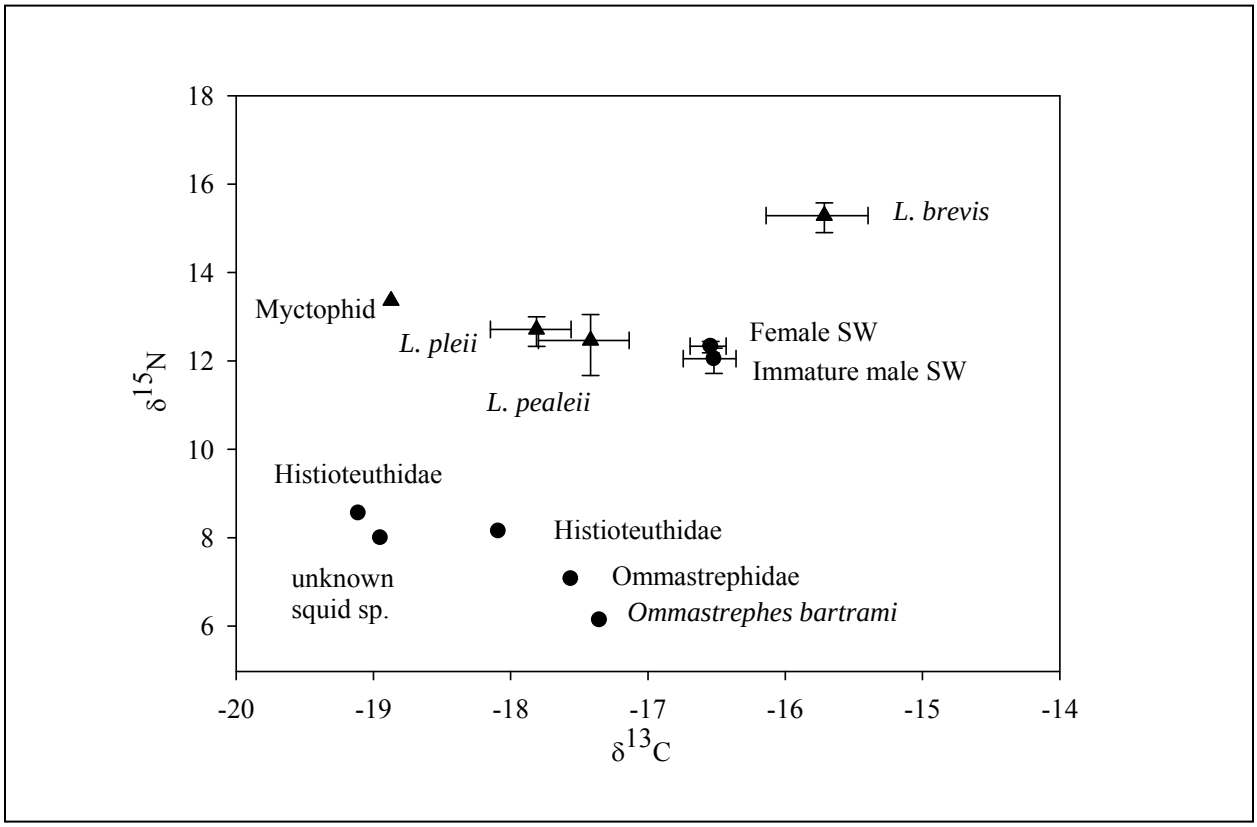

Figure 3. Stable isotope ratios (‰; mean ± 95% confidence intervals) from skin samples of free ranging sperm whales (SW; ●), muscle tissue samples of mesopelagic and neritic squid (*Loligo pealei, L. plei, Lolliguncula brevis*), and a myctophid collected in the northern Gulf of Mexico (▲)

5. Discussion

5.1 Relative Trophic Position of Sperm Whales

Isotopic signatures of female sperm whales from Mississippi Canyon (MC) and the northwest Gulf (NW) were similar. Satellite-telemetry studies have been conducted on individuals from which skin samples were obtained. Telemetry data indicated that groups of adult females have large home ranges but spend most of their time in smaller 'core' areas in Mississippi Canyon and the Rio Grande Slope (Mate and Ortega-Ortiz 2008). Females remained at the edge of the upper continental slope with affinity for particular areas on monthly scales (Mate and Ortega-Ortiz 2008). Moreover, although there was some overlap in the overall home range, the core areas were different for whales tagged (and biopsy sampled) in Mississippi Canyon than those tagged

in the Western Gulf (Mate and Ortega-Ortiz 2008). In the Galapagos Islands, Marcoux et al. (2007) reported isotopic differences between sympatric clans of sperm whales, but only one clan was identified in the northern Gulf of Mexico (Gordon et al. 2008). Because there is no evident oceanographic or biogeographic barrier between these areas, a comparable diversity and abundance of cephalopods and fish may exist at the isobathymetric boundary along the upper slope between depths of 200 and 1000 m. Therefore, it is possible that whales had similar diet between these two sites and fed at the same trophic level. Yet, the number of samples collected in the NW area represents only one year of collection compared with three years for the Mississippi Canyon (Table 1). Telemetry data showed that females did not spend much time in waters more than 2000 m deep (Mate and Ortega-Ortiz 2008) but groups of females with calves have been observed during surveys in that deep area of the lower continental slope (Mullin et al. 2004). No skin samples from female sperm whales from the lower continental slope region were analyzed in the present study. Whales from that region may have different home ranges (Mate and Ortega-Ortiz, 2008), diet composition and consequently trophic position. Further studies are needed to test whether females from the lower continental slope region have different relative trophic position than the ones from the upper slope.

The comparative variation in the relative trophic position between females and immature males from the northern GoM differed from previous findings in the Gulf of California (GC) (Ruiz-Cooley et al. 2004). In the GoM, immature males fed at a lower trophic position than females as indicated by the lower and significant differences in $\delta^{15}N$ between them, while in the GC immature males were higher in $\delta^{15}N$ than females, but no statistical differences were detected. This variation can be associated with length and age of immature males, range of foraging areas (horizontal and vertical) and prey size consumed. For example, in the northern GoM, many immature males tracked by satellite-telemetry moved to deeper waters and explored southern areas within the GoM, while females remained in the northern part (Mate and Ortega-Ortiz 2008). Immature males are likely to forage during their journey within the Gulf and may prey upon other prey species that are not accessible to females.

The isotopic differences between sperm whales and mesopelagic squid from the family Ommastrephidae, such as *Ommastrephes bartramii*, and squid from the family Histiotheutidae (Figure 3) suggest potential trophic-relationships between them, although a representative sample size of these squid species for each area (i.e. Mississippi Canyon and NW) is needed to evaluate this relationship. Our squid sample size is low, so caution should be taken with our interpretation. The overall predator-prey isotopic enrichment recognized in marine systems varied from 0.8 to 1.5‰ for $\delta^{13}C$ (DeNiro and Epstein 1978, Fry and Sherr 1984) and 2.5 to 3.5‰ for $\delta^{15}N$ (Minagawa and Wada 1984, Wada et al. 1987, Fry 1988). For consumers raised on a high-protein diet, the average trophic enrichment is 3.3‰ for $\delta^{15}N$ (McCutchan et al. 2003). Currently, the isotopic difference between sperm whale skin and cephalopod muscle has not been rigorously tested. In this study, the $\delta^{13}C$ isotopic difference between sperm whales and these mesopelagic squid species (1.6 ± 0.36 ‰, Figure 3) may suggest a predator-prey relationship. However the difference in $\delta^{15}N$ between sperm whales and these squid was higher (4.3 ± 0.58 ‰ for $\delta^{15}N$) than the overall predator-prey difference for $\delta^{15}N$. Using archival tags or D-tags, Miller et al. (2004a) found that sperm whales from the northern Gulf of Mexico produce creaks (echolocation signals used for foraging) mainly at depths between 490 and 742 m. Because our mesopelagic cephalopod species were collected in areas and at depths (400 to 600 m) where

sperm whales forage in the Gulf of Mexico (Miller et al. 2004a, Wormouth 2006), sperm whales may feed on these cephalopod species but of larger sizes than the ones that we analyzed. Stomach content analysis of four stranded sperm whales from the northern GoM revealed that *Histioteuthis* sp. had the highest percentage of occurrence (Barros 2003 cited by Wormouth 2006). Our results recognized *Histioteuthis* sp. as a potential prey, but squid with mantle lengths larger than 20 cm ML are crucial to evaluate its role as a potential prey. Additionally, more efforts are needed to collect other mesopelagic cephalopod species from sperm whale foraging areas to evaluate the importance of these species in the sperm whale diet.

5.2 Temporal Variation

Comparable $\delta^{13}C$ and $\delta^{15}N$ values among years indicate that sperm whales maintained a similar diet composition throughout the period from 2002 to 2005. While each of these observations is the result of the diet over a period prior to sample collection, the length of that period is currently not well defined. Based on the turnover rate of sloughed skin from other odontocetes and the thickness of sperm whale skin, Ruiz-Cooley et al. (2004) suggested that stable isotope ratios from sloughed skin samples of sperm whale may reflect the diet assimilated over a period of at least 72 days. Seasonal changes in the C- and N-isotopic signatures in pilot whales were recorded in the Strait of Gibraltar (De Stephanis et al. 2008). These seasonal differences in isotopic ratios may suggest that stable isotope ratios from pilot whale skin change at rate of about four months. It is likely that adult sperm whales have slower metabolism rates than pilot whales. Hence, if sperm whales would have changed their diet composition significantly between years, we would have detected changes in their skin isotopic signatures. In the Gulf of California, no detectable differences were observed in the isotopic values of sperm whale skin over three years of study (1997 to 1999), including one year (1997) in which an El Niño event occurred (Ruiz-Cooley et al. 2004). In contrast, the skin isotopic values of sperm whales varied significantly in the Galapagos area in a longer-term study (i.e. in 1989, 1991 and 1995) and in Chile between 1993 and 2000 (Marcoux et al. 2007). Our study analyzed skin samples of sperm whales collected 17 years later, long after the oil industry began in deep areas in 1985 (French et al. 2006). A comparison of the isotopic signatures for sperm whales before and after oil industry expansion would have enabled us to evaluate potential changes in the sperm whale relative trophic position in association with the developing oil industry from the northern GoM. Unfortunately, we lacked samples from prior to oil development.

Potential impacts from oil industry development on the sperm whale population are poorly understood. Modeling studies obtained from D-tagged whales exposed to controlled airgun emissions from seismic survey vessels revealed that these emissions may have an effect on sperm whales (Madsen et al. 2006), such as on their foraging effort (Miller et al. 2005, DeRuiter et al. 2008). Currently, it is unknown how seismic operations or activities of the oil industry influence mesopelagic cephalopod populations. Information on this aspect is of particular importance since mesopelagic squid are the main prey of sperm whales (Kawakami 1980, Wormouth 2006). As squid grow, some pelagic species occupy deeper waters (Rodhouse and Nigmatullin 1996), so larger sized squid are likely to occur in deeper waters of the mesopelagic zone. Miller et al. (2008) revealed that D-tagged sperm whales foraged both along the seafloor

and at mid-depth in the water column in the northern Gulf, areas where higher quality prey patches are likely to occur. Since cephalopods change rapidly as a result of anthropogenic activities and environmental variables (Rodhouse and Nigmatullin 1996), airgun emissions or other anthropogenic activities may potentially affect the distribution of squid in the mid-water column or in the deep-ocean. For instance, Engås et al. (1996) demonstrated that fish populations were severely affected by seismic emissions from airguns on the North Cape Bank in the Barents Sea; the abundance and catch rates of cod and haddock stocks were reduced by almost 50%. Clearly, directed efforts are needed to understand the potential effects of the oil industry on meso- and bathypelagic squid communities (sperm whale prey).

The resighting of individual sperm whales over periods of years, genetic findings indicating a unique GoM population, information on acoustic dialects, and satellite-telemetry results all support the hypothesis that at least some female sperm whales exhibit site-fidelity to the GoM coastal basins (Reeves and Notarbartolo di Sciara 2006, Mate and Ortega-Ortiz, 2008, Jochens et al. 2008, Engelhaupt et al., 2009). If so, changes in sperm whale diet composition (e.g. feeding on smaller sized squid or in prey patches with lower density) may result in a lower caloric gain per unit foraging effort and could reduce feeding success and consequently fitness. A reduction in feeding success of sperm whales would hinder allocation of energy to other biological activities such as reproduction and growth. Therefore, we recommend continuing monitoring the relative trophic position of sperm whales using stable isotope analysis and beginning of efforts to understand potential effects of the oil industry on mesopelagic cephalopod communities. A high concern would arise if sperm whales maintained their home ranges but the $\delta^{15}N$ in skin decreased through time.

5.3 Spatial Variation

When comparing the isotopic signatures of sperm whales from the GoM and the Gulf of California (GC), we were expecting distinct isotopic values, since sperm whale prey composition varies by area (Kawakami 1980). In the Gulf of California, sperm whales prey on the jumbo Humboldt squid *Dosidicus gigas* (Ruiz-Cooley et al. 2004 and 2006), whereas in the GoM they seem to prey on squid of the families Ommastrephidae and Histiotheutidae (Barros 2003; Figure 3). However, we observed contrasting differences in the $\delta^{13}C$ and $\delta^{15}N$ of sperm whales from the central Gulf of California and northern GoM. The isotopic ratios of sperm whales from the northern GoM were significantly lower (7.41 % for $\delta^{15}N$ and 2.74 % for $\delta^{13}C$) compared to ratios from the GC. We found no differences in the $\delta^{13}C$ and $\delta^{15}N$ values from skin lipid extracted with a volume of chloroform and methanol 1:1 versus 2:1 respectively. Therefore, we are confident that the small variation in this method did not have an effect on the resultant variation in isotopic ratios of sperm whale skin from these two oceanic basins.

At first glance, the isotopic difference between GoM and GC may suggest that sperm whales from the GoM occupy a lower trophic position than those in the GC, since enrichment in ^{15}N from prey to predators allows the $\delta^{15}N$ value to serve as an indicator of trophic position, whereas the $\delta^{13}C$ value traces origin of primary productivity (De Niro and Epstein 1978 and 1981, Minawaga and Wada 1984, Fry 1988, Rau et al. 1992). If we arbitrarily assumed that a

difference of 3 % for $\delta^{15}N$ represents one trophic level, sperm whales from the GoM would be 2.5 trophic levels lower than whales from the GC. Because sperm whales from the GC feed on larger jumbo Humboldt squid of approximately 60 to 70 cm mantle length (Ruiz-Cooley et al. 2004, Davis et al. 2007), then sperm whales from the GoM would be feeding on tiny squid or fish that may be part of the zooplankton; this is very unlikely.

Inferences about trophic position using stable isotopes in studies that lack samples at the base of the food web can be misleading. For instance, latitudinal variation in $\delta^{13}C$ values from plankton exist, e.g., a $\delta^{13}C$ decrease from tropical latitudes toward the poles, presumably due to gradients in temperature and CO_2 concentration as a function of latitude (Rau et al. 1982). Using both C and N isotope ratios allows for discrimination between trophic positions within the same system but also between aquatic systems such as freshwater, estuaries and marine ecosystems (Owens 1987). In marine systems, these biochemical tracers are also helpful to differentiate between offshore versus inshore marine animals (e.g. for $\delta^{13}C$, Rau et al. 1983). A geographic isotopic variation in $\delta^{15}N$ between oceanic areas has been documented in squid (Takai et al. 2000, Ruiz-Cooley et al. *in press*), sea turtles (Wallace et al. 2006) and sperm whales (Marcoux et al. 2007). This geographic variability in $^{15}N/^{14}N$ from animal tissues suggest that N can be used to differentiate geographic subpopulations due to differences in the physical and biochemical characteristics among systems (Wallace et al. 2006, Ruiz-Cooley et al. *in press)*. Hence, the distinct organic and isotopic pool derived from the variability in biochemical cycles of C and N between the GoM and GC may influence significantly isotopic differences between these populations.

For instance, we also observed that $\delta^{15}N$ values from skin samples of sperm whales and muscle tissue samples from two small squid species (*Loligo plei* and *L pealei*) were remarkably similar, but the $\delta^{13}C$ values differed. The δ^{15} N from *Lolliguncula brevis* muscle tissues was even higher than sperm whale isotopic signatures, but there was an overlap in the $\delta^{13}C$ values (Figure 3). These three squid species generally occupy warm, shallow, and inshore waters of the northern GoM and occur on the edge of the continental shelf at depths between 40 and 250 m (Hanlon et al. 1983). *L. plei* is often found at depths greater than 75 m, while *L. brevis* often occurs in shallower waters, for example in Galveston Bay at depths from 1 to 20 m (Hanlon et al. 1983). In contrast, sperm whales are distributed in the northern Gulf of Mexico in areas with depths between 200 to 2000 m (Hansen et al. 1995, Davis and Fargion 1996, Davis et al. 2000) while their prey would occupy the mesopelagic ocean at depths greater than 400 m (Miller et al. 2004a). There is no overlap in range between these three inshore squid species and sperm whales. Therefore, the stable isotopic signatures of these cephalopod species reflect the metabolism of N and C from inshore shallow waters of the northern Gulf, whereas isotopic ratios of sperm whales reflect their diet in offshore deep waters. The resultant $\delta^{15}N$ and $\delta^{13}C$ from specimens from inshore and shallow water systems (*Loligo* species) and offshore pelagic systems (sperm whales and mesopelagic squid) (Figure 3) illustrate the importance of collecting tissues samples from predator and prey from the same area and time; accurate inferences of trophic relationships using stable isotope analysis depend on that information.

6. Recommendations

This study provides baseline information regarding the relative trophic position of sperm whales in the northern Gulf of Mexico. Stable isotope analysis is a useful tool to infer aspects of the feeding ecology of sperm whales, but more effort is needed to evaluate temporal shifts in their trophic position. This is particularly important due to the fact that sperm whales from the northern Gulf of Mexico appear to exhibit unique characteristics. Recent findings based on mtDNA and nuclear DNA suggest that this 'stock' is genetically distinct from those whales comprising the neighboring North Atlantic Ocean stock (Engelhaupt et al. 2009), they belong to a unique acoustic clan (Gordon et al. 2008), and they have a smaller body length compared with other areas (Jochens et al. 2008). Seismic airguns used in energy exploration may have a negative impact on sperm whale feeding ecology (Miller et al. 2005, DeRuiter et al. 2008). Therefore, it is of great importance to establish a continual evaluation of the relative trophic position of sperm whales from the northern GoM and to investigate the relative trophic position of the offshore groups. Stable isotope analysis provides a feasible method to accomplish this goal. However, we need to invest more effort to investigate the diversity, distribution and abundance of mesopelagic cephalopods, especially in the core areas recognized by Mate and Ortega-Ortiz (2008). We need to understand the function of these areas in the biology of sperm whales. In particular, a large sample of the large sized mesopelagic squid (e.g. > 30 cm mantle length) or fish concurrent with skin samples of sperm whales would allow us to evaluate trophic relationships. Additionally, more effort is needed to collect and analyze samples from mid-water cephalopods as well as from particulate organic material or plankton concurrent with tissue samples of sperm whales and squid to determine trophic level and their changes through time. This is crucial to distinguish (1) if sperm whales from the northern Gulf have a lower trophic level than whales from the Gulf of California or (2) if the variability in the biochemical cycle of N and C between areas is responsible for the observed spatial isotopic variation.

7. References

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Appendix A. Stable Isotopic Ratios from Skin Samples Collected in the Northern Gulf of Mexico from 2002 to 2005 (tag # corresponds to Oregon State University S-tag codes)

Year	Date	Latitude	Longitude	$\delta^{15}N$	$\delta^{13}C$	Tag #	Gender
2002	24-Jun-02	28.056	-89.669	11.84039	-16.5913		Female
2002	24-Jun-02	28.012	-89.696	12.97605	-13.9777	2505660	Female
2002	02-Jul-02	28.866	-88.496	12.28513	-16.5473	2505678	Female
2002	02-Jul-02	28.884	-88.478	12.66605	-16.2659		Female
2002	03-Jul-02	28.794	-88.808	12.00501	-16.2022		Female
2002	03-Jul-02	28.705	-88.764	12.54042	-16.6176	2505709	Female
2002	03-Jul-02	28.84	-88.692	12.24767	-16.5741	2505670	Female
2002	03-Jul-02	28.85	-88.673	12.36314	-16.7803	2505720	Female
2002	03-Jul-02	28.799	-88.699	12.15242	-16.5428	2505670	Female
2002	03-Jul-02	28.808	-88.684	12.39353	-16.4660	2505669	Female
2002	03-Jul-02	28.805	-88.698	12.20104	-16.7156	2505701	Female
2002	06-Jul-02	28.426	-89.05	12.01514	-16.9087		Female
2002	07-Jul-02	28.701	-88.665	12.68226	-16.7197		Female
2002	07-Jul-02	28.742	-88.875	12.42291	-15.9152		Female
2002	23-Aug-02	28.301	-89.519	12.41075	-16.6135		Female
2002	23-Aug-02	28.301	-89.519	12.74912	-16.7662		Female
2002	25-Aug-02	28.78	-88.693	12.49078	-16.7833		Female
2002	26-Aug-02	28.686	-88.944	12.3368	-16.0506		Female
2002	28-Aug-02	28.651	-89.005	12.65186	-16.5463		Female
2002	28-Jun-02	29.208	-87.178	11.22545	-16.2477	2505648	Male
2002	01-Jul-02	28.958	-88.109	11.79328	-16.4635	2505685	Male
2002	01-Jul-02	28.956	-88.109	11.67627	-16.5388	2505650	Male
2002	01-Jul-02	28.951	-88.113	12.16255	-16.7065	2505726	Male
2002	01-Jul-02	28.903	-88.099	11.67627	-16.7126	2505725	Male
2002	06-Jul-02	28.445	-88.991	12.04604	-16.9258		Male
2002	08-Jul-02	28.992	-88.239	12.43101	-16.6388		Male
2002	23-Aug-02	28.239	-89.562	12.04098	-16.6226		Male
2002	29-Aug-02	28.832	-88.6	12.87525	-16.3791		Male
2003	02-Jun-03	27.265	-93.825	12.391	-16.6711		Female
2003	02-Jun-03	27.399	-93.885	12.90716	-16.3666		Female
2003	02-Jun-03	27.399	-93.885	11.77355	-16.8785		Female
2003	03-Jun-03	27.387	-93.811	12.47052	-16.0769		Female
2003	04-Jun-03	27.423	-93.842	12.1943	-16.6445		Female
2003	05-Jun-03	27.455	-93.003	12.174	-16.6671		Female
2003	08-Jun-03	27.527	-90.736	11.5395	-17.0643		Female

Appendix A. Stable Isotopic Ratios from Skin Samples Collected in the Northern Gulf of Mexico from 2002 to 2005 (tag # corresponds to Oregon State University S-tag codes) (continued)

Year	Date	Latitude	Longitude	$\delta^{15}N$	$\delta^{13}C$	Tag#	Gender
2003	08-Jun-03	27.535	-90.713	11.87179	-16.9582		Female
2003	08-Jun-03	27.592	-90.755	12.20915	-16.8953		Female
2003	05-Jul-03	28.637	-88.7452	12.69543	-16.5195	2800843	Female
2003	05-Jul-03	28.698	-88.7648	12.9318	-16.6446	2805719	Female
2003	08-Jul-03	28.61	-88.938	11.9668	-16.6629	2505669	Female
2003	09-Jul-03	28.5083	-89.034	11.8805	-16.6133	2505719	Female
2003	09-Jul-03	28.5487	-88.9961	12.121	-16.6371	2810820	Female
2003	09-Jul-03	28.4822	-89.0432	12.9945	-16.6533	2800829	Female
2003	11-Jul-03	28.6016	-88.8991	12.83219	-16.4296	2800833	Female
2003	11-Jul-03	28.7873	-88.2583	12.29019	-16.6530		Female
2003	11-Jul-03	28.7873	-88.2583	11.74	-16.4300	2805678	Female
2003	11-Jul-03	28.805	-88.7221	12.518	-16.7100	2800826	Female
2003	02-Jun-03	27.399	-93.885	12.393	-16.3414		Male
2003	05-Jun-03	27.411	-92.995	12.18686	-16.5893		Male
2003	11-Jul-03	28.7965	-88.763	11.5143	-16.6595	2800827	Male
2004	06-Jun-04	28.2664	-89.4285	12.215	-16.6705	3205660	Female
2004	06-Jun-04	28.2467	-89.6693	13.562	-16.5114	3202083	Female
2004	07-Jun-04	28.0311	-89.9294	12.18939	-16.8889	3205670	Female
2005	11-Jun-05	26.567	-95.732	12.36516	-16.1567	3700836	Female
2005	15-Jun-05	26.954	-95.137	12.09	-16.8100		Female
2005	18-Jun-05	26.976	-95.408	12.00096	-16.7995		Female
2005	18-Jun-05	26.96	-95.398	11.50252	-16.8586		Female
2005	18-Jun-05	26.974	-95.361	11.93866	-16.9036		Female
2005	21-Jun-05	26.922	-95.451	12.63059	-15.9920	3705644	Female
2005	21-Jun-05	26.931	-95.484	11.82722	-16.4862	3700846	Female
2005	21-Jun-05	26.928	-95.488	12.81902	-16.0829	3701386	Female
2005	21-Jun-05	26.909	-95.485	11.82	-16.6040	3705726	Female
2005	24-Jun-05	27.274	-93.845	11.95486	-16.7227	3705701	Female
2005	24-Jun-05	27.311	-93.858	12.28715	-16.8945		Female
2005	26-Jun-05	27.299	-93.768	12.2213	-16.7500	3700847	Female
2005	27-Jun-05	27.502	-94.554	12.48369	-16.4064		Female
2005	27-Jun-05	27.537	-94.511	12.28006	-16.6479		Female
2005	28-Jun-05	27.084	-95.074	11.70463	-16.4943		Female
2005	28-Jun-05	27.066	-95.124	12.8018	-16.7480		Female
2005	11-Jun-05	26.545	-95.799	12.05212	-16.2340		Male
2005	15-Jun-05	26.974	-95.141	12.62856	-15.8020		Male

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.