Project 7 Final Report

Reducing Conflicts Between Fisheries and Protected Species in North Carolina: Stable Isotope Analysis of the Diet of Pilot Whales

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Project Background

The most acute conservation problem currently facing marine mammals is bycatch, the unintended capture of animals in fishing gear (Read et al. 2006). The problem of bycatch is particularly challenging when marine mammals remove captured fish from fishing gear, a process known as depredation. Depredation and bycatch are common features of many pelagic and demersal longline fisheries throughout the world (Gilman et al. 2006). Depredation results in increased cost and lost revenue for the fishery due to a reduction in the quantity and value of catch ad damage to fishing gear. In addition, marine mammals may become entangled and die in fishing gear while engaging in depredation.

The Pelagic Longline Take Reduction Team (PLTRT) was convened by the National Marine Fisheries Service in 2005 to address the bycatch of pilot whales (*Globicephala spp.*) in the Atlantic pelagic longline fishery. This fishery primarily targets bigeye (*Thunnus obesus*) and yellowfin (*Thunnus albacores*) tuna. The diet of short-finned pilot whales (*G. macrohynchus*) is believed to be comprised mostly of squid (Mintzer et al. 2008), but pilot whales are known to take advantage of foraging opportunities presented by fishing gear (e.g. Gannon et al. 1997) including pelagic longline catches. At the present time we do not understand the prevalence of this behavior at a population level or whether certain sex or age classes, social groups, or individuals preferentially engage in depredation in this fishery.

Stable carbon and nitrogen isotope analysis is a powerful technique that can be used to address many questions concerning foraging ecology, habitat use, diet composition, and tropic ecology (see reviews by Hobson 1999; Kelly 2000; Newsome et al. 2010). The stable isotopic composition of the tissues of animal reflects the average isotopic composition of its assimilated diet, although isotope enrichment occurs between an animal and its food (DeNiro and Epstein 1978). ^{13}C enrichment is estimated to be 1-2‰ per trophic level due to carbon isotopic fractionation during assimilation or respiration (DeNiro and Epstein 1978; Peterson and Fry 1987). ^{15}N enrichment between trophic levels is 3-4‰ per trophic level, mainly due to the preferential excretion of ^{14}N in urine (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987).

As the enrichment in ^{15}N is relatively large and predictable between predator and prey, it can serve to identify an animal's trophic position within a community (Minagawa and Wada 1984; Fry 1988; Hobson and Welch 1992; Rau et al 1992; Lesage et al. 2001). ^{13}C enrichment along the food chain is relatively small and more variable but, instead, it reflects sources of primary production (Rau et al. 1992; Lesage et al. 2001). Carbon isotopes can provide information about the type of foraging habitat, from inferences regarding the sources of carbon (Ramsay and Hobson 1991; France 1995; Smith et al. 1996; Clementz and Koch 2001).

Isotope ratios are expressed in delta (δ) notation as parts per mil (‰) where δ is the isotope ratio of the sample relative to a standard using the following equation:

$$\delta^{h}X = [(R_{sample}/R_{standard})-1] \times 1000$$

where X is the element, h is the mass of the heavy isotope and R_{sample} and $R_{standard}$ are the heavy and light isotope ratios ($^{13}C/^{12}C$) or $^{15}N/^{14}N$) of the sample and standard, respectively (Newsome et al. 2010). The accepted standards are carbonates from Vienna Pee Dee Belemnite limestone for $\delta^{13}N$ (Newsome et al. 2010).

Objectives

The main objective for our project was to investigate the prevalence of depredation in pilot whales found in the Cape Hatteras Special Research Area (CHSRA), North Carolina. Pilot whales typically feed on squid (Mintzer et al. 2008), but if they regularly supplement their diet with tuna from longlines, we should observe elevated $\delta^{15}N$ signatures in their tissues. To address this question, we sampled pilot whales over several years and from multiple pods to determine the general prevalence of depredation in this population. Preliminary analysis of photo-identification images indicate that some of the pilot whales have been observed over multiple seasons during several years, and thus may be resident in the CHSRA.

Methods

Sample Collection
We collected skin samples of short-finned pilot whales during research cruises in the CHSRA from 2006 to 2010. We used a remote biopsy sampling system to obtain a small skin sample, using a projectile dart equipped with a specialized stainless-steel sampling tip launched from a modified crossbow with a 150-lb pull strength (Figure 7-1). Our sampling efforts focused on distinctive adult individuals, avoiding mature females with small dependent calves, to avoid double sampling any individual pilot whale; a photographer documented each biopsy attempt to identify the individual sampled.

The biopsy samples were initially collected to determine species identification (long-finned or short-finned pilot whale) and were stored in vials containing dimethyl sulfoxide (DMSO) saturated with NaCl, which is used for preserving tissue samples for generic analysis. Beginning in 2008 we sub-sampled each biopsy sample, with half of the sample stored in DMSO and the other half of the sample frozen at -20°C for stable isotope analysis. During a research cruise in 2010 in the CHSRA we also biopsied offshore bottlenose dolphins (*Tursiops truncatus*) to examine population structure in these dolphins. All of these samples were frozen at -20°C.

We also collected skin samples from stranded pilot whales. A mass stranding of 33 short-finned pilot whales occurred along the Outer Banks of North Carolina in January, 2005. Necropsies and sample collection were performed on 27 of these animals (Hohn et al. 2006). We obtained skin samples from 24 of these animals and three other short-finned pilot whales that stranded individually along the northern portion of the Outer Banks from 2005-2010. All samples from stranded animals were frozen at -20°C.

We obtained samples of bigeye and yellowfin tuna at Etheridge Seafood, Wanchese, North Carolina from pelagic longline vessels returning from fishing trips in the CHSRA in October 2006 and May 2007. Each fish was weighed and a hollow metal probe was inserted behind the pectoral fin, removing a core of sin and muscle used to judge the quality of the fish. We collected 69 samples from bigeye tuna and 77 samples from yellowfin tuna; all samples were placed in individual vials and frozen at -20°C. During October, 2007 we also obtained a 25 lb box of squid from a seafood distributor in Wanchese, North Carolina. These are local squid that the longline fishermen use as bait and are likely long-finned squid (*Loligo pealeii*) commonly referred to as *Loligo*.

Sample Preparation and Analysis

There is considerable variability in how cetacean tissues are prepared for stable isotope analysis, particularly in terms of sample preservation and whether or not lipid is extracted from the samples prior to analysis (Table 7-1). Lipids are known to be depleted in ^{13}C compared to proteins and carbohydrates and typically have more negative $\delta^{13}C$ values than proteins or carbohydrates (DeNiro and Epstein 1977; Post et al. 2007). Many, but not all, cetacean researchers extract lipid prior to analysis of stable isotopes (Table 1). In addition, several publications have suggested that it is possible to correct for the effects of DMSO preservation on isotopic signatures via lipid extraction on preservation effects of $\delta^{13}C$ and $\delta^{15}N$ values of pilot whales, we designed a matched sample experiment using skin samples from 10 pilot whales from the mass stranding in 2005. We chose these animals because of the relatively large skin samples available, because multiple sub-samples were required from each specimen for this experiment. Each piece of skin was sub-sampled, which one portion preserved in DMSO and the other portion frozen. Barrow et al. (2008) found that samples stored in DMSO for one to 30 days had different preservation effects than those stored for 60 days. All of our archived samples had been stored in DMSO for more than three years, so we allowed the samples to remain in DMSO for a minimum of 60 days.

The samples were then rinsed with de-ionized water, freeze-dried and further sub-sampled, with half of each sample receiving no further treatment and the other half subjected to lipid extraction. Each of these latter sub-samples was triple rinsed in de-ionized water and then lipids were extracted using a chloroform and methanol solvent (2:1 v/v), following the protocol of Lesage et al. (2010). We placed skin tissues in glass tubes with 8-10ml of the solvent, shook them for 10 minutes and stored them overnight. The solvent was removed the next day via pipette and a fresh 10ml of solvent was added. We repeated this procedure three times. Following lipid extraction, all 40 samples (20 lipid extracted, 20 with no lipid extracted) were homogenized either by use of a ball mill or mechanical chopping. We then weighed the samples and sealed them in tin capsules and sent them to the Duke Environmental Isotope Laboratory (DEVIL), in Durham, North Carolina, to be analyzed by isotope mass spectrometry.

After examining the results of the matched sample experiment (see below) we decided to extract lipids from all remaining samples. Following lipid extraction, a sub-sample (n=20) of the samples was run through a nitrogen evaporator to confirm that all lipids had been successfully extracted. The samples were homogenized and then weighed and sealed in tin capsules and analyzed by isotope mass spectrometry at DEVIL, Durham, North Carolina.

We divided the tuna samples into two seasonal categories (fall and spring) and three size class categories (small, medium, and large) and randomly selected five samples from each group, resulting in 30 samples of bigeye tuna and 30 samples of yellowfin tuna. We also randomly selected 20 squid samples for analysis. The selected samples were thawed and dried in a drying oven at 60°C for a minimum of five days to a stable weight. We then homogenized samples with mortar and pestle,

extracted the lipids, weighed and sealed them in tin capsules. These samples were also analyzed by isotope mass spectrometry at DEVIL, Durham, North Carolina. To confirm that the tuna were correctly identified to species at the time the samples were collected we performed genetic analyses on a subsample of the tuna samples (19 bigeye and 23 yellowfin tuna). We sequenced a portion of the mitochondrial cytochrome b gene using methods described in Bartlett and Davidson (1991). The genetic tests confirmed morphological species identifications for all samples.

We analyzed the results of the matched sample experiment with a two-way crossed ANOVA. Potential differences in stable isotope signatures caused by gender, age class, and indications of past fishery interactions were examined using a non-parametric Wilcoxon test. Inter-species differences in stable isotope value were also tested with Wilcoxon tests. We performed all statistical tests using JMP 8.0 statistical software.

Results

We obtained skin samples from 96 short-finned pilot whales, including 69 biopsies and 27 samples from stranded animals (Figure 7-2 and Table 7-2). We confirmed the identity of all specimens as short-finned pilot whales by molecular analysis in the laboratory of Dr. Patricia Rosel (NMFS/SEFSC). This genetic analysis also indicated that our sample included three sets of duplicate samples, with two samples obtained from three separate whales; we average isotope values for these individuals, resulting in a total of 93 samples. Eleven pilot whale biopsy samples collected during May 2008 were processed without lipid extraction and were excluded from further analysis.

Effects of Sample Preservation and Lipid Extraction
The results from our matched sample experiment demonstrated that preservation method did not have an effect on either $\delta^{13}C$ ($p = 0.672$) or $\delta^{15}N$ values ($p = 0.129$). Lipid extracted samples, however, had significantly enriched $\delta^{13}C$ ($p = 0.0001$) compared to non-lipid extracted samples but lipid extraction did not significantly affect $\delta^{15}N$ values ($p = 0.841$; Table 3). There was no interaction between preservation and lipid extraction for $\delta^{13}C$ ($p = 0.236$) or $\delta^{15}N$ ($p = 0.547$).

P*ilot Whales and Potential Prey*
There were significant differences in $\delta^{13}C$ values ($p < 0.0001$) and $\delta^{15}N$ values ($p < 0.0001$) between pilot whales and their potential prey (Figure 7-3). Short-finned pilot whales had the highest $\delta^{13}C$ signatures but a similar $\delta^{15}N$ signature as that of bigeye tuna. *Loligo* had the lowest $\delta^{13}C$ value and a $\delta^{15}N$ value intermediate between the two tuna species. Yellowfin tuna had a slightly more enriched $\delta^{13}C$ value than *Loligo* but the lowest $\delta^{15}N$ value. Bigeye tuna had the most enriched $\delta^{13}C$ signature and the highest $\delta^{15}N$ signature of the three potential prey species (Table 7-4).

Effects of Gender in Pilot Whales
We confirmed the sex of 41 female and 41 male short-finned pilot whales using genetic analysis (P. Rosel, pers. comm.). There was a significant difference in $\delta^{15}N$ values ($p = 0.011$) but not in $\delta^{13}C$ values ($p = 0.137$) between the sexes. Male short-finned pilot whales had significantly higher $\delta^{15}N$ values (12.2 ± 0.8) than female short-finned pilot whales (11.7 ± 0.8).

Effects of Age Class in Pilot Whales
We examined differences in isotopic values among adults, subadults and calves in 26 stranded pilot whales. We assigned age class using the criteria provided in Hohn et al. (2006). We excluded one sample

(RT22) because of inconsistencies in the source data concerning length. We found no significant differences in either $\delta^{13}C$ ($p = 0.382$) or $\delta^{15}N$ values ($p = 0.547$) among age classes (Table 5).

Effects of Past Fishery Interactions in Pilot Whales

We also investigated the effect of past fishery interactions on the isotopic signatures of the 27 stranded pilot whales. Ten of these whales (37%) had physical evidence of past fishery interactions, including broken teeth and healed line scars on their mandibles, bodies, or other appendages (Hohn et al. 2006; Figure 7-4). Seventeen animals had no evidence of previous fishery interactions. Pilot whales of all age classes showed evidence of past fishery interactions (Table 7-6). We found no significant differences in either $\delta^{13}C$ ($p = 0.978$) or $\delta^{15}N$ values ($p = 0.513$) between pilot whales with evidence of previous fishery interactions and those with no evidence of past interactions.

Comparisons with Bottlenose Dolphins

There were significant differences in both $\delta^{13}C$ ($p < 0.0001$) and $\delta^{15}N$ ($p = 0.0.17$) between the offshore bottlenose dolphins and short-finned pilot whales (Figure 7-5). Pilot whales had significantly enriched $\delta^{13}C$ values compared to bottlenose dolphins and significantly higher $\delta^{15}N$ values.

Discussion

The short-finned pilot whales and bigeye tuna we sampled exhibited similar $\delta^{15}N$ values, suggesting that depredation of bigeye tuna (the primary target species of the pelagic longline fishery) is not a widespread behavior in this population. This finding supports reports of the intermittent occurrence of depredation by participants in this fishery (Captain D. Hemilright, pers. comm.). Interestingly, we found no significant differences in $\delta^{15}N$ values between whales with evidence of past fishery interactions and those that did not. These results are similar to those found by Abend and Smith (1997) who compared stable isotope signatures between long-finned pilot whales caught in fishing gear versus stranded animals and found no difference in $\delta^{13}C$ or $\delta^{15}N$ values between the two groups. Cetacean skin has a tissue turnover rate of approximately two to three months (Hicks et al. 1985) and isotopic values reflect diet assimilated during that period. None of the stranded animals we sampled had fresh wounds from fishery interactions, so perhaps it is not surprising that these individuals did not have $\delta^{15}N$ values indicative of recent depredation. Nevertheless, this finding suggests that the occurrence of depredation is intermittent, even amongst individuals that engage in this behavior.

Nevertheless, more than one-third of the stranded animals had evidence of previous interactions with fishing gear, including individuals of all age classes. This suggests that interactions with fishing gear are relatively common and that many animals survive these entanglements and hooking. Of course it is impossible to determine what proportion of entangled and hooked individuals succumb to their injuries. In addition to the pelagic longline fishery, several other fisheries operate in the CHSRA, including a commercial greenstick fishery, a charter fishery and a recreational troll fishery, all targeting tuna. During our research cruises we observed many pilot whale groups in close proximity to these fishing vessels (Figure 7-6) and observed pilot whales trailing monofilament fishing line on several occasions (Figure 7-7).

Male short-finned pilot whales had significantly higher $\delta^{15}N$ values, but similar $\delta^{13}C$ values, when compared to female whales. de Stephanis et al. (2008) found no significant difference in $\delta^{13}C$ or $\delta^{15}N$ values between male and female long-finned pilot whales in the Strait of Gibraltar. Kiszka et al. (2010)

found no statistically significant differences between males and female short-finned pilot whale in the central South Pacific, but males had higher $\delta^{13}C$ and $\delta^{15}N$ signatures than females. Our results do not indicate that male and female pilot whales feed at different trophic levels, because the mean difference in $\delta^{15}N$ between males and females was less than 1‰ and the increase in $\delta^{15}N$ between trophic levels is typically 3-4‰. Our findings do, however, indicate that the diets of the two sexes differ. We plan to explore differences in the foraging behavior or male and female whales using data collected with digital acoustic tags (DTags) that record depth, sound and orientation (Johnson and Tyack 2003).

We found no significant differences in isotopic signatures amongst three age classes of stranded pilot whales. This result is somewhat surprising because other studies have found that nursing marine mammals have lower $\delta^{13}C$ and higher $\delta^{15}N$ values than adults (Hobson and Sease 1998; Knoff et al. 2008; Fernández et al. 2011). It is possible that the stranded animals identified as calves were no longer nursing. Lactation was not recorded for any of the stranded whales (Hohn et al. 2006), nor was milk reported in the stomachs of the calves. One calf had no hard parts in its stomach, one had seagrass and the other two had prey parts representative of the diet of mature pilot whales (Mintzer et al. 2008). We acknowledge that our designations of age classes is relatively crude and limited by small sample sizes; a more sophisticated analysis would require age estimates from these individuals, but such estimates are not yet available.

We observed significant differences in isotopic signatures between short-finned pilot whales and bottlenose dolphins in the CHSRA. The pilot whales had significantly enriched $\delta^{13}C$ compared to the bottlenose dolphins. Both species co-occur in the same environment, so this difference may be due to pilot whales foraging deeper in the water column than bottlenose dolphins. $\delta^{13}C$ values increase with depth and proximity to organic matter sources near the sea floor (France 1995; Hobson 1999; Kiszka et al. 2010). Short-finned pilot whales are known to forage at depths exceeding 1000m (Aguilar Soto et al. 2008) and pilot whales we equipped with DTags in this area have foraged at depths up to 1044m (A.J. Read, unpublished data). We do not have dive records for the bottlenose dolphins in our study area, but Corkeron and Martin (2004) tagged two offshore bottlenose dolphins off eastern Australia and found that the dolphins spent two-thirds of their time in water less than 5m deep and dove to a maximum of 155m. If bottlenose dolphins in the CHSRA exhibit similar behavior, differences in foraging depth may explain the higher $\delta^{13}C$ values found in pilot whales.

The pilot whales also had significantly higher $\delta^{15}N$ values than the bottlenose dolphins, but the mean difference in $\delta^{15}N$ was only 0.5‰. Offshore bottlenose dolphins exhibit a relatively catholic diet; prey items include pelagic fish, especially in the family Myctophidae, and cephalopods, including *Ornithoteuthis antillarum*, *Illex* spp., *Histioteuthis* spp. and *Octopus* spp. (Barros and Stolen 2001; Hoelzel et al. 1998). Minzter et al. (2008) examine the stomach contents of the short-finned pilot whales that mass stranded in North Carolina in 2005 and found that the stomachs contained prey remains from nine cephalopod families and one fish species; the largest prey item was a *Taonius pavo* squid with a mantle length of 393mm. Aguilar Soto et al. (2008) suggested that short-finned pilot whales may employ foraging sprints at depth to capture relatively large, high caloric squid prey. The ability of pilot whales to capture prey that are larger and have higher nutritional quality perhaps explains their higher $\delta^{15}N$ signatures relative to offshore bottlenose dolphins.

Unfortunately, we did not have access to any of the cephalopod species found commonly in the stomachs of stranded pilot whales in North Carolina, as none of these species are harvested commercially. Mintzer et al. (2008) found *Loligo* prey remains in the stomachs of stranded whales, but with a relatively low frequency of occurrence, and concluded that *Loligo* does not constitute a

substantial portion of the diet of short-finned pilot whales in this area. This is in contrast to the Pacific, where *Loligo* comprise a large portion of the prey items in the stomachs of stranded animals (Sinclair 1992).

Our stable isotope results indicate that neither bigeye nor yellowfin tuna are important components of the diet of short-finned pilot whales. The pilot whales and bigeye tuna were feeding at the same trophic level, as evidenced by their similar $\delta^{15}N$ signatures. Both of these predators had a mean $\delta^{15}N$ value 1.8‰ greater than the yellowfin tuna, likely due to differences in their prey. The bigeye tuna we sampled were substantially larger than yellowfin tuna; the average weight of the bigeye tuna was 33kg compared to 19kg for the yellowfin. This difference in size may result in resource partitioning with the larger bigeye tuna foraging at greater depths and consuming larger prey than the smaller yellowfin (Menard et al. 2007) which, in turn, would be reflected in their isotopic signatures. This hypothesis is supported by observations from other areas; Potier et al. (2004) found that epipelagic fish comprised the majority of fish prey of yellowfin tuna in the Indian Ocean, while mesopelagic fish dominated the diet of bigeye tuna.

Conclusion

The results of our research indicate that preserving short-finned pilot whale skin samples in DMSO for relatively short periods (60 days) did not have an effect on δ13C or δ15N values. These findings are in contrast with other studies that have found effects of DMSO preservation on δ13C and δ15N values in a variety of cetacean species including beluga whales (*Delphinapterus leucas*), harbor porpoise (*Phocoena phocoena*), minke whales (*Balaenoptera acutorostrata*), fin whales (*Balaenoptera physalus*) (Lesage et al. 2010), and humpback whales (*Megaptera novaeangliae*) (Todd et al. 1997). Our finding that lipid extracted samples were enriched in δ13C relative to non-extracted samples is consistent with other research (Post et al. 2007; Lesage et al. 2010). We conclude that the effects of preservation in DMSO are variable and perhaps complex, but recommend extracting lipid from samples used for stable isotope analysis of cetacean tissues.

In conclusion, we found no evidence that tuna are an important component of the diet of short-finned pilot whales in the CHSRA. Nor did we find any indication that individual pilot whales consistently engaged in depredation, even including those with evidence of past fishery interactions. This is despite our success in sampling animals from many different pods, from both sexes and several age classes. The indications of past fishery interactions on many of the stranded pilot whales supports the idea that depredation, perhaps on a variety of commercial and recreational fishing gears, may be a *widespread* behavior throughout this population. However, the lack of any stable isotope signature reflecting recent depredation in any of the whales we sampled leads us to conclude that individual whales engage in this behavior only *infrequently*.

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Figure 7-1. Collecting a skin sample from a short-finned pilot whale in the Cape Hatteras Special Research Area using remote biopsy sampling.

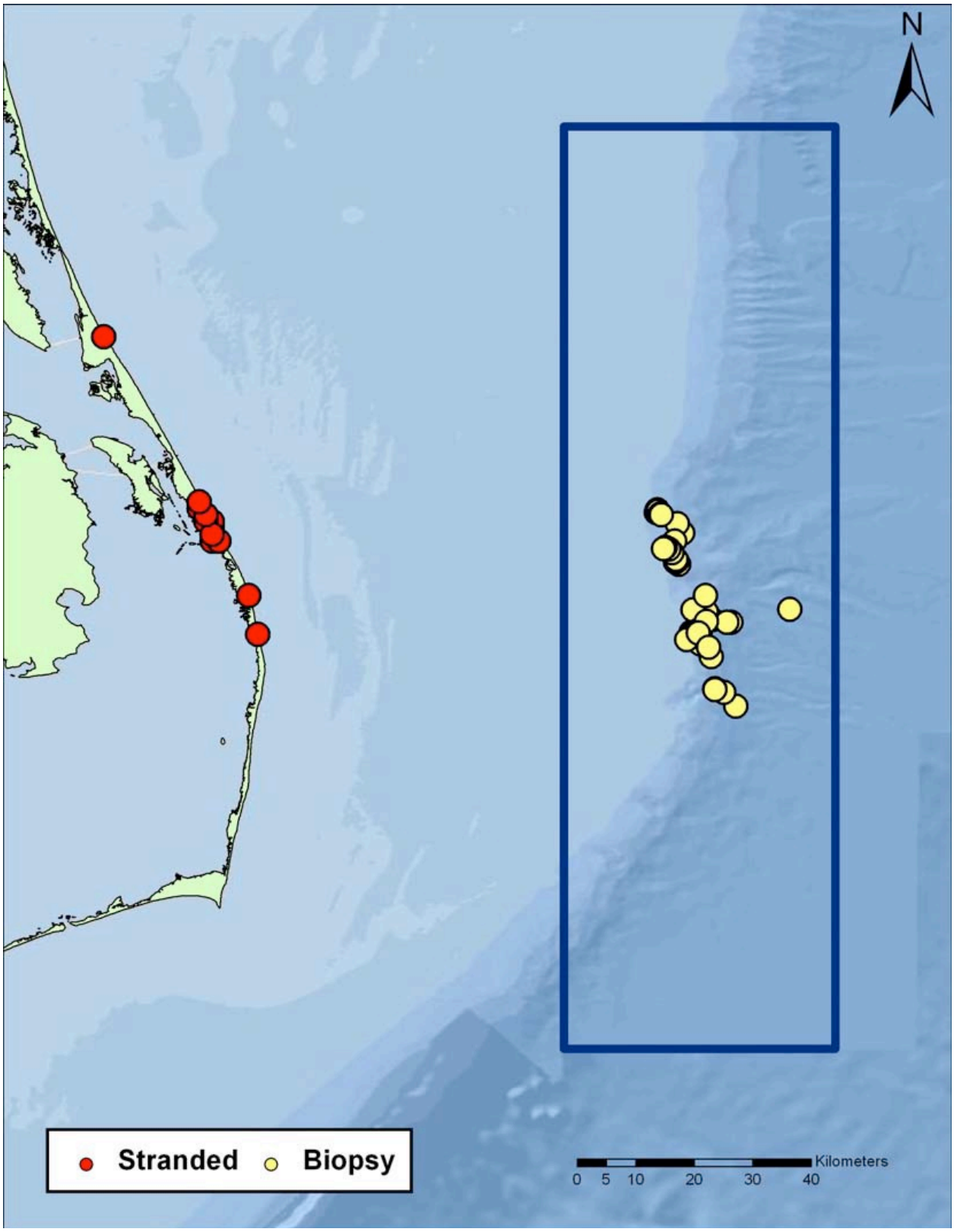

Figure 7-2. Locations of skin samples of short-finned pilot whales. The borders of the Cape Hatteras Special Research Area are outlined in the blue rectangle.

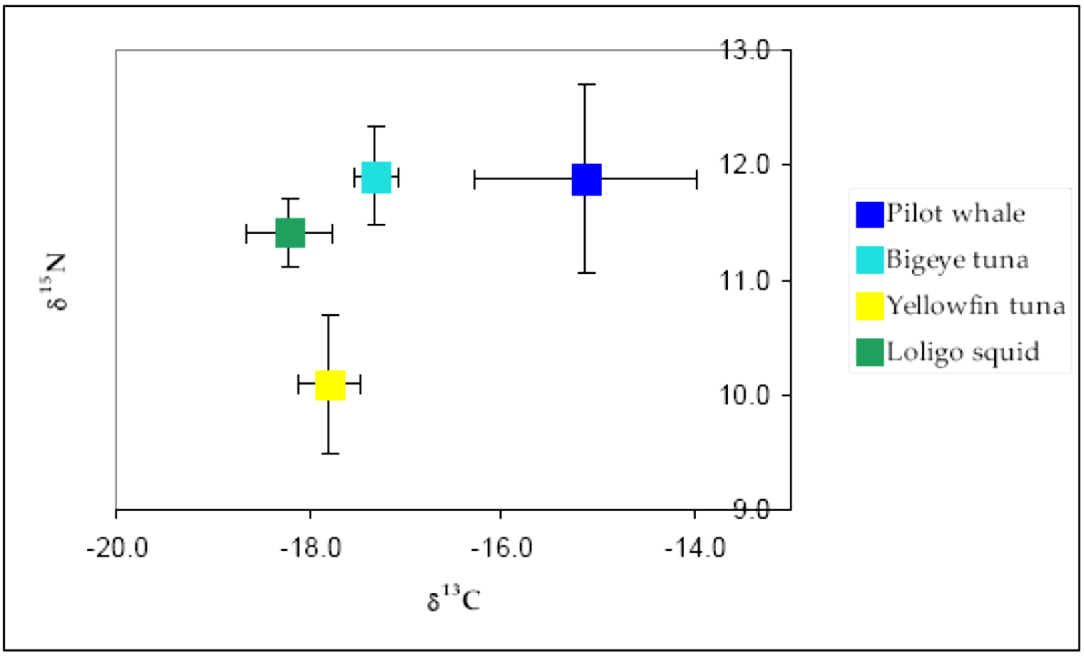

Figure 7-3. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) mean (± SD) values for short-finned pilot whales (n = 83), bigeye tuna (n = 30), yellowfin tuna (n = 30) and Loligo squid (n = 20). Isotope values are expressed in ‰.

Figure 7-4. Healed scars of past fishery interaction on a short-finned pilot whale stranded on the Outer Banks, NC in January 2005.

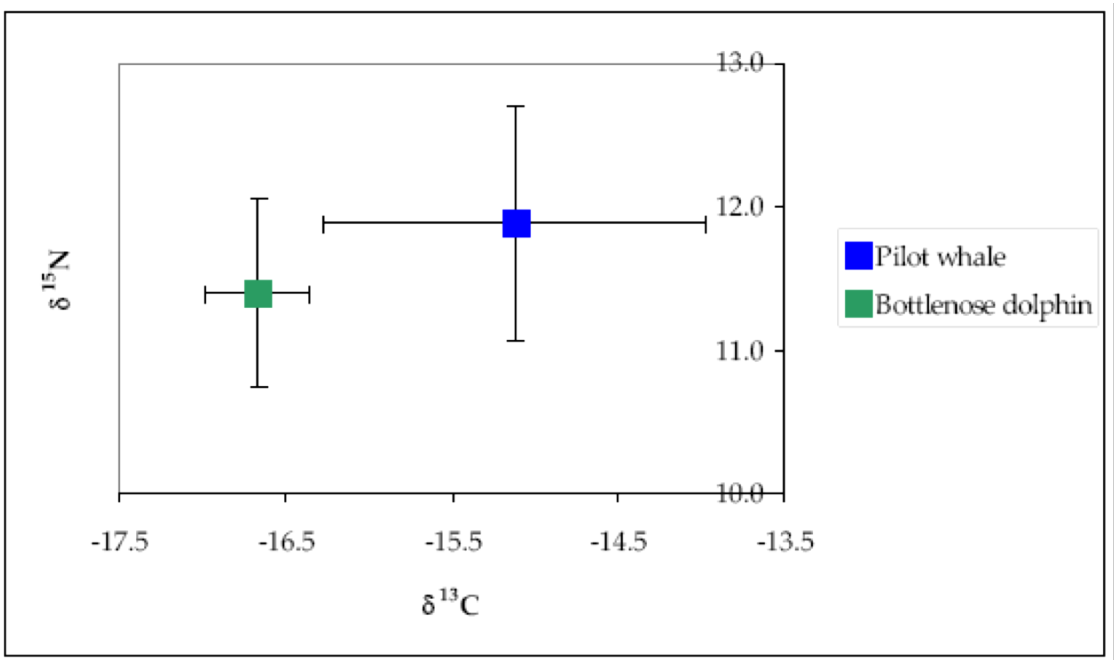

Figure 7-5. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) mean (± SD) values for short-finned pilot whales (n = 83) and offshore bottlenose dolphins (n = 14). Isotope values are expressed in ‰.

Figure 7-6. Pilot whales in close association with a commercial charter vessel in the Cape Hatteras Special Research Area.

Figure 7-7. Short-finned pilot whale in the Cape Hatteras Special Research Area with monofilament line trailing from its dorsal fin.

Table 7-1. Cetacean stable isotope studies including species studied, sample preservation method and whether lipid extraction was performed on samples.

Authors	Year	Species	Preservation Method	Lipid Extraction
Abend and Smith	1995	Long-finned pilot whale	Not reported	No
Abend and Smith	1997	Long-finned pilot whale	Frozen	No
Todd et al.	1997	Humpback whale	DMSO or frozen	Yes
Das et al.	2000	Striped dolphin, common dolphin	Frozen	Yes
Ruiz-Cooley et al.	2004	Sperm whale	DMSO	Yes
Lusseau and Wing	2006	Bottlenose dolphin	Frozen	No
Marcoux et al.	2007	Sperm whale	DMSO	Yes
de Stephanis et al.	2008	Long-fined pilot whale	Frozen	Yes
Witteveen et al.	2009	Humpback whale	DMSO or ethanol or frozen	Yes
Kiszka et al.	2010	Spinner dolphin, rough-toothed dolphin, short-finned pilot whale, melon-headed whale	Ethanol then frozen	Yes
Ohizumi and Miyazaki	2010	Dall's porpoise	Frozen	No
Fernández et al.	2011	Bottlenose dolphin	Frozen	Yes

Table 7-2. Sample source and preservation method for all pilot whale skin samples used in this research.

Date	Source	Number of samples	Preservation
January 2005	Stranding	24	Frozen
May 2005	Stranding	1	Frozen
September 2006	CHSRA research cruise	6	DMSO
May 2007	CHSRA research cruise	27	DMSO
August 2007	CHSRA research cruise	16	DMSO
May 2008	CHSRA research cruise	11	Frozen and DMSO
July 2008	Stranding	1	Frozen
February 2010	Stranding	1	Frozen
July 2010	CHSRA research cruise	6	Frozen
September 2010	CHSRA research cruise	1	Frozen
Total		**94**	

Table 7-3. Mean (± SD) stable isotope values for the four treatments in the matched sample experiment examining effects of preservation method and lipid extraction on stable isotope values. Isotope values are expressed in ‰.

Treatment	$\delta^{13}C$	$\delta^{15}N$
DMSO not lipid extracted	-17.1 ± 0.7	12.4 ± 0.5
Frozen not lipid extracted	-16.6 ± 0.5	12.3 ± 0.5
DMSO lipid extracted	-16.6 ± 0.9	12.5 ± 0.4
Frozen lipid extracted	-15.9 ± 1.0	12.1 ± 0.3

Table 7-4. Mean (± SD) stable isotope values for short-finned pilot whales and their potential prey. Isotope values are expressed in ‰.

Species	Number of animals	Mean $\delta^{13}C$ (± SD)	Mean $\delta^{15}N$ (± SD)
Short-finned pilot whale	83	-15.1 ± 1.1	11.9 ± 0.8
Bigeye tuna	30	-17.3 ± 0.2	11.9 ± 0.4
Yellowfin tuna	30	-17.8 ± 0.3	10.1 ± 0.6
Loligo squid	20	-18.2 ± 0.4	11.4 ± 0.3

Table 7-5. Mean $\delta^{13}C$ and $\delta^{15}N$ values for 26 stranded short-finned pilot whales classified by age class. Isotopic values are expressed in ‰.

Age class	Number of animals	Mean $\delta^{13}C$ (± SD)	Mean $\delta^{15}N$ (± SD)
Adult	16	-15.7 ± 1.0	11.3 ± 1.0
Subadult	6	-16.3 ± 0.5	11.7 ± 1.2
Calf	4	-15.4 ± 1.0	11.3 ± 0.6

Table 7-6. Age class and fishery interaction status for the 27 stranded short-finned pilot whales.

Age class	Number of animals	Number with fishery interactions	Percent with fishery interactions
Adult	17	7	41
Subadult	6	2	33
Calf	4	1	25
Total	**27**	**10**	**37**