PROTECTED SPECIES MONITORING IN NAVY OPAREAS OFF THE US ATLANTIC COAST

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Introduction

This report describes results from a multi-institutional monitoring project intended to provide information on the species composition, population identity, density and baseline behavior of marine mammals and sea turtles present in Navy range complexes along the Atlantic coast. This program began in 2007, with baseline aerial and vessel surveys and a passive acoustic monitoring program in Onslow Bay, North Carolina and has since expanded to include study areas off Jacksonville, Florida and Cape Hatteras, North Carolina. In Onslow Bay, six years of monitoring have yielded a comprehensive picture of the density, distribution and abundance of marine mammals and sea turtles and provided new insights into residency patterns among pelagic delphinid cetaceans in this region. More than four years of monitoring in Jacksonville have provided similar information on the density and distribution of marine mammals and sea turtles in this area. In Cape Hatteras, almost three years of surveys have provided preliminary information on the complex patterns of distribution and diversity of the marine mammals and sea turtles in this highly productive area. The current report builds on this past body of work and describes monitoring activities, including vessel surveys, photo-identification and biopsy sampling, that occurred in these three areas between January and December 2013.

1. Cape Hatteras

Methods

Study Area

The study area within the Virginia Capes (VACAPES) OPAREA is located east of Cape Hatteras, NC. The study area incorporates a large portion of the Cape Hatteras Special Research Area (CHSRA), designated by NOAA Fisheries as a management tool to facilitate research and mitigation of interactions between odontocete cetaceans and a pelagic longline fishery. The study area is approximately 16000 km^2 in extent and straddles the continental shelf break, including both shelf and pelagic waters (Figure 1.1). The survey area excludes nearshore coastal waters where the spatial distribution and abundance of coastal bottlenose dolphins (*Tursiops truncatus*) has been well established (Torres *et al*. 2003; Torres *et al.* 2005; Waring *et al.* 2013).

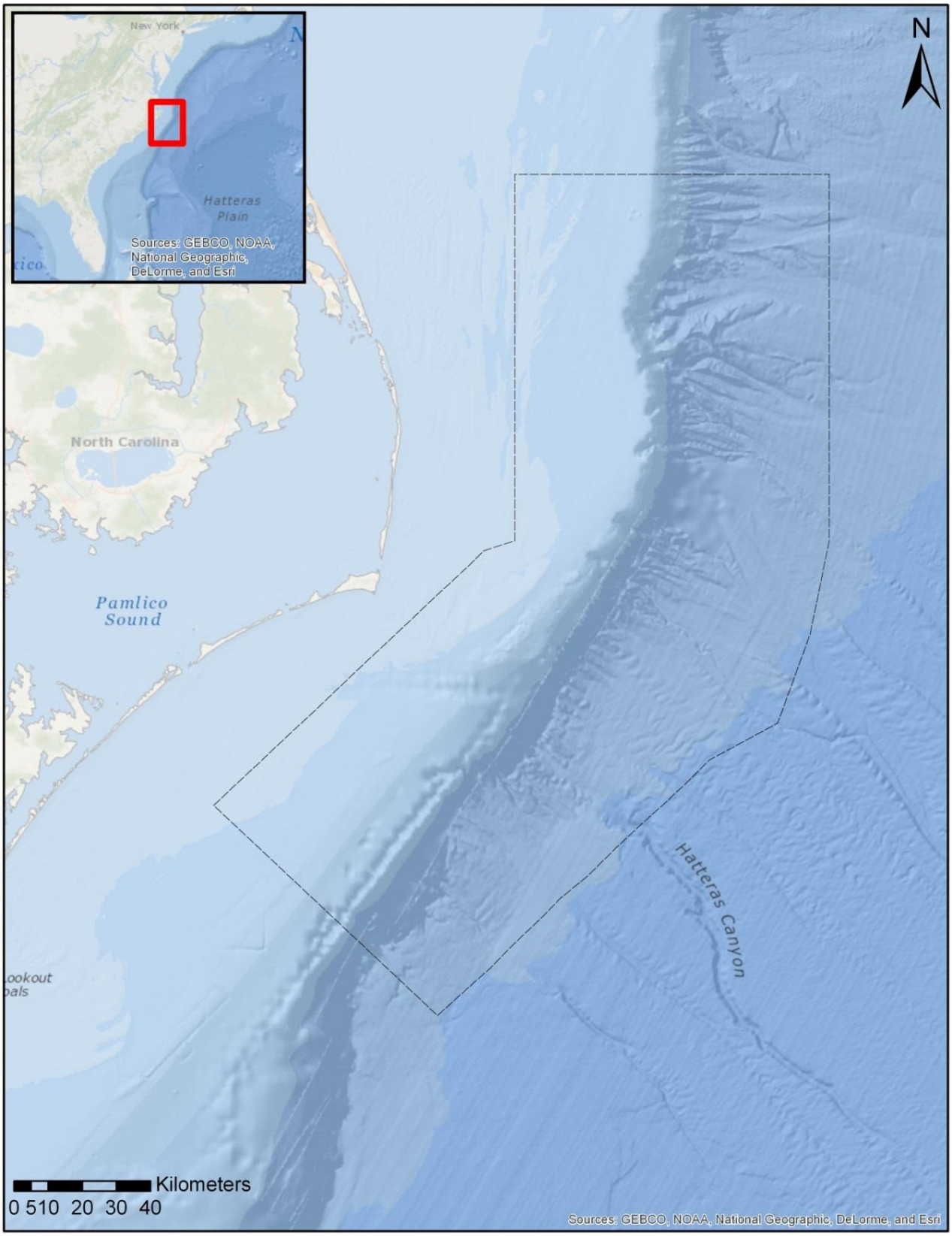

Figure 1.1. Map of the Cape Hatteras study area.

Visual Surveys

Vessel-based survey platforms provide a greater probability of sighting deep-diving species than aerial surveys (Barlow and Gisiner 2006). Shipboard observers are also more likely to be able to confirm species identity, particularly for animals that are difficult to distinguish from the air. Additionally, vessel-based platforms allow for biopsy sampling and photographic identification. Thus, we conducted vessel surveys to address questions of residency and population structure of cetacean species in the Cape Hatteras study area. Specifically, the objectives of these surveys were to: (1) document the distribution of marine mammals off Cape Hatteras; and (2) collect photo-identification images and biopsy samples from representative cetacean species in this area.

Figure 1.2. Vessel survey platform, the F/V *Samanna.*

We conducted surveys for cetaceans and other marine megafauna from the F/V *Samanna* during a single dedicated AFTT photo-ID and biopsy survey conducted in October 2013 (Figure 1.2). Additional surveys were conducted in the Cape Hatteras study area as part of the Deep Divers Project and are reported in the Deep Diver 2013 Report. Observations were made from the vessel's flying bridge by naked eye and 7x50 binoculars. Two observers (one port and one starboard) scanned constantly from straight ahead to 90° abeam either side of the track. The location, species and behavior of each cetacean group was recorded, along with the location and species for each turtle sighting. Environmental conditions (weather, sea state, depth and sea

surface temperature) were recorded at each sighting and whenever sighting conditions changed, using an iPad tablet and a linked GPS unit.

We used photo-identification and biopsy techniques to examine the use of the Cape Hatteras survey area by individual cetaceans. Thus, whenever possible, photographs of cetaceans for individual photo-identification were obtained; these photographs were also used to confirm species identification at each sighting and to compare identification features with those used by the aerial survey team. Photographs were obtained with Canon or Nikon digital SLR cameras (equipped with 100-400 mm zoom lenses) in 24-bit color at a resolution of 3072 X 2048 pixels and saved in .jpg format. Remote biopsy sampling methods were employed to collect small skin and blubber samples using a variety of 27 kg – 68 kg pull crossbows, depending on the species and sampling distance. Biopsy samples were obtained with a specialized 2.5 cm stainless biopsy tip attached to a modified bolt, typically fired from the bow of the survey vessel.

Data Analysis

Vessel survey effort and sighting data were mapped using *ArcGIS* 10.1. All vessel sighting data from January 2013 through December 2013 have been posted on the data repository OBIS-SEAMAP (http://seamap.env.duke.edu/).

Data Storage

All acoustic, visual survey and photographic data have been archived on digital media, and backed up on a Duke University network server.

Results

Survey Effort

On 04 October 2013, 63.4 km were surveyed during approximately 3.7 hours of dedicated marine mammal and sea turtle vessel surveys (Table 1.1, Figure 1.3).

Table 1.1. Dates, kilometers and hours surveyed during vessel surveys in the Cape Hatteras survey area, January 2013 – December 2013.

Date	Sea State	Km surveyed	Survey Time (hrs:min)	At Sea Time	Platform
4-Oct-13	2-3	63.4	3:44	10:25	F/V *Samanna*

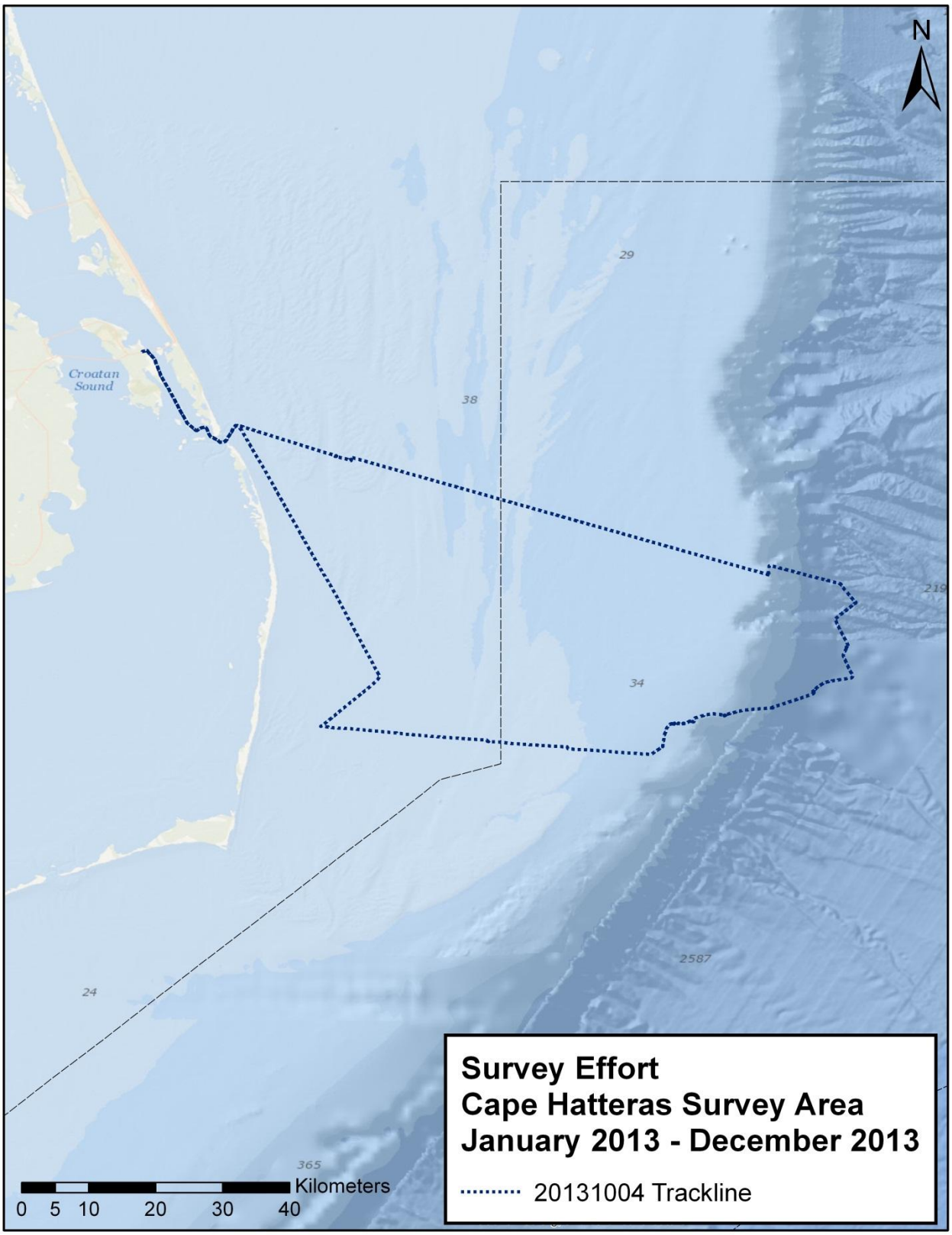

Figure 1.3. Survey effort in the Cape Hatteras study area, January 2013 – December 2013.

Marine Mammal and Sea Turtle Sightings

We recorded 9 cetacean sightings, including eight bottlenose dolphin groups and one fin whale (*Balaenoptera physalus*) (Tables 1.2 and 1.3, Figure 1. 4). There were no sea turtles observed during the AFTT surveys in the Cape Hatteras study area in 2013.

Table 1.2. Cetacean sightings observed during photo-id/biopsy vessel surveys in the Cape Hatteras survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Common Name	Group Size	Biopsy Samples	Images
4-Oct-13	11:06	35.38120	-74.94079	*T. truncatus*	Bottlenose dolphin	28	2	87
4-Oct-13	12:01	35.40535	-74.85950	*T. truncatus*	Bottlenose dolphin	10	0	16
4-Oct-13	12:15	35.41637	-74.79592	*T. truncatus*	Bottlenose dolphin	14	0	9
4-Oct-13	12:33	35.43773	-74.74117	*T. truncatus*	Bottlenose dolphin	14	1	16
4-Oct-13	13:12	35.49868	-74.69587	*T. truncatus*	Bottlenose dolphin	14	0	34
4-Oct-13	13:37	35.53869	-74.70773	*T. truncatus*	Bottlenose dolphin	40	0	4
4-Oct-13	14:09	35.58036	-74.70375	*T. truncatus*	Bottlenose dolphin	5	0	0
4-Oct-13	14:25	35.60011	-74.80185	*B. physalus*	Fin whale	1	1	37
4-Oct-13	14:25	35.59389	-74.80136	*T. truncatus*	Bottlenose dolphin	2	0	0

Table 1.3. Number of sightings and mean group size for each species observed during photo-id/biopsy vessel surveys in the Cape Hatteras survey area.

	Sightings				
Species	**2009**	**2011**	**2012**	**2013**	**Mean Group Size**
Balaenoptera physalus	0	0	1	1	2.0±1.4
Delphinus delphis	0	6	11	0	183.5±212.4
Globicephala macrorhynchus	9	33	52	0	39.7±83.4
Grampus griseus	1	2	2	0	10.8±13.3
Physeter macrocephalus	0	1	4	0	1.6±0.9
Stenella frontalis	0	8	2	0	66.8±88.1
Stenella/Delphinus mix	0	1	0	0	85.0±0.0
Tursiops truncatus	23	27	54	8	17.6±27.1
Tursiops/Stenella mix	0	1	0	0	100.0±0.0
Ziphius cavirostris	0	3	1	0	2.0±0.8
Unid. delphinid	1	0	3	0	4.7±3.1
Total:	**34**	**82**	**130**	**9**	

Distributions and Habitat Associations of Cetaceans and Sea Turtles

The distribution of marine mammal sightings is presented in Figures 1.4. Bottlenose dolphins and fin whales were encountered along the shelf break (Figures 1.5 and 1.6).

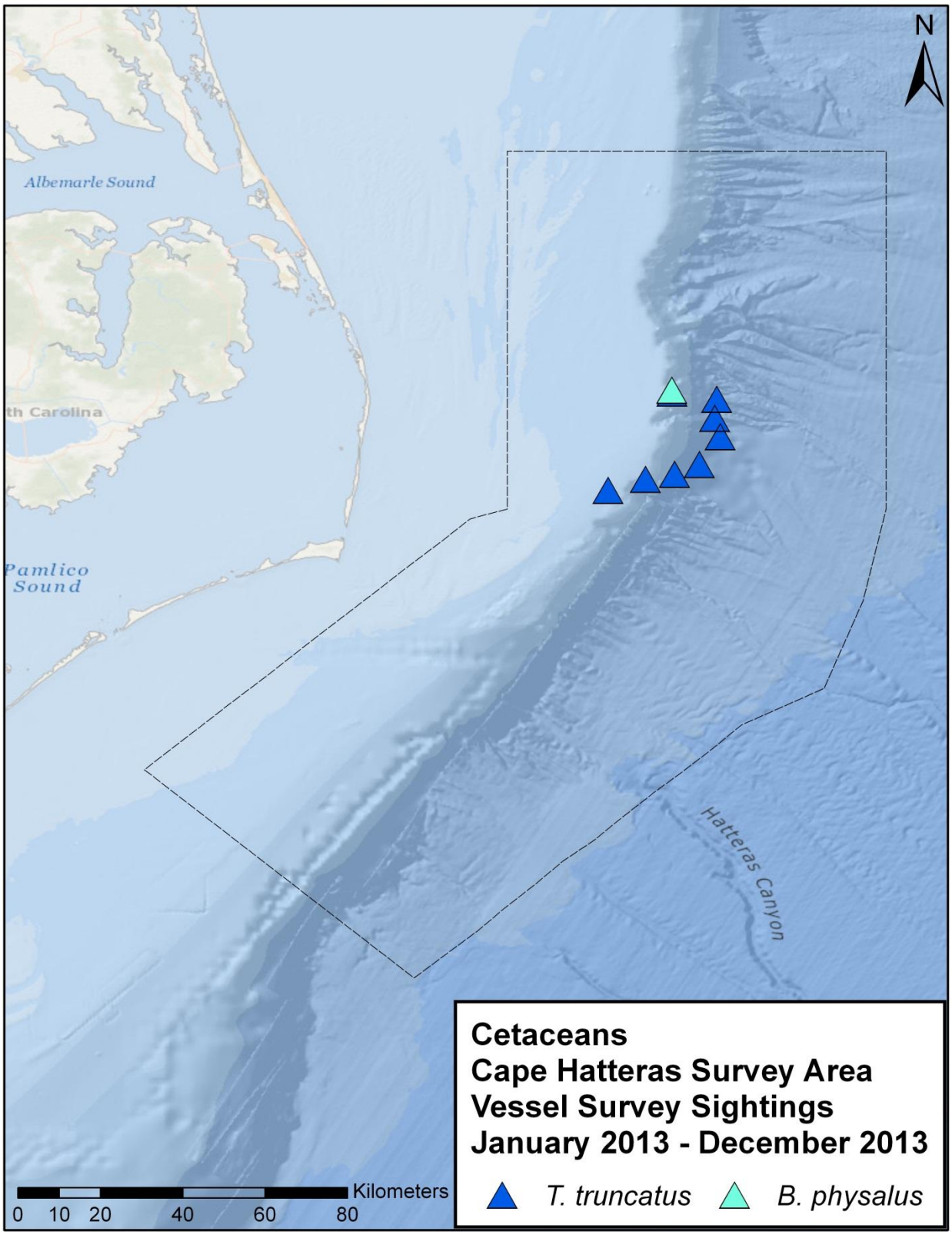

Figure 1.4. Distribution of all cetacean sightings made during vessel surveys in the Cape Hatteras survey area, January 2013 - December 2013.

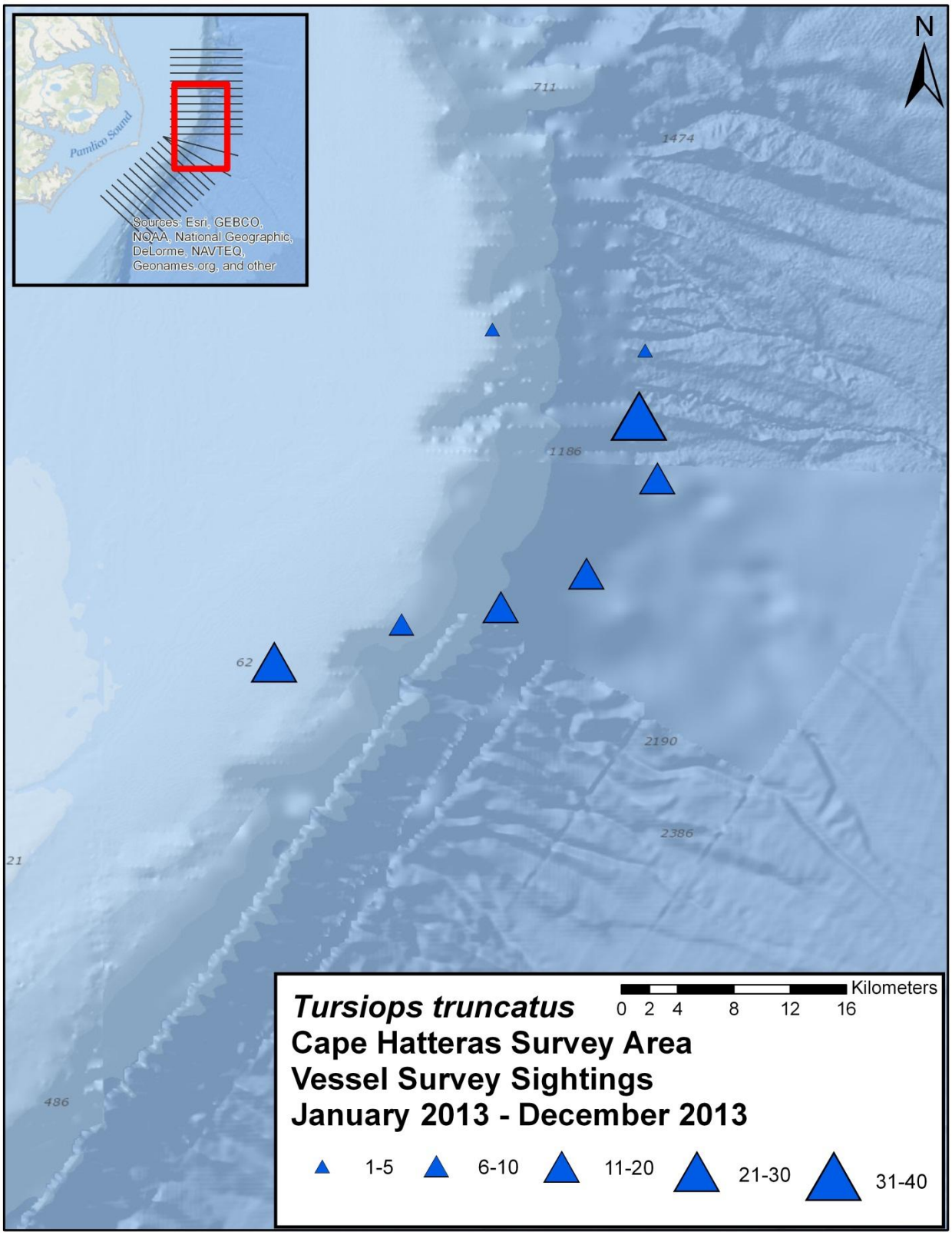

Figure 1.5. Distribution of bottlenose dolphin sightings indicating group size observed during vessel surveys in the Cape Hatteras survey area, January 2013 - December 2013.

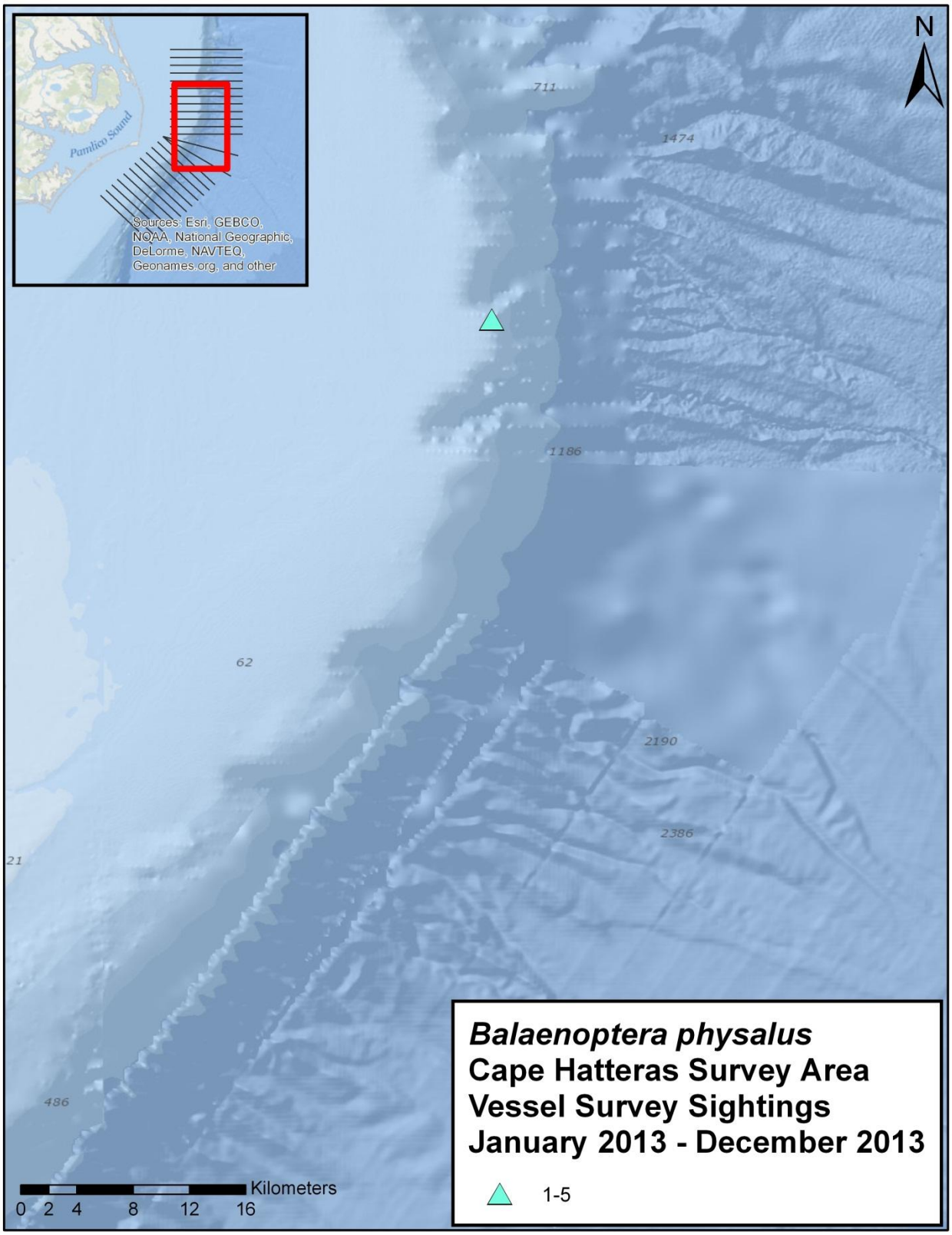

Figure 1.6. Distribution of fin whale sightings indicating group size observed during vessel surveys in the Cape Hatteras survey area, January 2013 - December 2013.

Biopsy Sampling

We collected biopsy samples from three bottlenose dolphins and one fin whale in the Cape Hatteras survey area in 2013 (Table 1.4, Figure 1.7). Genetic analysis of extracted DNA from bottlenose dolphin biopsy samples collected in the Cape Hatteras study area between May 2011 and July 2013 confirms that all of the sampled dolphins were of the offshore ecotype, suggesting that there is limited overlap between coastal and offshore populations in the study area. Skin samples collected after July 2013 will be analyzed for sex and population identity in the coming months. Voucher specimens of these samples will be archived with the Southeast Fisheries Science Center in Lafayette, LA.

Table 1.4. Biopsy samples collected in the Cape Hatteras survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Sample #
4-Oct-13	11:20	35.39319	-74.93014	*T. truncatus*	HJF_13_05
4-Oct-13	11:39	35.39400	-74.91683	*T. truncatus*	HJF_13_06
4-Oct-13	12:37	35.43879	-74.74184	*T. truncatus*	ZTS_13_30
4-Oct-13	14:32	35.59665	-74.80201	*B. physalus*	HJF_13_07

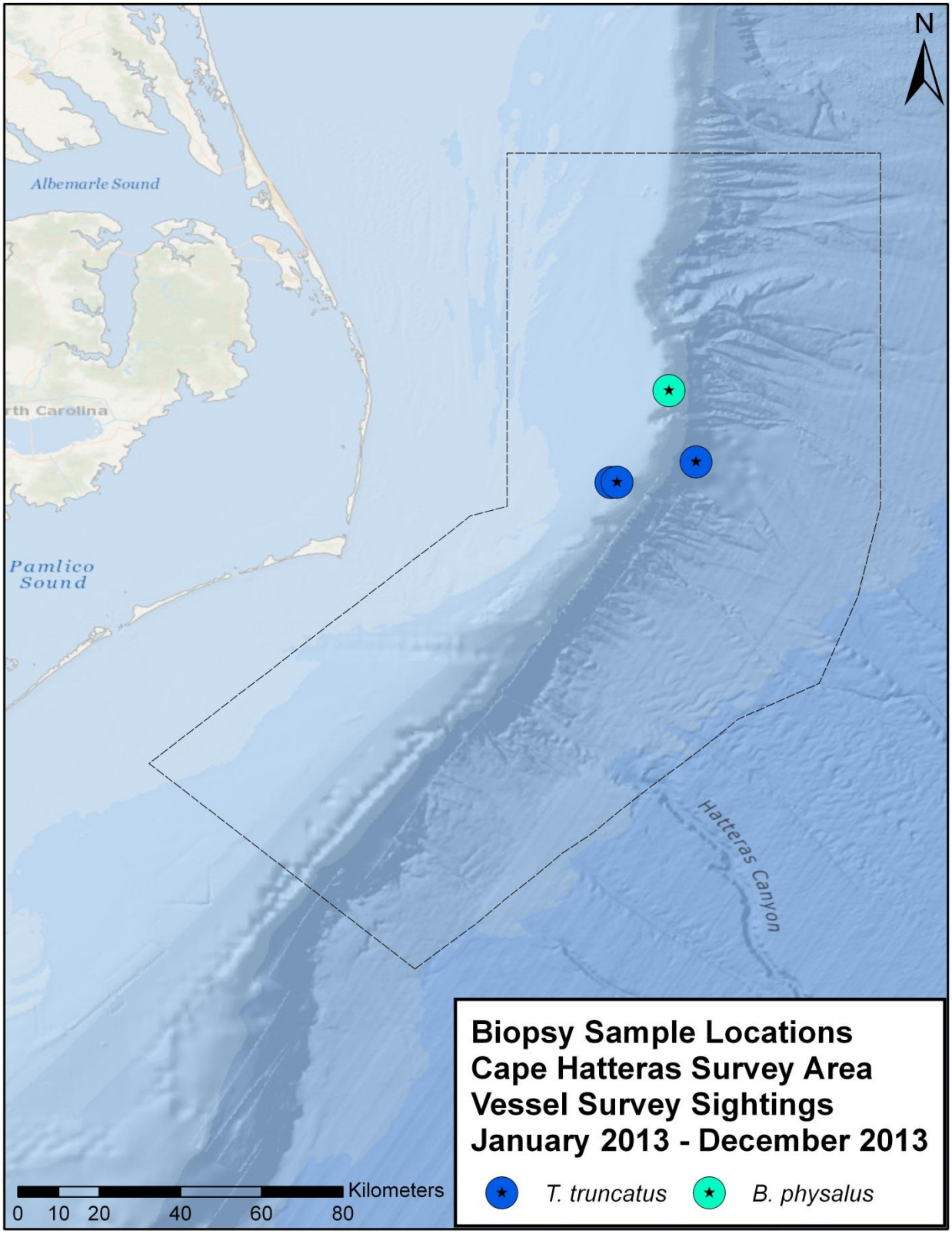

Figure 1.7. Locations of biopsy samples collected in the Cape Hatteras survey area, January 2013 – December 2013.

Photographic Effort – Cape Hatteras and Deep Divers

During the 4 October 2013 survey, we obtained 203 digital images for species identification and to identify biopsied individuals (Table 1.5). We made every attempt to photograph all animals encountered, both to validate species identification and to develop photo-identification catalogs for cetacean species in the Cape Hatteras survey area. The images taken of the bottlenose dolphins will be evaluated for quality and distinctiveness and compared to our existing catalogs. The images of the fin whale taken on this day were the first we have obtained of this species in our study area.

In addition, we obtained 1687 digital images for species confirmation and individual identification during the 2013 Deep Diver vessel surveys. Images of newly identified animals were added to existing catalogs and to newly developed catalogs for sperm whales (*Physeter macrocephalus*) and Cuvier's beaked whales (*Ziphius cavirostris*) (Table 1.6).

Photo-analysis of all images taken in the Cape Hatteras survey area is ongoing. To date, the photo-identification catalogs for *Tursiops truncatus*, *Globicephala macrorhynchus* and *Delphinus delphis* include 107, 229 and 20 individuals, respectively. Three of the 107 bottlenose dolphins photographed in the Cape Hatteras survey area have been seen multiple times (Table 1.6, Figure 1.8). Ttr 1-001 was first observed on 20 July 2009 and was re-sighted on 30 May 2011 and then again on 27 June 2011. Ttr 7-031 was photographed on two separate occasions in 2011, on 22 May and 22 June. Ttr 7-038 was also observed twice in 2011; it was initially seen on 29 May and was resighted on 27 June. In addition, we have made one match of a common

dolphin in the Cape Hatteras survey area; Dde 7-002 was first observed on 27 May 2007 and was re-sighted almost five years later on 15 March 2012 (Table 1. 6, Figure 1.8).

We have re-sighted a large proportion (22 of 229) of pilot whales included in our catalog (Table 1.5). These re-sightings span up to six years and several individuals have been observed on multiple occasions and in multiple seasons (Table 1.6, Figure 1.9).

We compared catalogs of short-finned pilot whales in the Cape Hatteras (n = 229) and Onslow Bay (n = 23) study areas, but found no matches between the two sites. In addition, we routinely compare images of stranded pelagic delphinids recovered by local stranding networks to our photo-identification catalogs, but have not yet made any matches.

Table 1.5. Summary of images collected during photo-ID/biopy and Deep Diver vessel surveys in the Cape Hatteras survey area, January 2013 - December 2013, with photo-identification catalog sizes and matches to date, and 2013 biopsy samples collected with the total number of biopsy samples collected to date in paranthesis.

Common Name	Scientific Name	Photos Taken	Catalog Size	Matches	Samples
		2013			2013 (Total)
Common dolphin	*D. delphis*	48	20	1	2 (7)
Short-finned pilot whale	*G. macrorhynchus*	649	229	22	10 (47)
Risso's dolphin	*G. griseus*	84	3	0	2 (2)
Sperm whale	*P. macrocephalus*	196	3	0	1 (1)
Atlantic spotted dolphin	*S. frontalis*	126	13	0	2 (8)
Bottlenose dolphin	*T. truncatus*	610	107	3	13 (37)
Cuvier's beaked whale	*Z. cavirostris*	140	1	0	2 (2)

Table 1.6. Photo-identification matches of species observed in the Cape Hatteras study area.

Cape Hatteras, NC								
ID	**Sex**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Ttr 1-001					JUL		MAY, JUN	
Ttr 7-031							MAY, JUN	
Ttr 7-038							MAY, JUN	
Dde 7-002			MAY					MAR
Gma_1-001								MAY, JUN
Gma_1-002							MAY	MAY
Gma_6-001	M						JUN	MAY
Gma_6-006	M		AUG					JUN
Gma_6-026	M			MAY				MAY
Gma_6-033	M							JUN*
Gma_7-002	M	SEP		MAY				JUN
Gma_7-003		SEP						JUN*
Gma_7-007	M	SEP*						
Gma_7-009							MAY	MAY
Gma_7-012								MAY, JUN
Gma_7-014								JUN*
Gma_7-017								JUN*
Gma_7-018								JUN*
Gma_7-026								JUN*
Gma_7-027								JUN*
Gma_7-055	F		MAY, AUG					
Gma_7-071	M			MAY				MAY
Gma_7-084	F							MAY, JUN
Gma_7-085	F							MAY, JUN
Gma_8-007								JUN*
Gma_8-016			MAY			JUL		

*Resighted within same month

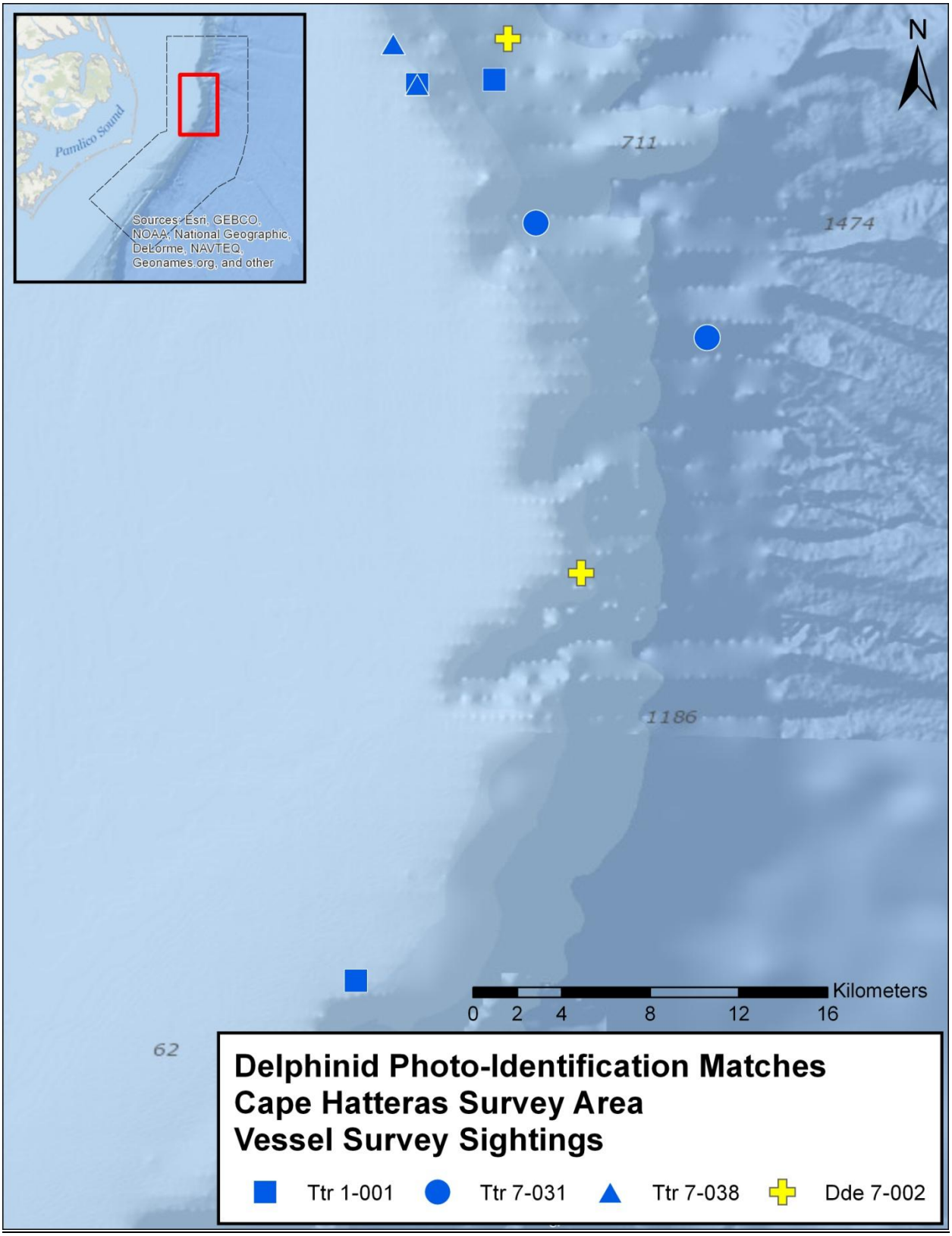

Figure 1.8. Photo-identification matches of other species observed in the Cape Hatteras study area.

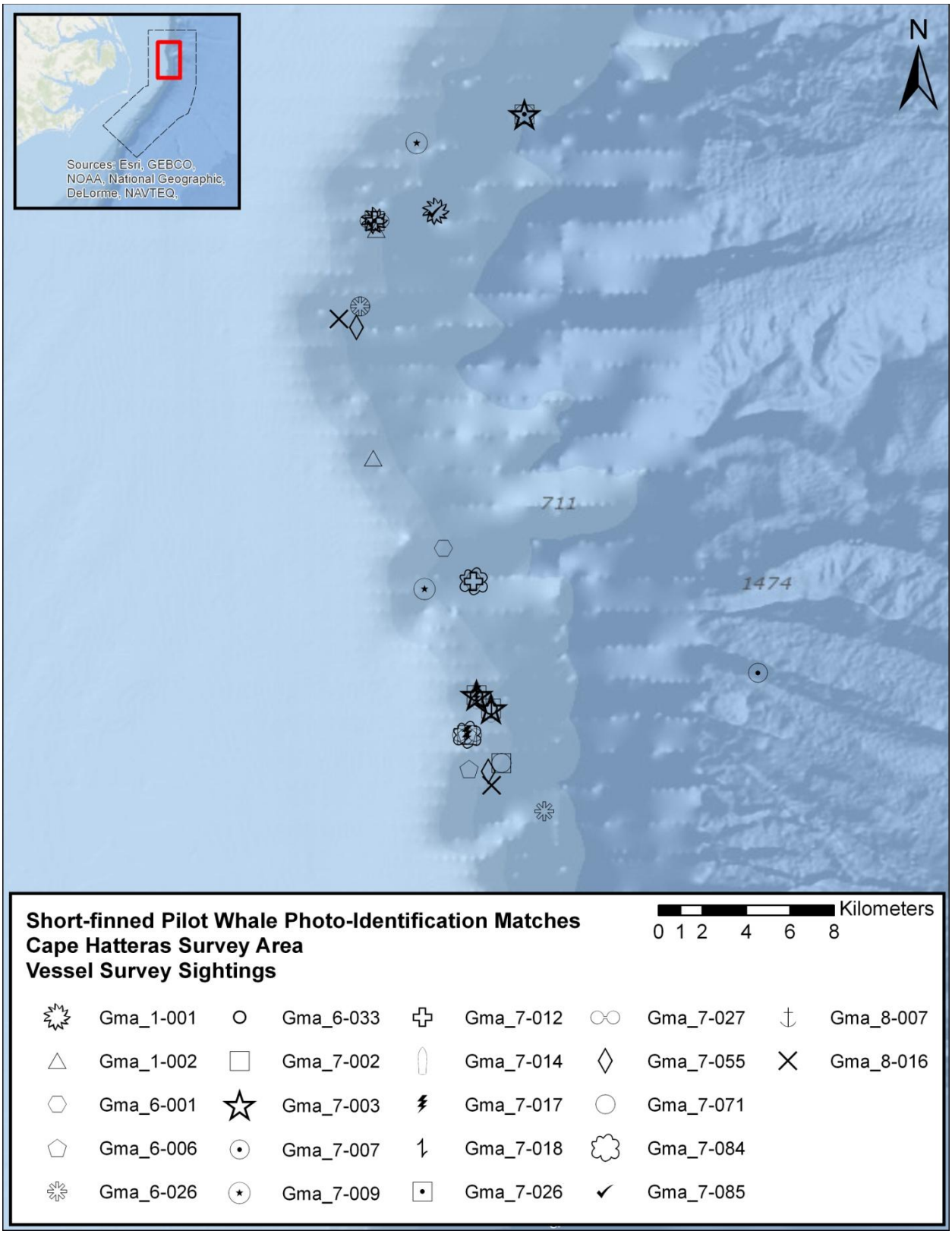

Figure 1.9. Photo-identification matches of short-finned pilot whales observed in the Cape Hatteras study area.

Summary Tables

Total survey effort conducted since the commencement of the monitoring program in the Cape Hatteras survey area in 2009 is reported in Table 1.7. The number of sightings and mean group size by species since 2009 is presented in Table 1.8. The number of biopsy samples collected and processed to date is reported in Table 1.9. Table 1.10 summarizes the catalog sizes and matches by species to date. These summaries include survey effort, photographic images and biopsy samples collected during both Deep Diver and photo-id and biopsy survey effort in 2013.

Table 1.7. Kilometers and hours surveyed to date in the Cape Hatteras, NC survey area.

	2009	2011	2012	2013	Total
Survey Hours	26.3	179.9	86.8	63.2	356.2
Km Surveyed	296.4	1097.4	1049.4	878.7	3321.9

Table 1.8. Number of sightings and mean group size for each species observed during vessel surveys in the Cape Hatteras survey area.

	Sightings				
Species	**2009**	**2011**	**2012**	**2013**	**Mean Group Size**
Balaenoptera physalus	0	0	1	2	2.3±1.2
Delphinus delphis	0	6	11	3	164.0±201.2
Globicephala macrorhynchus	9	33	52	32	36.5±75.9
Grampus griseus	1	2	2	3	8.9±10.6
Mesoplodon spp.	0	0	0	1	2.0±0.0
Physeter macrocephalus	0	1	4	3	1.8±0.9
Stenella frontalis	0	8	2	3	62.5±76.9
Stenella/Delphinus mix	0	1	0	0	85.0±0.0
Tursiops truncatus	23	27	54	38	17.6±24.6
Tursiops/Stenella mix	0	1	0	0	100.0±0.0
Ziphius cavirostris	0	3	1	2	2.5±1.1
Unid. beaked whale	0	0	0	4	2.3±1.9
Unid. delphinid	1	0	3	1	4.3±2.6
Total:	**34**	**82**	**130**	**92**	

Table 1.9. Biopsy samples collected and processed during all vessel surveys for 2013 in the Cape Hatteras survey area.

Common Name	Scientific Name	2011	2012	2013	# of Samples Processed
Fin whale	*B.physalus*	0	0	3	0
Common dolphin	*D. delphis*	0	5	2	0
Short-finned pilot whale	*G. macrorhynchus*	4	33	10	39
Risso's dolphin	*G. griseus*	0	0	2	0
Sperm whale	*P.macrocephalus*	0	0	1	0
Atlantic spotted dolphin	*S. frontalis*	6	0	2	1
Bottlenose dolphin	*T. truncatus*	14	10	13	31
Cuvier's beaked whale	*Z. cavirostris*	0	0	2	0

Table 1.10. Summary of images collected during all vessel surveys in the Cape Hatteras survey area, January 2013 - December 2013, with photo-identification catalog sizes and matches to date.

	2009-2012		2013		
Species	Catalog Size	Matches	Images	Catalog Size	Matches
Delphinus delphis	5	0	48	20	1
Globicephala macrorhynchus	136	14	649	229	22
Grampus griseus	1	0	84	3	0
Physeter macrocephalus	1	1	196	3	0
Stenella frontalis	0	n/a	126	13	0
Tursiops truncatus	78	1	610	107	3
Ziphius cavirostris	0	n/a	140	1	0

2. Onslow Bay

Methods

Study Area

The study area within the Cherry Point (CHPT) OPAREA consists of a rectangular box that is approximately 37% larger than the original proposed Undersea Warfare Training Range (USWTR); the USWTR area itself is 25 nm (46 km) long and 20 nm (37 km) wide, running approximately from NW to SE; Figure 2.1. We conducted vessel survey effort both inside and outside the original study area, with a focus on prevailing bathymetric and oceanographic features influencing the study area, such as the 200-m and 1000-m isobaths and the Gulf Stream front. These features are known to attract marine mammals and sea turtles, so we focused our survey effort on these features to maximize the number of encounters. As a result, we surveyed areas to the north and east of the originally proposed USWTR that we had not covered in previous years.

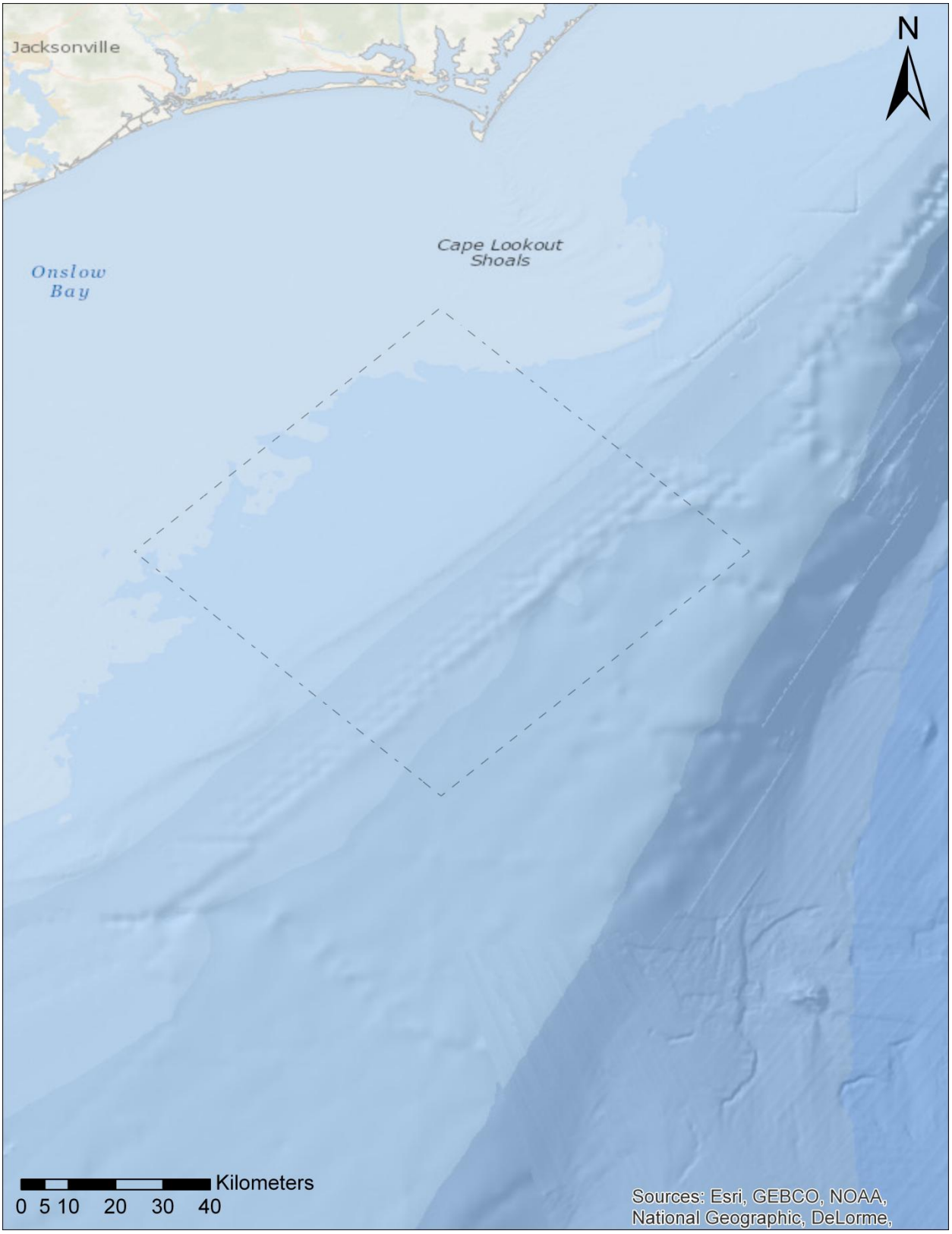

Figure 2.1. Map of the Onslow Bay study area.

Vessel Survey Data Collection

Visual Surveys

During this reporting period our vessel survey effort focused on addressing questions of residency and population structure of odontocetes, as our previous analysis of photo-identification data from Onslow Bay suggests a considerable level of residency in this area, despite a relatively low level of sampling.

We conducted visual surveys for marine mammals and sea turtles were conducted at a speed of approximately 10 knots. Two surveys were conducted from the F/V *Sensation* (Figure 2.2), a 16 m offshore fishing vessel which we have used extensively in the past. One survey was conducted from the F/V *Dancin' Outlaw*, which is very similar in size and configuration to the *Sensation.* We made observations from the flying bridge (5.0 m above waterline) by naked eye and 7x50 binoculars. Two observers (one port and one starboard) scanned constantly from straight ahead to 90° abeam either side of the trackline. We closed on all marine mammal sightings and recorded the location, species and behavior of each cetacean group. We surveyed turtles in passing mode, but recorded the location and species of all sea turtles. We recorded environmental conditions (weather, sea state, depth

Figure 2.2. Vessel survey platform, the F/V *Sensation.*

and sea surface temperature) at each sighting and whenever survey conditions changed. We recorded all sighting and environmental data on an iPad tablet connected to a GPS unit.

In addition, we examined the use of the Onslow Bay study area by individual cetaceans using photo-identification and biopsy techniques. We used photographs to confirm species identification and to compare identification features with those used by the aerial survey team. Photographs were taken with Canon or Nikon digital SLR cameras (equipped with 100-400 mm zoom lenses) in 24-bit color at a resolution of 3072 X 2048 pixels and saved in .jpg format. Remote biopsy sampling methods were employed to collect small skin and blubber samples using a variety of 27 kg – 68 kg pull crossbows, depending on the species and sampling distance. Biopsy samples were obtained with a specialized 2.5 cm stainless biopsy tip attached to a modified bolt, typically fired from the stern of the survey vessel.

Data Analysis

We mapped vessel survey effort and sightings using *ArcGIS* 10.1. All sighting data collected from January 2013 through December 2013 have been posted on the data repository OBIS-SEAMAP (http://seamap.env.duke.edu/).

Data Storage

All acoustic, visual survey, and photographic data have been archived on digital media and backed up on a Duke University network server.

Results

Vessel Survey Effort

Between 01 January 2013 and 31 December 2013, we conducted three surveys, covering 185.6 km and 14.6 hours of photo-identification and biopsy effort (Table 2.1, Figure 2.3). Surveys occurred in Beaufort Sea State (BSS) 0 to 4 and were focused along the 200m and 1000m shelf breaks (Figure 2.3).

Table 2.1. Dates, kilometers, and hours surveyed during vessel surveys in the Onslow Bay survey area, January 2013 – December 2013.

Date	Sea State	Km surveyed	Survey Time (hrs:min)	At Sea Time (hrs:min)	Platform
28-Jan-13	3-4	60.2	4:04	8:54	F/V *Sensation*
16-Jul-13	2-4	63.1	4:55	10:17	F/V *Dancin' Outlaw*
4-Oct-13	0-2	62.3	5:38	9:53	F/V *Sensation*

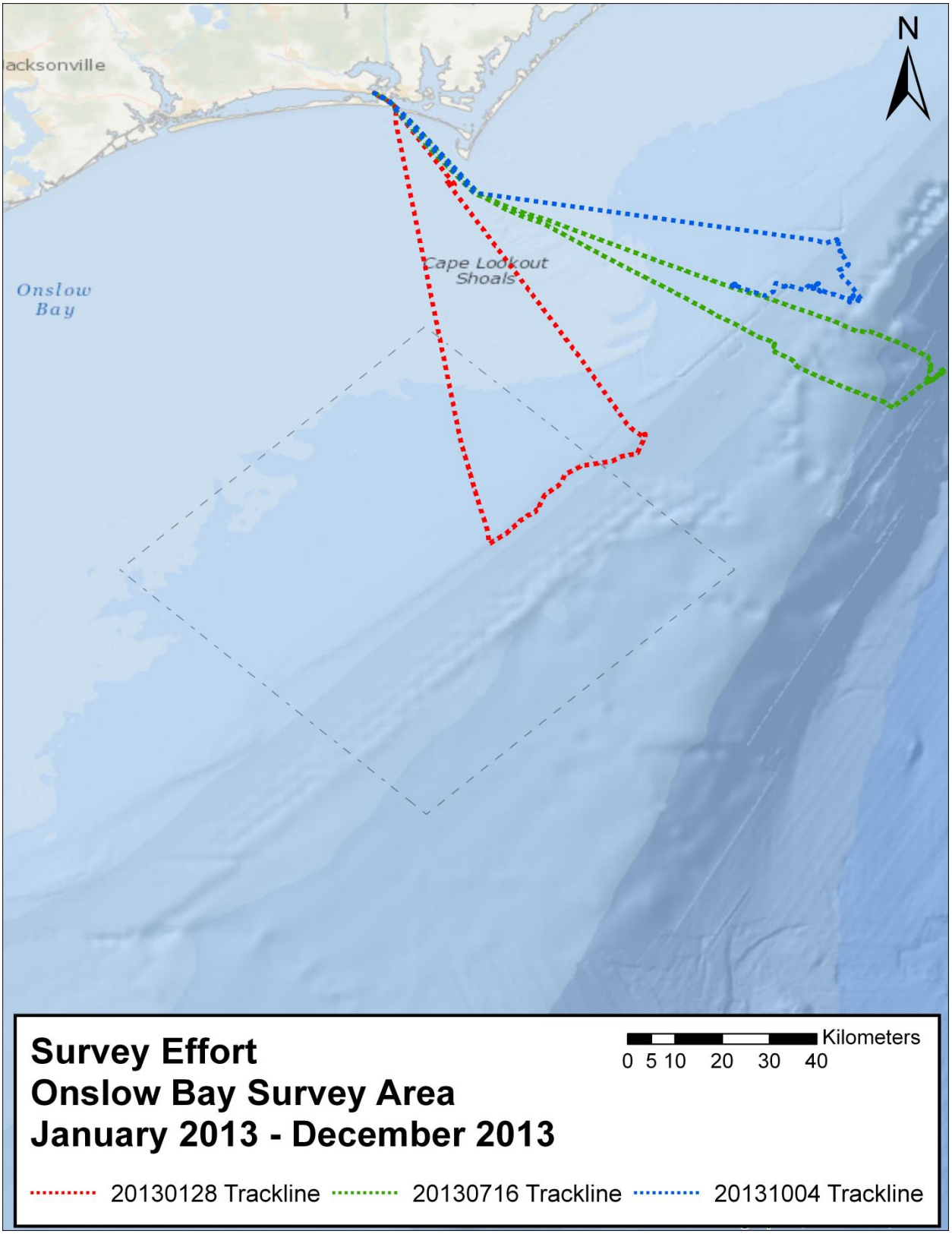

Figure 2.3. Survey effort in the Onslow Bay study area, January 2013 – December 2013.

Marine Mammal and Sea Turtle Sightings

We recorded seven cetacean sightings during vessel surveys in Onslow Bay during 2013, including: bottlenose dolphins (*Tursiops truncatus*; n = 6) and short finned pilot whales (*Globicephala macrorhynchus*; n = 1) (Table 2.2). The total number of sightings and mean group sizes from six years of survey effort are provided in Table 2.3. We also recorded a single loggerhead sea turtle (*Caretta caretta*) in Onslow Bay during 2013 (Table 2.4). Cumulative sightings of all sea turtles over all years are given in Table 2.5.

Table 2.2. Cetacean sightings observed during vessel surveys in the Onslow Bay survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Common Name	Group Size	Biopsy Samples	Images
16-Jul-13	9:03	34.26768	-75.79039	*T. truncatus*	Bottlenose dolphin	2	0	0
16-Jul-13	10:27	34.17333	-75.65895	*G. macrorhynchus*	Short-finned pilot whale	30	3	116
16-Jul-13	13:49	34.22807	-75.94833	*T. truncatus*	Bottlenose dolphin	10	1	35
4-Oct-13	9:36	34.34720	-75.89286	*T. truncatus*	Bottlenose dolphin	18	2	118
4-Oct-13	11:13	34.33552	-75.78858	*T. truncatus*	Bottlenose dolphin	20	2	119
4-Oct-13	12:35	34.39542	-75.80797	*T. truncatus*	Bottlenose dolphin	3	2	42
4-Oct-13	13:08	34.42345	-75.82513	*T. truncatus*	Bottlenose dolphin	5	0	17

Table 2.3. Number of cetacean sightings and mean group size for each species observed during Year 1 (June 2007 – June 2008), Year 2 (July 2008 – June 2009), Year 3 (July 2009 – June 2010), Year 4 (July 2010 – December 2011), Year 5 (January 2012 – December 2012) and Year 6 (January 2013 – December 2013) of vessel surveys in the Onslow Bay survey area.

	Sightings							
Species	Year 1	Year 2	Year 3	Year 4 Line Transect	Year 4 Photo-ID/Biopsy	Year 5 Photo-ID/Biopsy	Year 6 Photo-ID/Biopsy	Mean Group Size
Globicephala macrorhynchus	1	0	2	0	0	0	1	30.8±16.4
Grampus griseus	3	0	3	0	0	1	0	34.7±15.7
Mesoplodon spp.	0	0	0	0	0	2	0	2.5±2.1
Stenella frontalis	6	17	17	5	4	1	0	19.2±34.5
Steno bredanensis	0	0	1	0	0	0	0	27.0±0.0
Tursiops truncatus	23	14	29	6	1	7	6	11.1±13.0
Unid. delphinid	3	2	3	0	0	0	0	1.7±0.5
Unid. small whale	0	0	0	0	0	1	0	1.0±0.0
Total:	**36**	**33**	**55**	**11**	**5**	**12**	**7**	

Table 2.4. Sea turtle sightings made from vessel surveys in the Onslow Bay survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Common Name	Group Size
28-Jan-13	13:20	33.94439	-76.51210	*C. caretta*	Loggerhead sea turtle	1

Table 2.5. Number of sea turtle sightings and mean group size for each species observed during Year 1 (June 2007 – June 2008), Year 2 (July 2008 – June 2009), Year 3 (July 2009 – June 2010), Year 4 (July 2010 – December 2011), Year 5 (January 2012 – December 2012) and Year 6 (January 2013 – December 2013) of vessel surveys in the Onslow Bay survey area.

	Sightings							
Species	**Year 1**	**Year 2**	**Year 3**	**Year 4 Line Transect**	**Year 4 Photo-ID/Biopsy**	**Year 5 Photo-ID/Biopsy**	**Year 6 Photo-ID/Biopsy**	**Mean Group Size**
Caretta caretta	19	49	47	2	1	2	1	1.0±0.1
Dermochelys coriacea	0	0	2	0	0	0	0	1.0±0.0
Unid. sea turtle	1	0	1	0	0	0	0	1.0±0.0
Total:	**20**	**49**	**50**	**2**	**1**	**2**	**1**	

Distributions and Habitat Associations of Cetaceans and Sea Turtles

The distribution of marine mammals and sea turtles observed in Onslow Bay during 2013 is presented in Figures 2.4 through 2.7. The bottlenose dolphins and short-finned pilot whales were observed in deeper, offshore waters, well to the east of the original survey area (Figure 2.4). The sea turtle sighting was observed in relatively shallow waters over the continental shelf (Figure 2.7).

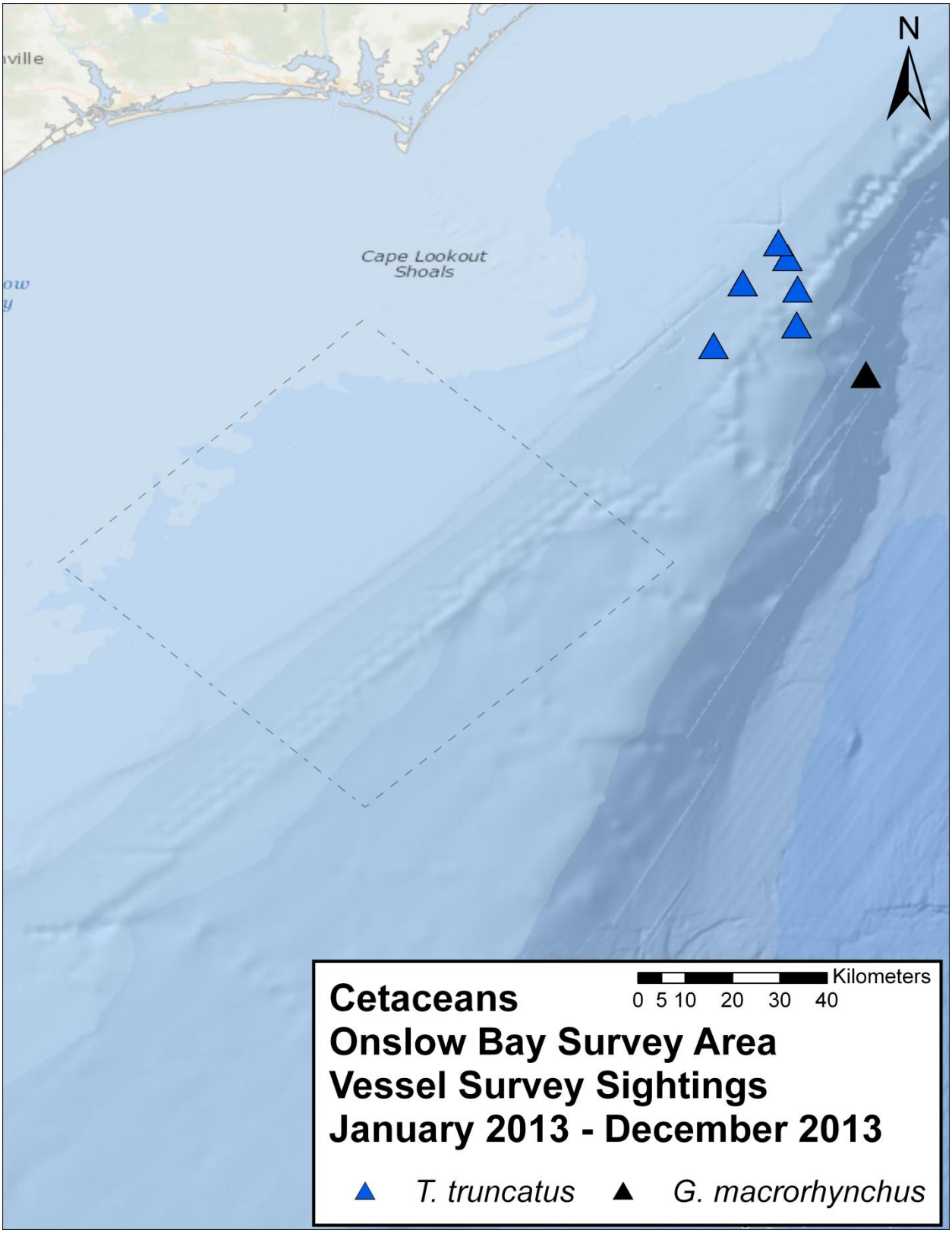

Figure 2.4. Distribution of all cetacean sightings made during vessel surveys in the Onslow Bay study area, January 2013 - December 2013.

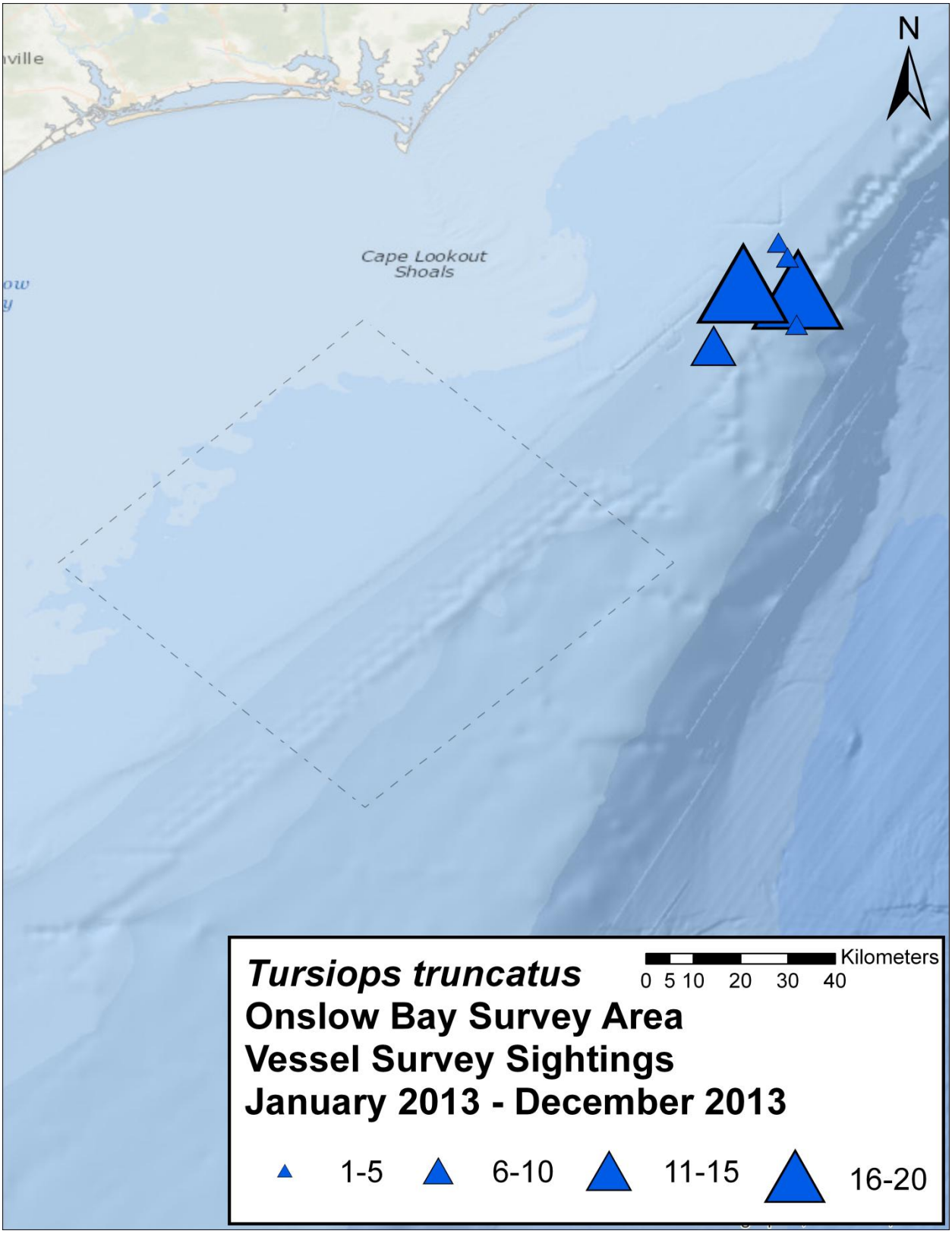

Figure 2.5. Distribution of bottlenose dolphin sightings indicating group size made during vessel surveys in the Onslow Bay study area, January 2013 – December 2013.

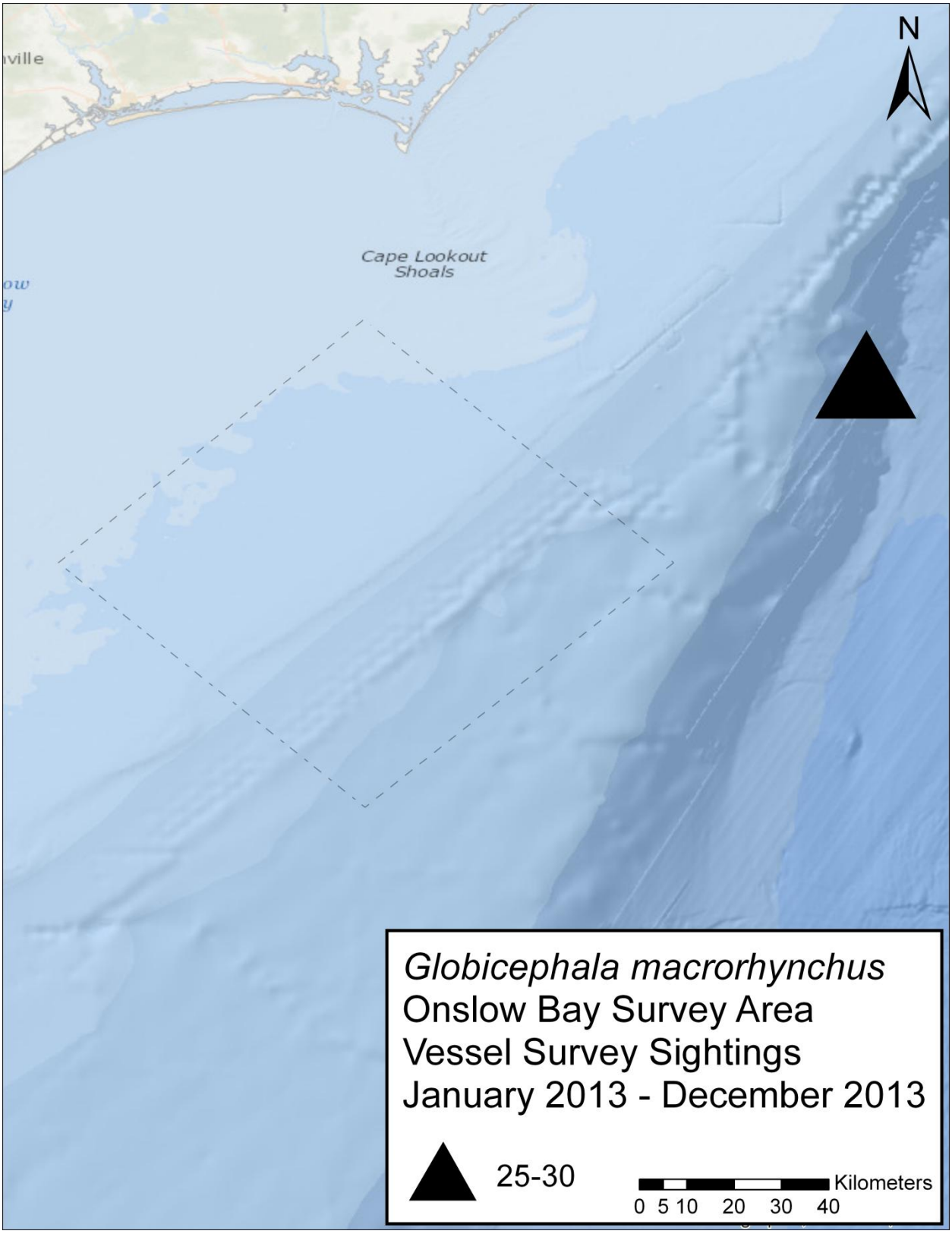

Figure 2.6. Distribution of short-finned pilot whale sightings indicating group size made during vessel surveys in the Onslow Bay study area, January 2013 – December 2013.

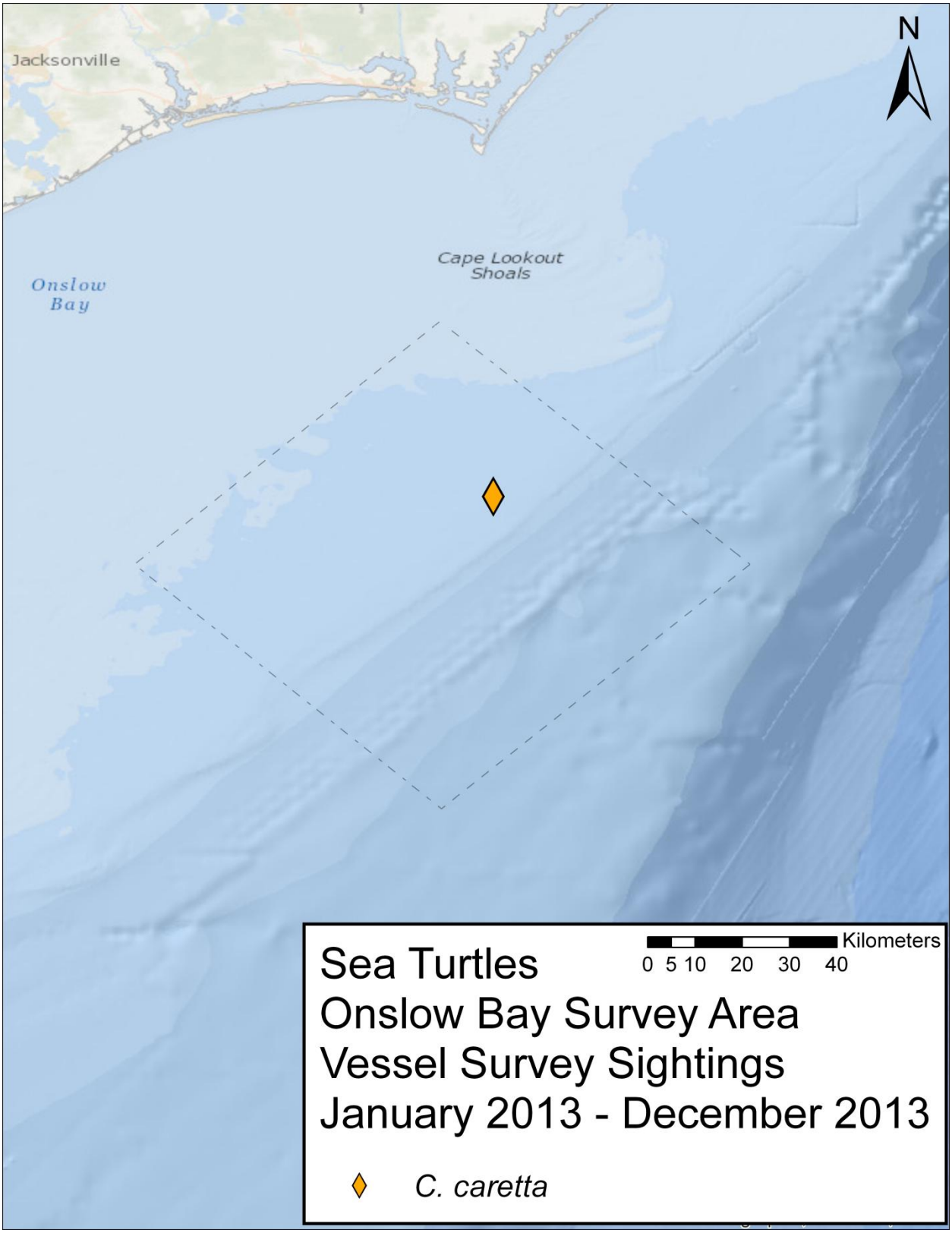

Figure 2.7. Distribution of sea turtle sightings made during vessel surveys in the Onslow Bay study area, January 2013 – December 2013.

Biopsy Sampling

We collected 10 biopsy samples in 2013 in Onslow Bay from bottlenose dolphins (n = 7), and short-finned pilot whales (n = 3) (Table 2.6, Figure 2.8). Genetic analysis of extracted DNA from bottlenose dolphin biopsy samples collected in Onslow Bay between May 2011 and July 2013 confirms that all of the sampled dolphins were of the offshore ecotype, suggesting that there is limited overlap between coastal and offshore populations in the study area. Skin samples collected after July 2013 will be analyzed for sex and population identity in the coming months. Voucher specimens of these samples are archived with the Southeast Fisheries Science Center in Lafayette, LA.

Table 2.6. Biopsy samples (and corresponding photo-identification codes) collected in the Onslow Bay survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Sample #	Photo-ID Code
16-Jul-13	10:41	34.17560	-75.64765	*G. macrorhynchus*	ZTS-13-020	Gma_8-003_OB
16-Jul-13	10:48	34.17890	-75.64468	*G. macrorhynchus*	ZTS-13-021	Gma_9-003_OB
16-Jul-13	11:03	34.18830	-75.63364	*G. macrorhynchus*	ZTS-13-022	Gma_7-007_OB
16-Jul-13	14:07	34.24412	-75.94815	*T. truncatus*	ZTS-13-023	Ttr_6-035_OB
4-Oct-13	9:48	34.34721	-75.89349	*T. truncatus*	AJR-13-004	No ID photo
4-Oct-13	9:58	34.35155	-75.88708	*T. truncatus*	AJR-13-005	Ttr_7-033_OB
4-Oct-13	11:22	34.32410	-75.80606	*T. truncatus*	AJR-13-006	No ID photo
4-Oct-13	11:38	34.33288	-75.80244	*T. truncatus*	AJR-13-007	Not distinctive
4-Oct-13	12:41	34.38503	-75.82321	*T. truncatus*	AJR-13-008	Not distinctive
4-Oct-13	12:48	34.38914	-75.81250	*T. truncatus*	AJR-13-009	No ID photo

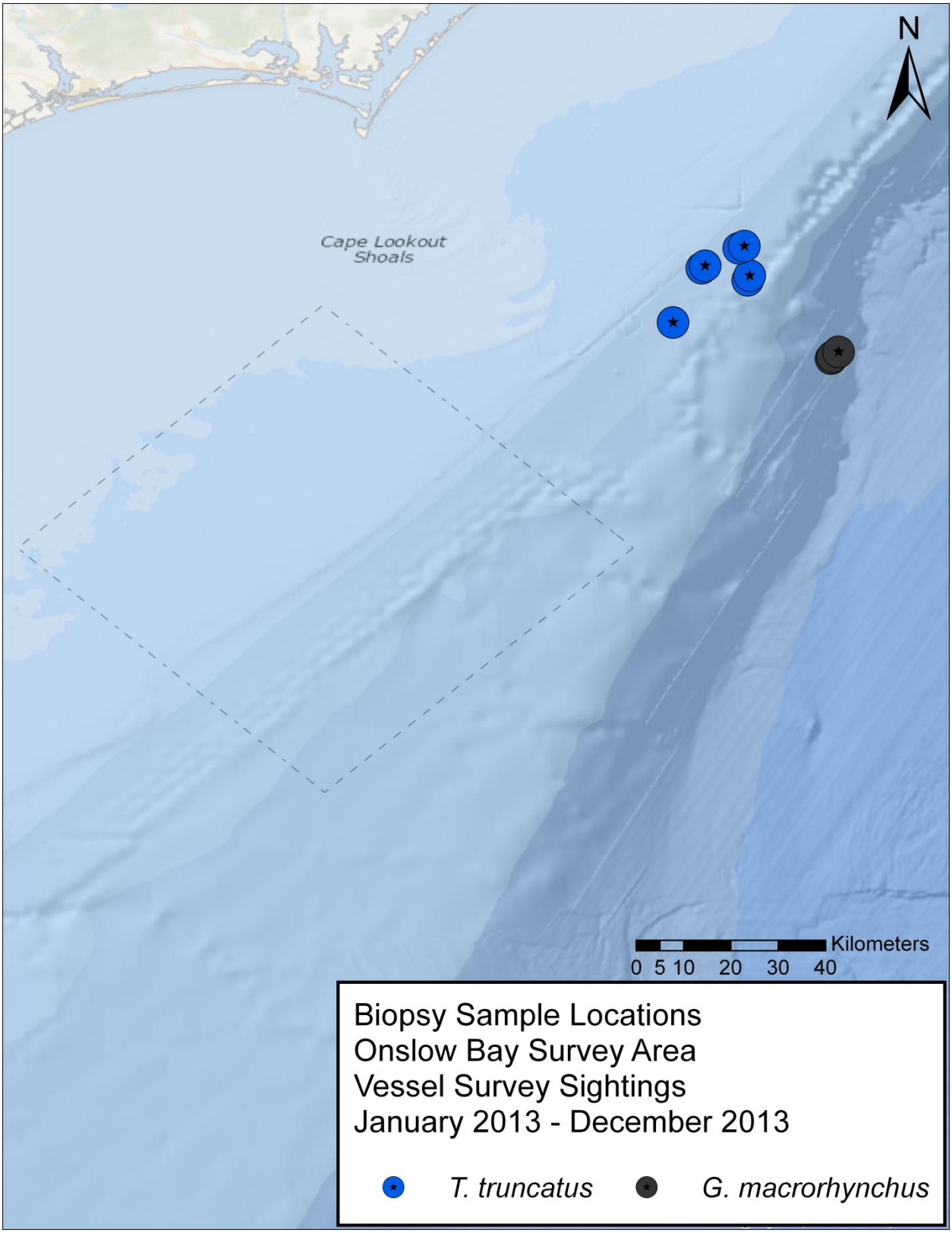

Figure 2.8. Locations of biopsy samples collected in the Onslow Bay study area, January 2013 – December 2013.

Photographic Effort

We obtained 437 digital images during 2013 for species confirmation and individual identification. We made every attempt to photograph all animals encountered, both to validate species identification and to augment photo-identification catalogs for cetacean species encountered in Onslow Bay. Individuals were identified to species in all seven encounters.

We added images of newly identified dolphins to existing photo-identification catalogs in Onslow Bay (Table 2.7). Photo-identification analysis is complete for all images taken through December 2013. Since the inception of the monitoring program in Onslow Bay in 2007, we have resighted eight bottlenose dolphins and four Atlantic spotted dolphins (Table 2.7 and Figure 2.9), representing approximately 6% of the catalogs for bottlenose dolphins (8 of 126) and 5% (4 of 78) for Atlantic spotted dolphins. Resightings of bottlenose dolphins and Atlantic spotted dolphins in Onslow Bay span up to six and ten years, respectively (Table 2.8). Two bottlenose dolphins (Ttr_7-015 and Ttr_8-009) were seen together in both April 2009 and 2010. One bottlenose dolphin (Ttr_1-004) has now been photographed on three separate occasions, in October 2009, April 2010 and January 2012 (Table 2.8). Furthermore, one Atlantic spotted dolphin (Sfr_8004) biopsied and photographed on 12 September 2011 was matched to an animal photographed on 28 June 2001 and on 24 June 2002 during surveys conducted in near-shore coastal waters of Onslow Bay (Figure 2.9). We also matched an additional Atlantic spotted dolphin from the same 12 September 2011 group to Sfr_9-023_MCB, photographed a month earlier on 19 August 2011 during surveys in the coastal waters off of Camp Lejeune, NC (Figure 2.9). Spotted dolphin Sfr 7-013 was first observed during an offshore AFTT Onslow Bay vessel survey on 12 September 2011 and was re-sighted this year on 25 July 2013 during an acoustic

vessel survey in coastal waters of Marine Corp Base Camp Lejeune. These numerous re-sightings over multiple years and across seasons supports the existence of considerable fine-scale population structure and some degree of residency for both bottlenose and spotted dolphins in Onslow Bay. To date, we have not resighted individuals of any other species, although the number of sightings and catalog sizes for these species are very small. In addition, we compared the photo-identification catalogs of bottlenose dolphins, Atlantic spotted dolphins and pilot whales from the Onslow Bay survey area with the Jacksonville and Cape Hatteras photo-identification catalogs for these species, but to date we have not made any matches. As we continue to augment our photo-identification catalogs and biopsy sample collections, matched genetic and photo-id data will be particularly useful for understanding population structure and site fidelity of odontocetes in Onslow Bay and other Navy OPAREAs. We regularly compare images of the dorsal fins of stranded pelagic cetaceans in North Carolina with our photo-identification catalogs for Onslow Bay, but to date there have been no matches.

Table 2.7. Summary of images collected during all vessel surveys in the Onslow Bay survey area, January 2013 through December 2013, with photo-identification catalog sizes and matches to date, and 2013 biopsy samples collected with the total number of biopsy samples collected to date in paranthesis.

Common Name	Scientific Name	Photos Taken	Catalog Size	Matches	Samples
		2013			2013 (Total)
Short-finned pilot whale	*G. macrorhynchus*	116	23	0	3 (3)
Risso's dolphin	*G. griseus*	n/a	22	0	0 (5)
Atlantic spotted dolphin	*S. frontalis*	n/a	78	4	0 (4)
Rough-toothed dolphin	*S. bredanensis*	n/a	12	0	0 (0)
Bottlenose dolphin	*T. truncatus*	321	126	8	7 (15)

Table 2.8. Photo-identification matches of species observed in the Onslow Bay study area.

Onslow Bay, NC										
ID	**2001**	**2002**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Ttr 1-004						OCT	APR		JAN	
Ttr 2-001				SEP						OCT
Ttr 4-002						SEP, OCT				
Ttr 6-010				SEP					JAN	
Ttr 6-018						APR	OCT			
Ttr 7-015^						APR	APR			
Ttr 8-009^						APR	APR			
Ttr 9-016					JUL	AUG				
Sfr 7-013								SEP		JUL
Sfr 8-004	JUN	JUN						SEP		
Sfr 9-013						AUG, OCT				
Sfr 9-023								AUG, SEP		

^Observed together in multiple sightings

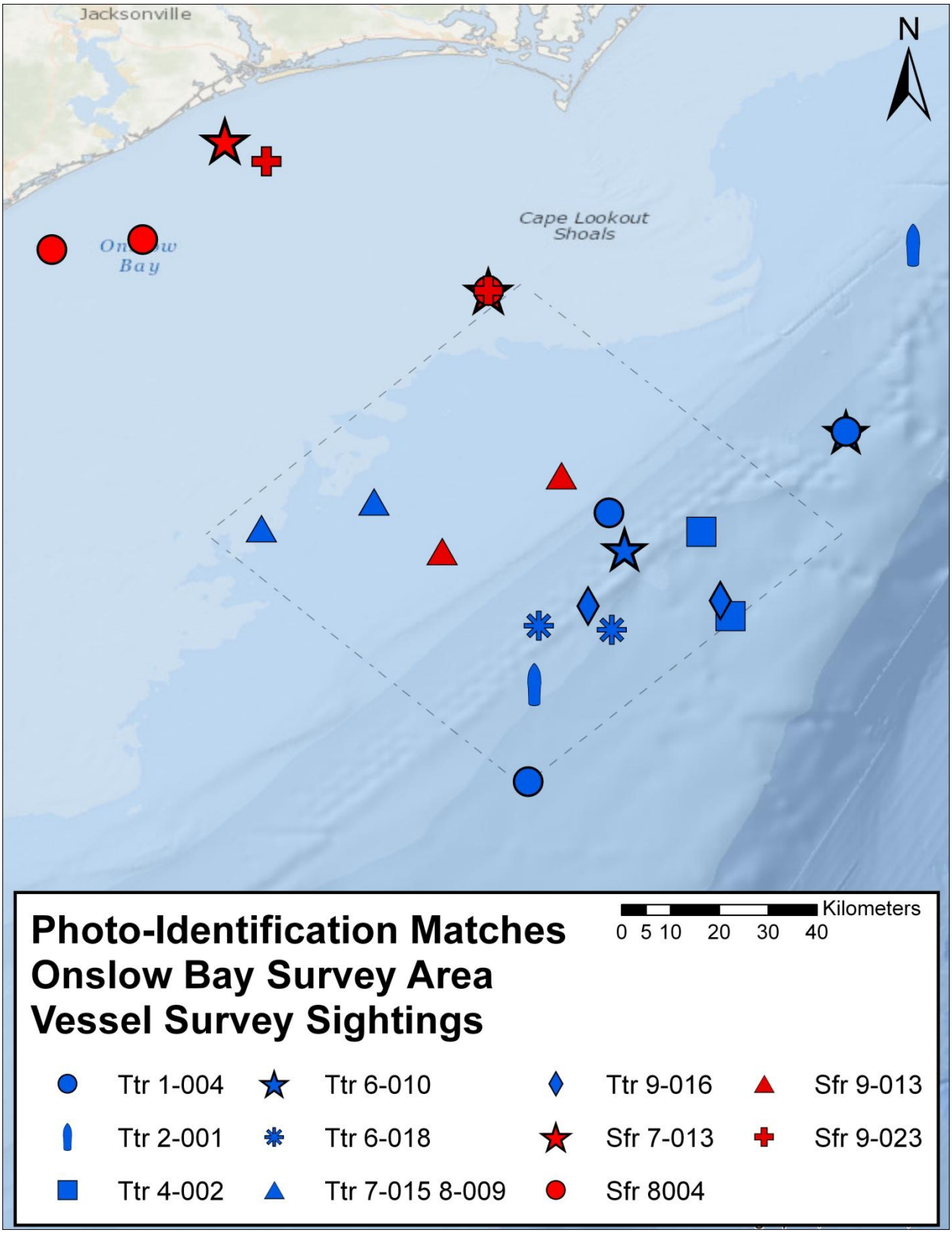

Figure 2.9. Locations of photo-identification matches in the Onslow Bay study area.

Summary Tables

Total survey effort conducted since the beginning of the monitoring program in the Onslow Bay survey area is reported in Table 2.9. Number of sightings and mean group size by species are presented in Table 2.10. The number of biopsy samples collected and processed to date is reported in Table 2.11. Table 2.12 summarizes the catalog sizes and matches by species to date and images taken during the reporting period in the Onslow Bay survey area.

Table 2.9. Kilometers and hours surveyed during Year 1 (June 2007 – June 2008), Year 2 (July 2008 – June 2009), Year 3 (July 2009 – June 2010), Year 4 (July 2010 – December 2011), Year 5 (January 2012 – December 2012) and Year 6 (January 2013 – December 2013) in the Onslow Bay survey area.

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Total
Survey Hours	170.7	109.0	105.7	53.6	31.5	14.6	485.1
Km Surveyed	2333.6	1742.4	1555.8	754.1	496.8	185.6	7068.3

Table 2.10. Number of cetacean sightings and mean group size for each species observed during Year 1 (June 2007 – June 2008), Year 2 (July 2008 – June 2009), Year 3 (July 2009 – June 2010), Year 4 (July 2010 – December 2011), Year 5 (January 2012 – December 2012) and Year 6 (January 2013 – December 2013) of vessel surveys in the Onslow Bay survey area.

	Sightings							
Species	**Year 1**	**Year 2**	**Year 3**	**Year 4 Line Transect**	**Year 4 Photo-ID/Biopsy**	**Year 5 Photo-ID/Biopsy**	**Year 6 Photo-ID/Biopsy**	**Mean Group Size**
Mesoplodon spp.	0	0	0	0	0	2	0	2.5±2.1
Globicephala macrorhynchus	1	0	2	0	0	0	1	30.8±16.4
Grampus griseus	3	0	3	0	0	1	0	34.7±15.7
Stenella frontalis	6	17	17	5	4	1	0	19.2±34.5
Tursiops truncatus	23	14	29	6	1	7	6	11.1±13.0
Steno bredanensis	0	0	1	0	0	0	0	27.0±0.0
Unid. delphinid	3	2	3	0	0	0	0	1.7±0.5
Unid. small whale	0	0	0	0	0	1	0	1.0±0.0
Total:	**36**	**33**	**55**	**11**	**5**	**12**	**7**	

Table 2.11. Biopsy samples collected and processed to date in the Onslow Bay survey area.

Common Name	Scientific Name	Year 4	Year 5	Year 6	# of Samples Processed
Short-finned pilot whale	*G.macrorhynchus*	0	0	3	0
Risso's dolphin	*G. griseus*	0	5	0	0
Atlantic spotted dolphin	*S. frontalis*	2	2	0	0
Bottlenose dolphin	*T. truncatus*	0	8	7	9

Table 2.12. Summary of images collected during all vessel surveys in the Onslow Bay survey area, January 2013 through December 2013, with photo-identification catalog sizes and matches to date.

	Year 1		Year 2		Year 3		Year 4		Year 5		Year 6		
Species	Catalog size	Matches	Catalog size	Matches	Catalog size	Matches	Catalog size	Matches	Catalog size	Matches	Images	Catalog size	Matches
Globicephala macrorhynchus	8	0	8	0	16	0	16	0	16	0	116	23	0
Grampus griseus	5	0	5	0	7	0	7	0	22	0	n/a	22	0
Stenella frontalis	3	0	29	0	49	1	68	2	78	3	n/a	78	4
Steno bredanensis	0	0	0	0	12	0	12	0	12	0	n/a	12	0
Tursiops truncatus	52	0	78	0	106	5	112	5	139	7	321	126	8

3. Jacksonville Vessel Surveys

Methods

Study Area

The study area within the Jacksonville OPAREA (JAX) is approximately 5728 km^2, surrounding the proposed USWTR site, which is approximately 1700 km^2 in area. The study area straddles the continental shelf break, including some of the Blake Plateau, and includes both shelf and pelagic waters (Figure 3.1).

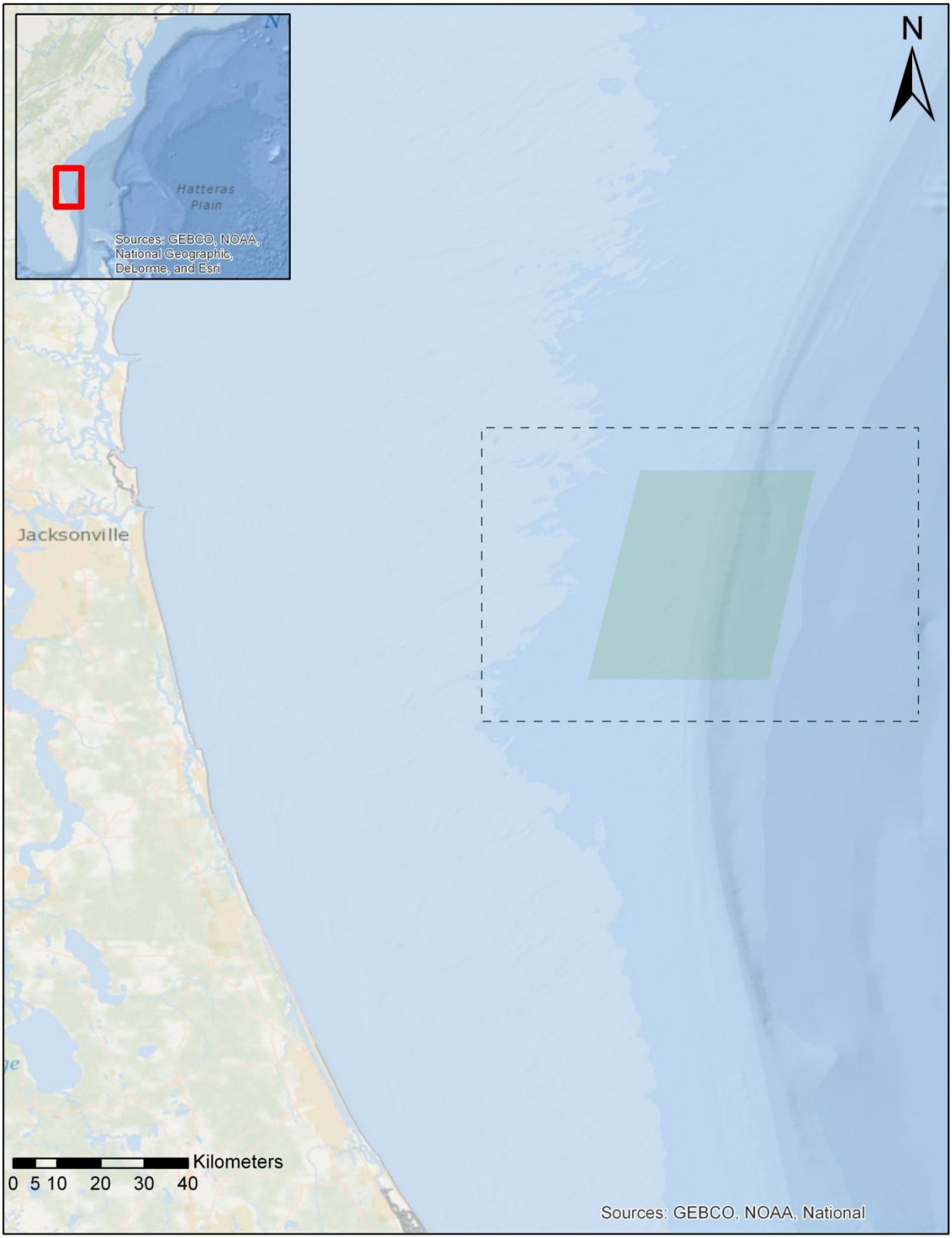

Figure 3.1. Map of the Jacksonville study area and the proposed USWTR site (shaded box).

Vessel Survey Data Collection

Visual Surveys

Vessel survey effort in the Jacksonville study area during 2013 focused on questions of residency and population structure of cetaceans. We conducted visual surveys at a speed of approximately 10 knots from several chartered sport fishing vessels; the F/V *Jodie Lynn*, F/V *Jodie Lynn II* and the F/V *Knot Tied Down* (Figure 3.2). We made observations from the vessel's flying bridge by naked eye and 7x50 binoculars. Two observers (one port and one starboard) scanned constantly from straight ahead to 90° abeam either side of the trackline. We closed on all cetacean sightings and recorded the location, species and behavior of each cetacean group. We surveyed turtles in passing mode, but recorded the location and species of all sea turtles. We recorded environmental conditions (weather, sea state, depth and sea surface temperature) at each sighting and whenever survey conditions changed. We recorded sighting and environmental data on an iPad tablet linked to a GPS unit. We examined use of the survey area by individual cetaceans using photo-identification and biopsy techniques. We also used photographs to confirm species identification at each sighting and to compare identification features with those used by the aerial survey team. We obtained photographs with Canon or Nikon digital SLR cameras (equipped with 100-400 mm zoom lenses) in 24-bit color at a resolution of 3072 X 2048 pixels and saved in .jpg format. Remote biopsy sampling methods were employed to collect small skin and blubber samples using a variety of 27 kg – 68 kg pull crossbows, depending on the species and sampling

Figure 3.2. Vessel survey platform, the F/V *Jodie Lynn II*.

distance. Biopsy samples were obtained with a specialized 2.5 cm stainless biopsy tip attached to a modified bolt, typically fired from the bow of the survey vessel.

Data Analysis

Vessel survey effort and sighting data were mapped using *ArcGIS* 10.1. All vessel sighting data collected from January 2013 through December 2013 have been posted on the data repository OBIS-SEAMAP (http://seamap.env.duke.edu/).

Data Storage

All acoustic, visual survey and photographic data have been archived on digital media, and backed up on a Duke University network server.

Results

Vessel Survey Effort

We conducted ten vessel surveys in 2013, totaling 1021.7 km, or 58.7 hours, of survey effort (Table 3.1). These surveys were conducted in Beaufort Sea States (BSS) 1 to 5 and were concentrated in the southern portion of the survey area and along the 200m shelf break (Figure 3.3).

Table 3.1. Dates, kilometers, and hours surveyed during vessel surveys in the Jacksonville survey area, January 2013 – December 2013.

Date	Sea State	Km surveyed	Survey Time (hrs:min)	At Sea Time (hrs:min)	Platform
5-Feb-13	2-3	109.0	7:09	10:48	F/V *Jodie Lynn*
6-Feb-13	1-3	130.0	6:56	10:58	F/V *Jodie Lynn*
9-May-13	2-4	68.0	4:55	11:06	F/V *Knot Tied Down*
10-May-13	1-2	156.0	7:47	11:09	F/V *Jodie Lynn*
15-May-13	2-3	170.0	8:03	11:34	F/V *Jodie Lynn*
17-May-13	1-3	107.0	7:08	11:39	F/V *Knot Tied Down*
18-Jul-13	1-4	101.0	6:16	10:16	F/V *Jodie Lynn II*
19-Jul-13	4-5	13.6	0:49	7:15	F/V *Knot Tied Down*
12-Sep-13	1-4	108.0	6:07	10:45	F/V *Jodie Lynn II*
13-Sep-13	4	59.1	3:29	8:32	F/V *Jodie Lynn*

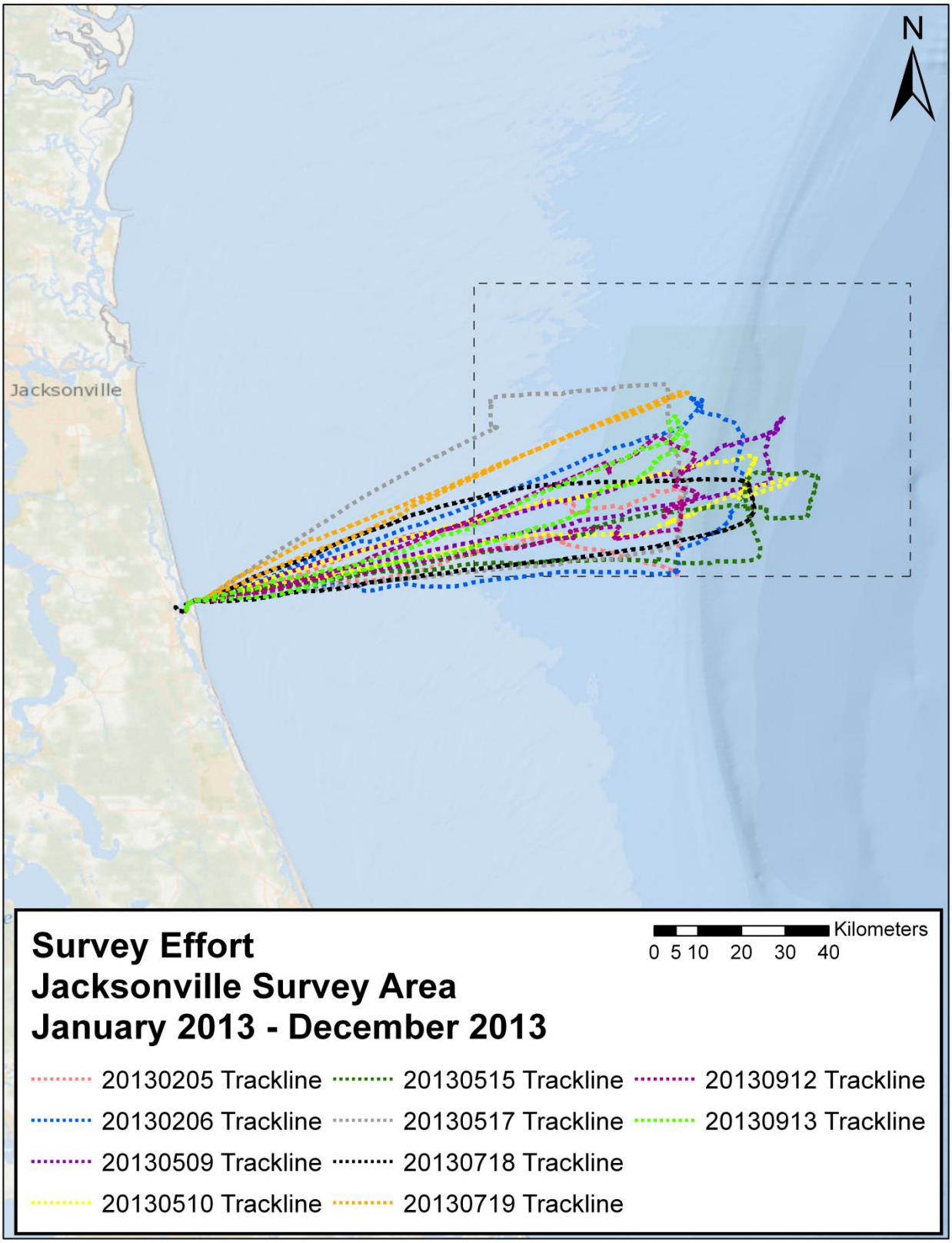

Figure 3.3. Survey effort during vessel surveys in the Jacksonville survey area, January 2013 – December 2013.

Marine Mammal and Sea Turtle Sightings

We recorded twenty-eight cetacean sightings of three species during these vessel surveys: bottlenose dolphins (*Tursiops truncatus*; n=15), Atlantic spotted dolphins (*Stenella frontalis*; n=9) and Risso's dolphins (*Grampus griseus*; n=1). In addition, we observed unidentified delphinids on three occasions (Tables 3.2 and 3.3). We encountered 35 sea turtles in the study area during 2013. Loggerhead sea turtles (*Caretta caretta*, n = 33) were by far the most frequently recorded species, with a single sighting of a leatherback sighting (*Dermochelys coriacea*) and one unidentified sea turtle (Tables 3.4 and 3.5).

Table 3.2. Cetacean sightings from vessel surveys in the Jacksonville survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Common Name	Group Size	Biopsy Samples	Images
5-Feb-13	7:42	30.01048	-80.69933	Unidentified delphinid		1	0	0
5-Feb-13	8:12	30.02640	-80.63286	*T. truncatus*	Bottlenose dolphin	5	0	9
5-Feb-13	9:06	30.04527	-80.52871	*S. frontalis*	Atlantic spotted dolphin	35	2	71
5-Feb-13	10:12	30.01444	-80.41601	*T. truncatus*	Bottlenose dolphin	3	0	14
5-Feb-13	14:16	30.11721	-80.55792	*T. truncatus*	Bottlenose dolphin	2	0	34
6-Feb-13	12:21	30.31270	-80.23669	*T. truncatus*	Bottlenose dolphin	3	1	60
6-Feb-13	14:40	30.20568	-80.47242	*S. frontalis*	Atlantic spotted dolphin	6	0	5
9-May-13	9:43	30.12613	-80.23081	*S. frontalis*	Atlantic spotted dolphin	7	0	10
9-May-13	11:07	30.20289	-80.09539	*T. truncatus*	Bottlenose dolphin	2	0	0
10-May-13	9:04	30.05184	-80.34094	*T. truncatus*	Bottlenose dolphin	2	1	17
10-May-13	9:35	30.08043	-80.29186	*T. truncatus*	Bottlenose dolphin	4	2	102
10-May-13	12:33	30.12571	-80.15334	*G. griseus*	Risso's dolphin	10	0	187
10-May-13	14:46	30.18638	-80.27247	*T. truncatus*	Bottlenose dolphin	4	0	5
10-May-13	14:53	30.17742	-80.30686	*S. frontalis*	Atlantic spotted dolphin	6	0	0
10-May-13	15:37	30.11497	-80.57033	Unidentified delphinid		1	0	0
15-May-13	8:44	29.99532	-80.32777	*S. frontalis*	Atlantic spotted dolphin	3	0	0
15-May-13	12:05	30.08442	-80.09579	*T. truncatus*	Bottlenose dolphin	5	0	24
15-May-13	14:43	30.06316	-80.45593	*S. frontalis*	Atlantic spotted dolphin	2	0	30
17-May-13	10:18	30.17351	-80.27912	*T. truncatus*	Bottlenose dolphin	3	0	5
17-May-13	11:19	30.26000	-80.29536	*T. truncatus*	Bottlenose dolphin	2	0	0
17-May-13	14:51	30.27849	-80.66583	*S. frontalis*	Atlantic spotted dolphin	16	3	186
18-Jul-13	8:46	30.15313	-80.53125	*T. truncatus*	Bottlenose dolphin	6	1	34
18-Jul-13	15:06	29.97150	-80.75553	Unidentified delphinid		6	0	0
12-Sep-13	8:49	30.05714	-80.50322	*T. truncatus*	Bottlenose dolphin	2	0	6
12-Sep-13	9:29	30.06313	-80.40074	*T. truncatus*	Bottlenose dolphin	7	0	46
12-Sep-13	13:38	30.17769	-80.42123	*S. frontalis*	Atlantic spotted dolphin	8	1	43
13-Sep-13	9:07	30.10248	-80.46825	*S. frontalis*	Atlantic spotted dolphin	2	0	0
13-Sep-13	11:17	30.29086	-80.28055	*T. truncatus*	Bottlenose dolphin	2	0	0

Table 3.3. Number of cetacean sightings and mean group size for each species observed during Year 1 (July 2009 – June 2010), Year 2 (July 2010 – December 2011), Year 3 (January 2012 – December 2012) and Year 4 (January 2013 – December 2013) of vessel surveys in the Jacksonville survey area.

	Sightings				
Species	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Mean Group Size**
Globicephala macrorhynchus	3	0	0	0	33.3±17.6
Grampus griseus	2	0	0	1	17.6±15.0
Stenella frontalis	24	17	14	9	9.5±10.1
Tursiops truncatus	15	10	23	15	4.8±4.4
Unid. delphinid	12	1	4	3	2.7±1.2
Total:	**56**	**28**	**41**	**28**	

Table 3.4. Sea turtle sightings from vessel surveys in the Jacksonville survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Common Name	GroupSize
5-Feb-13	10:20	30.01533	-80.41139	*C. caretta*	Loggerhead sea turtle	1
5-Feb-13	11:42	30.04310	-80.27983	*C. caretta*	Loggerhead sea turtle	1
5-Feb-13	12:18	30.13869	-80.27102	*C. caretta*	Loggerhead sea turtle	1
6-Feb-13	8:44	29.97329	-80.28801	*C. caretta*	Loggerhead sea turtle	2
6-Feb-13	11:53	30.31084	-80.23349	*C. caretta*	Loggerhead sea turtle	1
6-Feb-13	12:09	30.32487	-80.23354	*C. caretta*	Loggerhead sea turtle	1
9-May-13	13:15	30.23012	-80.19310	*C. caretta*	Loggerhead sea turtle	1
9-May-13	13:41	30.23012	-80.19310	*C. caretta*	Loggerhead sea turtle	1
10-May-13	14:33	30.19750	-80.22066	*C. caretta*	Loggerhead sea turtle	2
10-May-13	14:50	30.18117	-80.29117	*C. caretta*	Loggerhead sea turtle	1
10-May-13	15:05	30.16135	-80.36582	*C. caretta*	Loggerhead sea turtle	1
10-May-13	15:13	30.14826	-80.41781	*C. caretta*	Loggerhead sea turtle	1
15-May-13	8:13	29.99634	-80.54265	*C. caretta*	Loggerhead sea turtle	1
15-May-13	13:24	30.10073	-80.24569	*C. caretta*	Loggerhead sea turtle	1
15-May-13	14:15	30.08335	-80.37773	*C. caretta*	Loggerhead sea turtle	1
15-May-13	14:24	30.07989	-80.40252	*C. caretta*	Loggerhead sea turtle	1
15-May-13	15:23	30.04543	-80.50269	*C. caretta*	Loggerhead sea turtle	1
15-May-13	15:31	30.04213	-80.52418	*C. caretta*	Loggerhead sea turtle	1
17-May-13	10:18	30.17036	-80.27370	*C. caretta*	Loggerhead sea turtle	1
17-May-13	12:41	30.35545	-80.40221	*C. caretta*	Loggerhead sea turtle	1
17-May-13	13:09	30.34683	-80.47280	*C. caretta*	Loggerhead sea turtle	1
17-May-13	13:28	30.33991	-80.52218	*C. caretta*	Loggerhead sea turtle	1
17-May-13	13:33	30.33989	-80.52486	*C. caretta*	Loggerhead sea turtle	2
17-May-13	13:40	30.34066	-80.55042	*C. caretta*	Loggerhead sea turtle	1
17-May-13	13:58	30.33865	-80.59685	*C. caretta*	Loggerhead sea turtle	1
17-May-13	14:28	30.33103	-80.66562	*C. caretta*	Loggerhead sea turtle	1
17-May-13	14:34	30.31304	-80.66438	*D. coriacea*	Leatherback sea turtle	1
18-Jul-13	9:57	30.15816	-80.39938	*C. caretta*	Loggerhead sea turtle	1
18-Jul-13	14:07	30.01799	-80.38518	*C. caretta*	Loggerhead sea turtle	1
18-Jul-13	14:17	30.00943	-80.44466	*C. caretta*	Loggerhead sea turtle	1
12-Sep-13	8:30	30.040283	-80.53717	Unid. Sea Turtle		1
12-Sep-13	10:24	30.04575	-80.378305	*C. caretta*	Loggerhead sea turtle	1
12-Sep-13	14:16	30.180565	-80.465072	*C. caretta*	Loggerhead sea turtle	1
13-Sep-13	10:15	30.194978	-80.468246	*C. caretta*	Loggerhead sea turtle	1
13-Sep-13	11:20	30.29295	-80.28293	*C. caretta*	Loggerhead sea turtle	1

Table 3.5. Number of sea turtle sightings and mean group size for each species observed during Year 1 (July 2009 – June 2010), Year 2 (July 2010 – December 2011), Year 3 (January 2012 – December 2012) and Year 4 (January 2013 – December 2013) of vessel surveys in the Jacksonville survey area.

	Sightings				
Species	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Mean Group Size**
Caretta caretta	49	23	41	33	1.1±0.3
Dermochelys coriacea	5	6	4	1	1.0±0.0
Lepidochelys kempii	1	0	1	0	1.0±0.0
Unid. sea turtle	3	8	3	1	1.0±0.0
Total:	**58**	**37**	**49**	**35**	

Distributions and Habitat Associations of Cetaceans and Sea Turtles

The distribution of marine mammals and sea turtles in the Jacksonville study area is presented in Figures 3.4 through 3.9. Similar to our observations from previous years, bottlenose dolphins were encountered throughout the survey area, including deeper pelagic waters (Figure 3.5), whereas Atlantic spotted dolphins were restricted to the relatively shallow shelf waters (Figure 3.6). All sea turtles were observed in relatively shallow waters over the continental shelf (Figure 3.9).

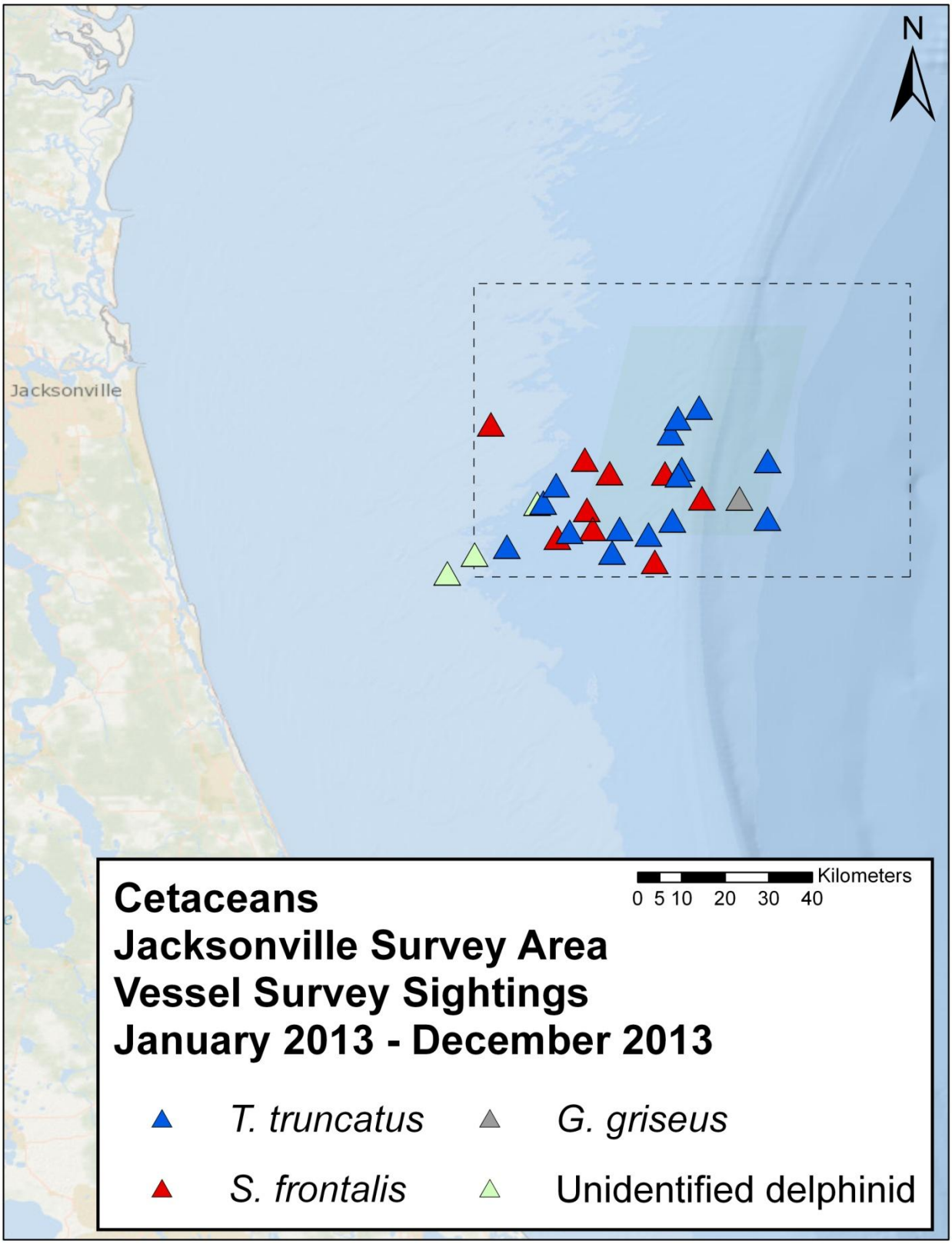

Figure 3.4. Distribution of all cetacean sightings made during vessel surveys in the Jacksonville study area, January 2013 – December 2013.

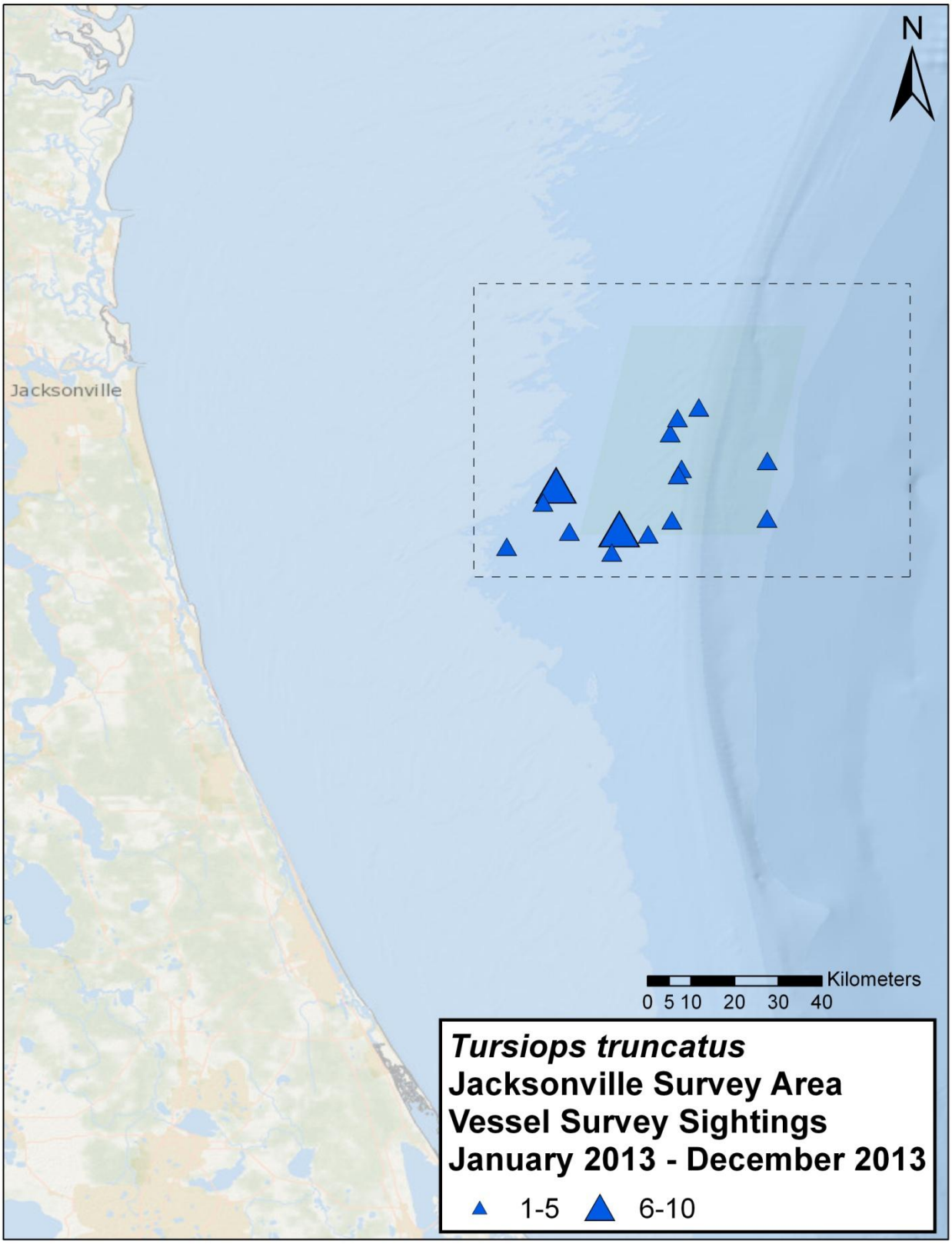

Figure 3.5. Distribution of bottlenose dolphin sightings indicating group size made during vessel surveys in the Jacksonville study area, January 2013 – December 2013.

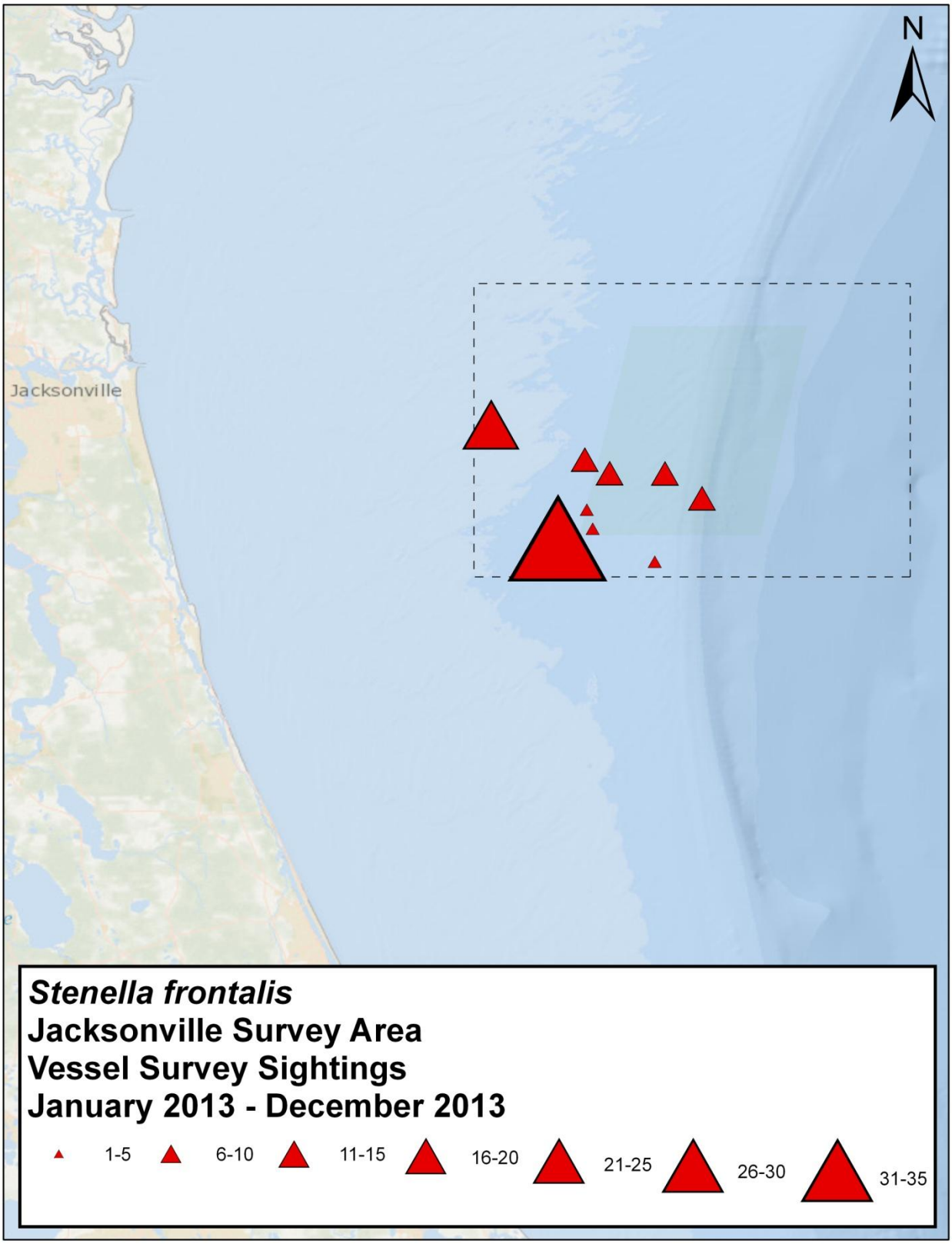

Figure 3.6. Distribution of Atlantic spotted dolphin sightings indicating group size made during vessel surveys in the Jacksonville study area, January 2013 – December 2013.

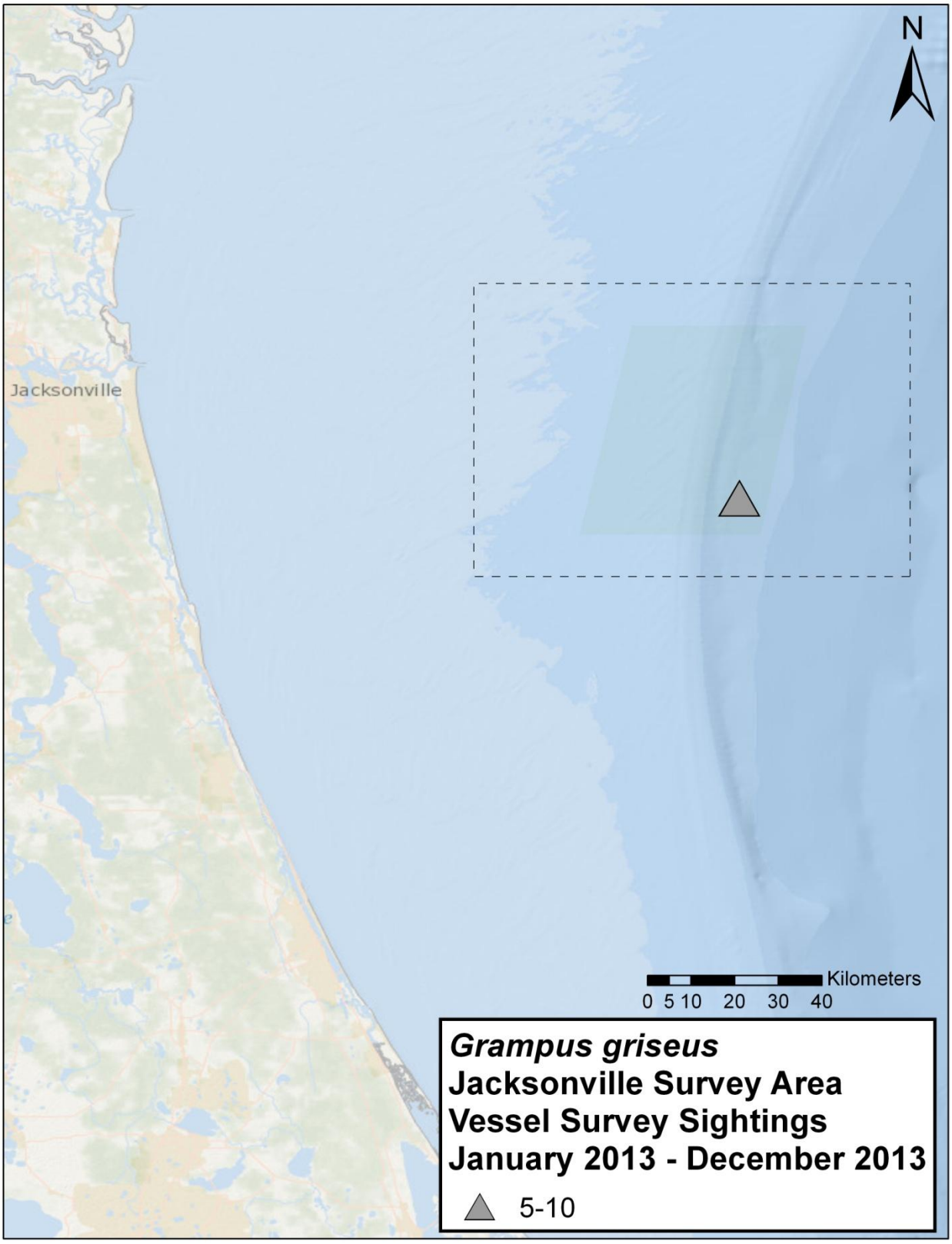

Figure 3.7. Distribution of Risso's dolphin sightings indicating group size made during vessel surveys in the Jacksonville study area, January 2013 – December 2013.

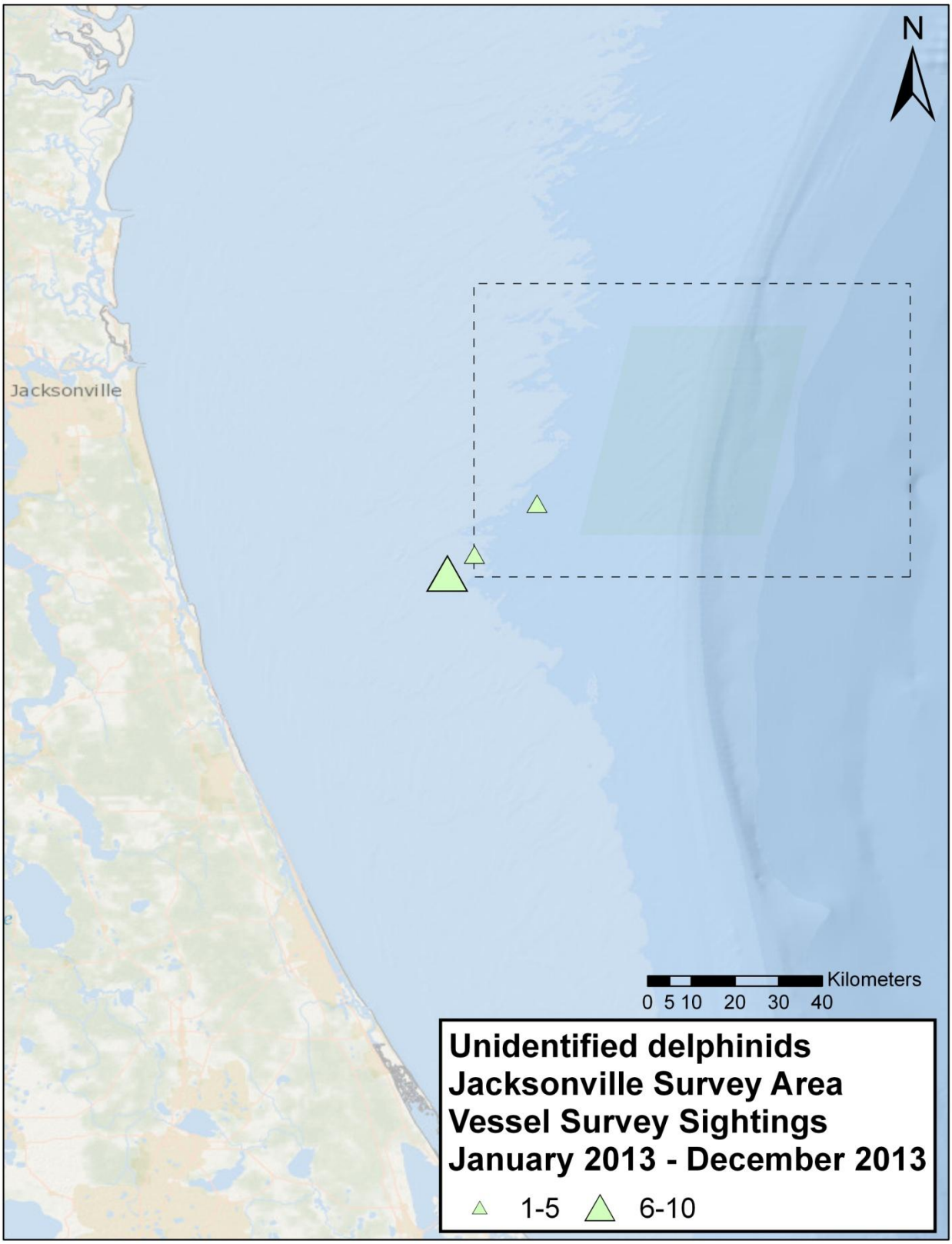

Figure 3.8. Distribution of unidentified delphinid sightings indicating group size made during vessel surveys in the Jacksonville survey area, January 2013 – December 2013.

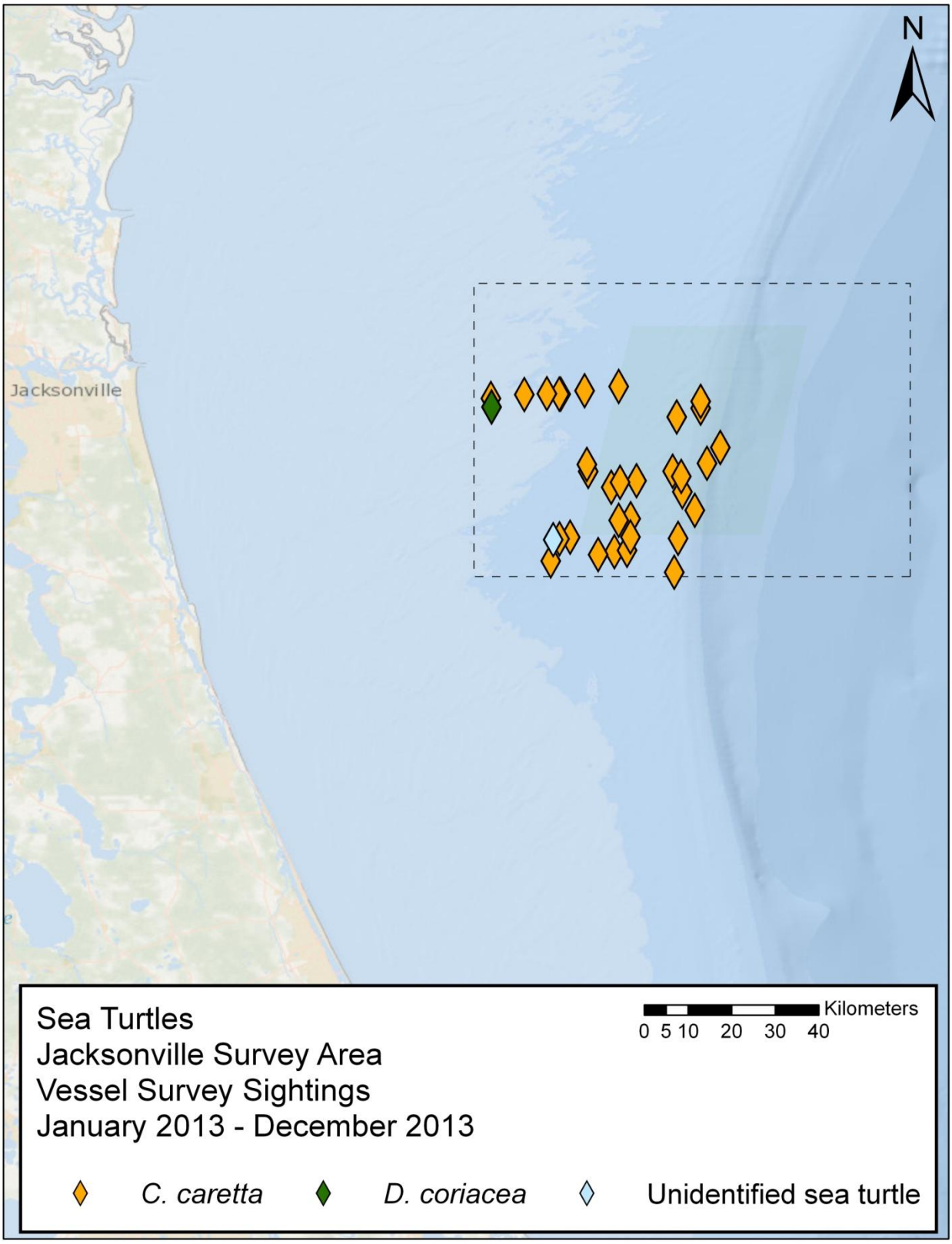

Figure 3.9. Distribution of sea turtle sightings made during vessel surveys in the Jacksonville survey area, January 2013 – December 2013.

Biopsy Sampling

We collected 12 biopsy samples in the Jacksonville survey area during 2013 from: Atlantic spotted dolphins *(n* = 6); bottlenose dolphins (*n* = 5); and Risso's dolphins (*n* = 1) (Table 3.6, Figure 3.10). Genetic analysis of extracted DNA from bottlenose dolphin biopsy samples collected in the Jacksonville survey site between May 2011 and July 2013 confirms that all of the sampled dolphins were of the offshore ecotype, suggesting that there is limited overlap between coastal and offshore populations in the study area. Skin samples collected after July 2013 will be analyzed for sex and population identity in the coming months. Voucher specimens of these samples are archived with the Southeast Fisheries Science Center in Lafayette, LA.

Table 3.6. Biopsy samples collected in the Jacksonville survey area, January 2013 – December 2013.

Date	Time	Latitude	Longitude	Species	Sample #	Photo-ID Code
5-Feb-13	9:14	30.03963	-80.52446	*S. frontalis*	HJF-13-001	Not distinctive
5-Feb-13	9:21	30.03616	-80.52062	*S. frontalis*	HJF-13-002	Sfr 2-009
6-Feb-13	13:45	30.33014	-80.25067	*T. truncatus*	DMW-13-001	Ttr 6-006
10-May-13	9:10	30.05559	-80.33879	*T. truncatus*	ZTS-13-001	Not distinctive
10-May-13	9:55	30.07555	-80.29203	*T. truncatus*	ZTS-13-002	Not distinctive
10-May-13	9:58	30.07555	-80.29203	*T. truncatus*	ZTS-13-003	No ID photo
10-May-13	12:49	30.14541	-80.14754	*G. griseus*	ZTS-13-004	Not distinctive
17-May-13	15:01	30.27046	-80.65457	*S. frontalis*	DMW-13-002	Not distinctive
17-May-13	15:06	30.27166	-80.65774	*S. frontalis*	DMW-13-003	Sfr 9-018
17-May-13	15:12	30.27167	-80.65780	*S. frontalis*	DMW-13-004	Sfr 9-017
18-Jul-13	8:58	30.15254	-80.53394	*T. truncatus*	ZTS-13-024	Ttr 2-004
12-Sep-13	13:45	30.19008	-80.42313	*S. frontalis*	HJF-13-004	Not distinctive

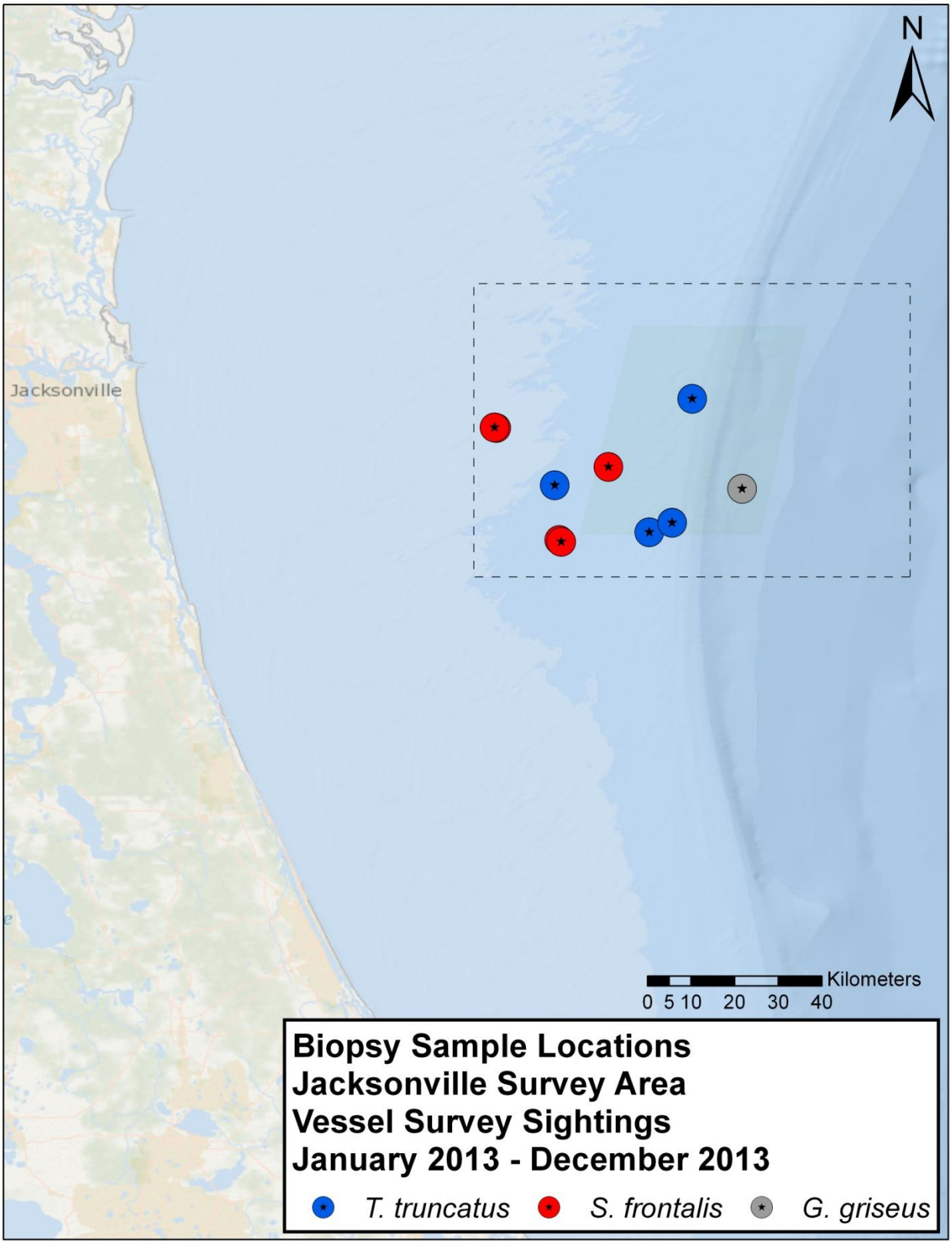

Figure 3.10. Locations of biopsy samples collected in the Jacksonville survey area, January 2013 – December 2013.

Photographic Effort

We obtained 1014 digital images for species confirmation and individual identification during 2013. We added images of newly identified dolphins to existing catalogs (Table 3.7); photo-identification analysis is now complete for all images taken through December 2013. Photo-identification cataloges for *T. truncatus* and *S. frontalis* currently consist of 52 and 77 individuals, respectively. We have resighted two individual spotted dolphins within the Jacksonville study area (Figure 3.11). Sfr 3-001 was observed first on 10 October 2010 and again on 19 March 2011; Sfr 8-005 was photographed during surveys on two consecutive days: 18 March 2011 and 19 March 2011 (Table 3.8). In addition, we resighted two bottlenose dolphins together on 25 January 2012 and 18 July 2013 (Table 3.8, Figure 3.11). We compared the photo-identification catalogs of bottlenose dolphins, Atlantic spotted dolphins and short-finned pilot whales from Onslow Bay, NC and Jacksonville, FL but we found no matches between the two study areas.

Table 3.7. Summary of photographs taken of animals in the Jacksonville survey area, January 2013 through December 2013, with photo-identification catalog sizes and number of matches (2009-2013), and biopsy samples collected in 2013 with the total number of samples in parenthesis.

Common Name	Scientific Name	Photos Taken	Catalog Size	Matches	Samples
		2013			2013 (Total)
Short-finned pilot whale	*G. macrorhynchus*	n/a	23	0	0 (0)
Risso's dolphin	*G. griseus*	187	7	0	1 (1)
Atlantic spotted dolphin	*S. frontalis*	345	77	2	6 (25)
Bottlenose dolphin	*T. truncatus*	356	52	2	5 (17)

Table 3.8. Photo-identification matches of bottlenose dolphins and Atlantic spotted dolphins observed in the Jacksonville, FL study area.

Jacksonville, FL					
ID	2009	2010	2011	2012	2013
Ttr 2-004^				JAN	JUL
Ttr 6-010^				JAN	JUL
Sfr 3-001		OCT	MAR		
Sfr 8-005			MAR		

^Observed together in multiple sightings

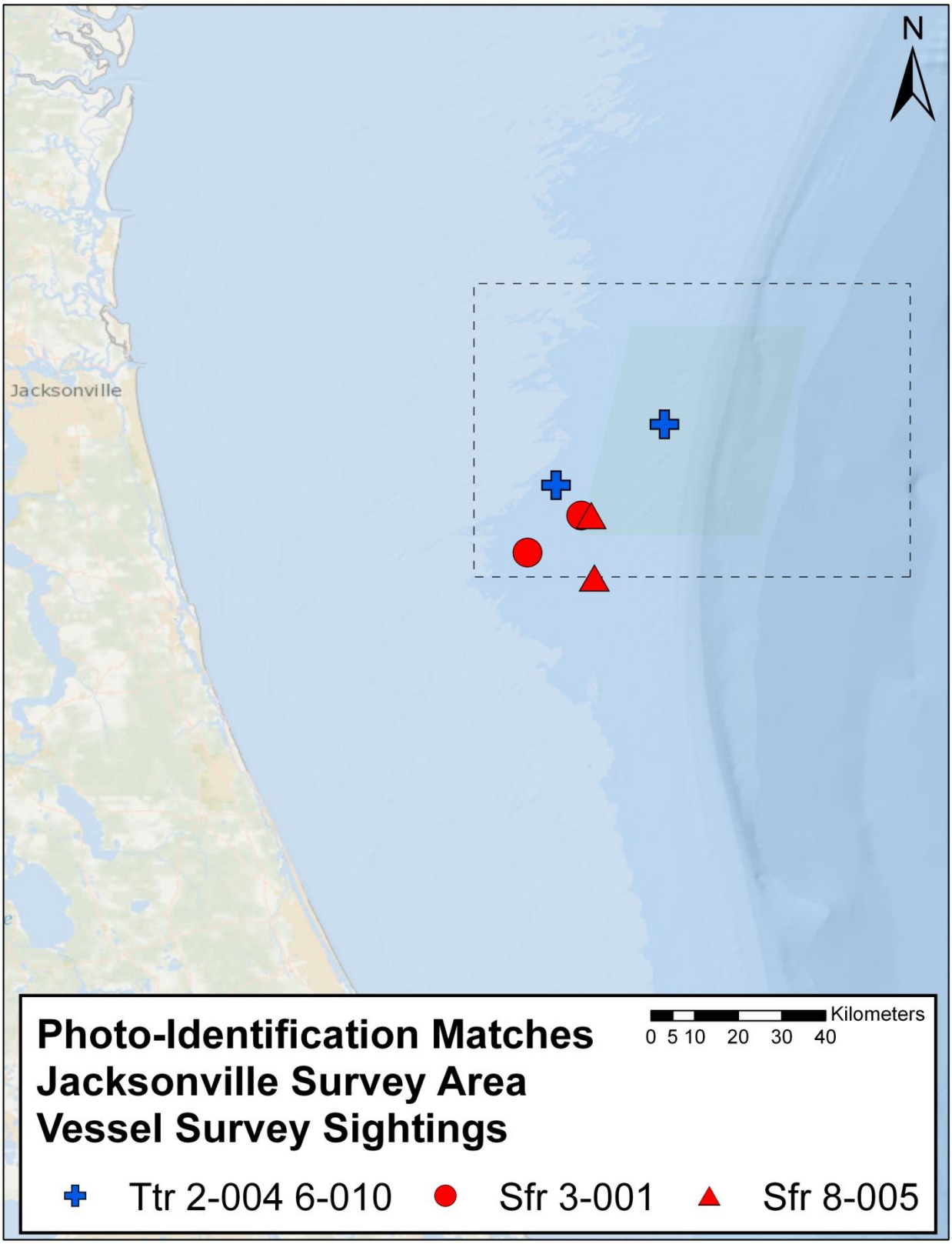

Figure 3.11. Locations of matched dolphins within the Jacksonville survey area.

Summary Tables

Total survey effort conducted since the beginning of the monitoring program in the Jacksonville survey area is reported in Table 3.9. Number of sightings and mean group size by species are presented in Table 3.10. The number of biopsy samples collected and processed to date is reported in Table 3.11. Table 3.12 summarizes the catalog sizes and matches by species to date and images taken during the reporting period in the Jacksonville survey area.

Table 3.9. Kilometers and hours surveyed during Year 1 (July 2009 – June 2010), Year 2 (July 2010 – December 2011), Year 3 (January 2012 – December 2012) and Year 4 (January 2013 – December 2013) in the Jacksonville survey area.

	Year 1	Year 2	Year 3	Year 4	Total
Survey Hours	96.3	49.3	58.6	58.7	262.9
Km Surveyed	1638.2	847.3	937.4	1021.7	4444.6

Table 3.10. Number of cetacean sightings and mean group size for each species observed during Year 1 (July 2009 – June 2010), Year 2 (July 2010 – December 2011), Year 3 (January 2012 – December 2012) and Year 4 (January 2013 – December 2013) of vessel surveys in the Jacksonville survey area.

	Sightings				
Species	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Mean Group Size**
Globicephala macrorhynchus	3	0	0	0	33.3±17.6
Grampus griseus	2	0	0	1	17.6±15.0
Stenella frontalis	24	17	14	9	9.5±10.1
Tursiops truncatus	15	10	23	15	4.8±4.4
Unid. delphinid	12	1	4	3	2.7±1.2
Total:	**56**	**28**	**41**	**28**	

Table 3.11. Biopsy samples collected and processed to date in the Jacksonville survey area.

Common Name	Scientific Name	Year 2	Year 3	Year 4	# of Samples Processed
Risso's dolphin	*G. griseus*	0	0	1	0
Atlantic spotted dolphin	*S. frontalis*	0	19	6	0
Bottlenose dolphin	*T. truncatus*	0	12	5	15

Table 3.12. Summary of images collected during all vessel surveys in the Jacksonville survey area, January 2013 - December 2013, with photo-identification catalog sizes and matches to date.

	Year 1		Year 2		Year 3		Year 4		
Species	Catalog Size	Matches	Catalog Size	Matches	Catalog Size	Matches	Images	Catalog Size	Matches
Globicephala macrorhynchus	0	0	0	0	0	0	n/a	23	0
Grampus griseus	1	0	1	0	1	0	187	7	0
Stenella frontalis	0	0	41	0	60	2	345	77	2
Tursiops truncatus	0	0	21	0	41	0	356	52	2

4. Analysis of Biopsy Samples

Molecular Analysis

We commenced molecular analysis of cetacean biopsy samples collected as part of this program in June 2013. This analysis is intended to provide information on population identity and structure of cetaceans encountered during survey efforts. This work is coordinated closely with the molecular laboratory of Dr. Patricia Rosel at the NMFS Southeast Fisheries Science Center.

Genetic Variation in Short-finned Pilot Whales

In the first phase of this work, our analysis concentrated on an investigation of genetic variation across the mitochondrial control region in short-finned pilot whales and bottlenose dolphins. Genetic variation in the mitochondrial control region is one of the primary tools used to differentiate populations of marine mammals and other vertebrates. However, previous studies of the five-prime end of the mitochondrial control region in short-finned pilot whales have identified little genetic variation in this species, hindering attempts to describe population structure in this species. Our project extended sequences across the entire mitochondrial control region in an effort to identify additional variation that might be used to differentiate among short-finned pilot whales in the study area. We extracted DNA from 39 short-finned pilot whale biopsy samples, and sequenced 819 base pairs from the 3 prime end of the mitochondrial control region. Polymerase chain reactions and sequencing were conducted using the primers L16061 and H00651. Four variable sites were identified in the 819-base-pair region (Figure 4.1). However, variation was very rare at three of the four sites; variants at these sites were observed in only a single sample. Overall, the results indicate very little genetic variation across the entire mitochondrial control region in short-finned pilot whales.

Position	216	281	428	719	
Consensus	T	C	A	C	**N**
	.	.	.	.	**26**
	.	.	G	.	**11**
	.	.	G	T	**1**
	C	T	G	.	**1**

Figure 4.1. Four variable sites were identified in the 819 base-pairs of sequence from the 3' end of the mitochondrial control region.

Bottlenose Dolphin Ecotypes

We completed DNA extraction and sequencing of bottlenose dolphin biopsy samples to identify offshore and coastal morphotypes in August 2013. We distinguished offshore and coastal morphotypes by aligning sequences from the mitochondrial control region to known variant sequences. We extracted DNA from 55 bottlenose dolphin biopsy samples and 489 base pairs of the mitochondrial control region were amplified and sequenced using the primers L15824 and H16498. This dataset included all bottlenose dolphins sampled in Onslow, Jacksonville, and Cape Hatteras between May 2011 and July 2013, except for one sample (DMW-13-001) that had insufficient tissue (Figure 4.2). Dr. Rosel confirmed that all of the sampled dolphins were of the offshore ecotype (Figure 4.3). These data suggest that there is little overlap between coastal and offshore populations in the sample areas. We plan to examine photographs of these 55 offshore bottlenose dolphins to describe their morphology and patterns of pigmentation and determine whether we can use external features to identify dolphins of this ecotype in the field.

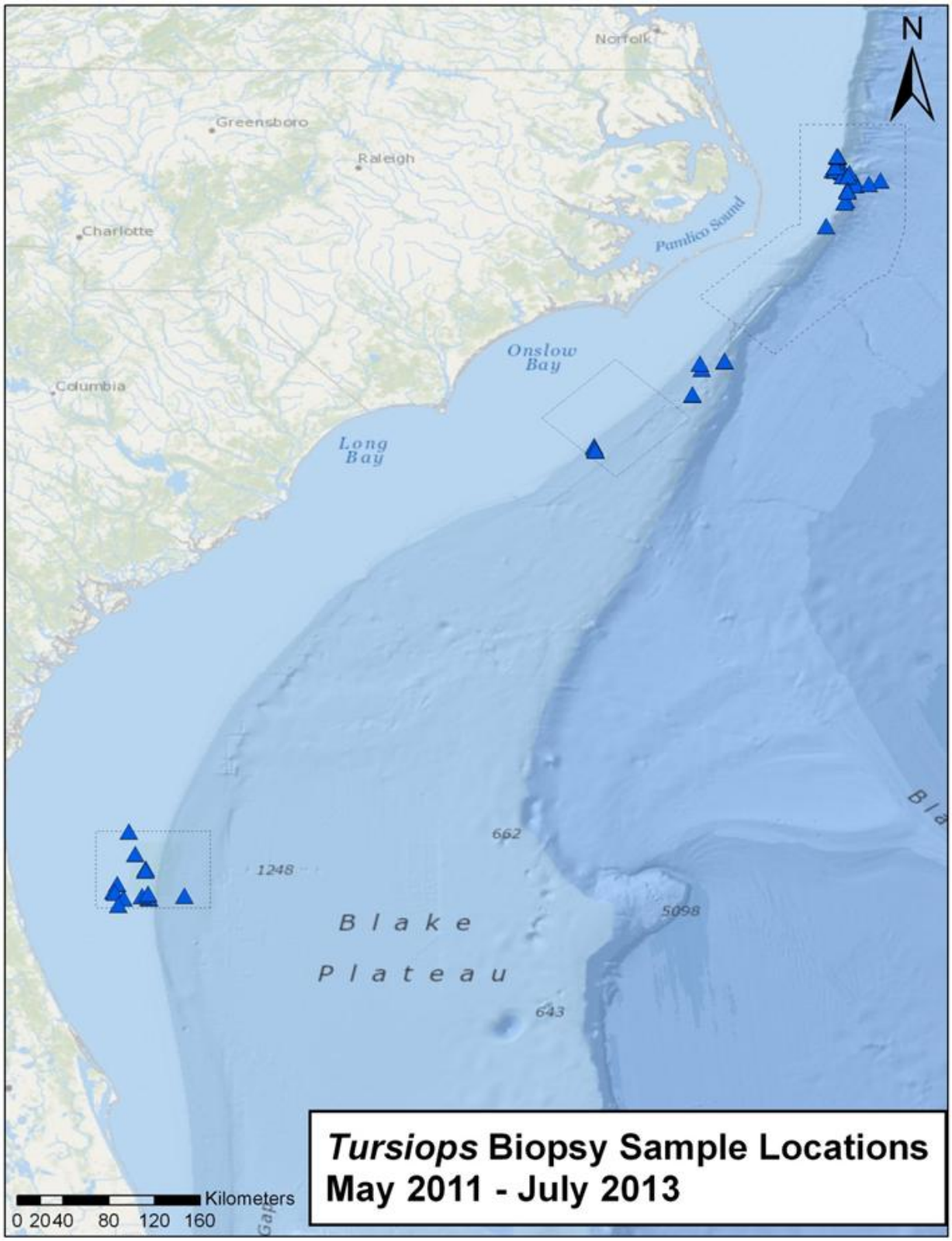

Figure 4.2. Locations of all bottlenose dolphins sampled in Cape Hatteras, Onslow, and Jacksonville between May 2011 and July 2013.

Position	14	84	96	161	168	173	180	212	256	268	297	308	311	314	328	332	337	341	342	345	346	356	366	379	392	420	454				
Consensus	T	G	C	T	G	C	T	C	A	C	T	T	G	C	T	C	C	C	T	C	C	T	G	A	C	C	C	Hat	Ons	Jax	**N**
	.	.	.	.	A	.	.	T	.	T	.	.	.	.	C	.	.	.	C	T	.	.	A	.	.	.	T	2	0	0	**2**
	.	.	.	.	A	.	.	T	.	T	.	.	.	.	C	T	.	.	C	T	.	.	A	.	.	.	T	6	4	0	**10**
	.	.	.	.	A	.	.	T	.	T	.	.	.	.	C	T	.	.	C	T	.	C	A	.	.	.	T	3	1	0	**4**
	.	.	.	.	A	.	C	T	.	T	.	.	.	.	C	T	.	.	C	T	.	.	A	.	.	.	T	8	1	0	**9**
	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	T	1	1	0	**2**
	.	.	.	.	A	.	.	T	.	.	.	.	.	T	.	.	.	.	C	.	.	.	A	.	.	.	T	3	0	0	**3**
	.	.	.	C	A	.	.	T	.	.	.	.	.	T	.	.	.	.	C	.	.	.	A	.	.	.	T	1	0	0	**1**
	.	.	.	.	A	.	.	T	.	.	.	.	.	T	C	.	.	.	C	.	.	.	A	.	.	.	T	0	0	1	**1**
	.	.	T	.	A	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T	T	.	1	1	0	**2**
	.	.	T	.	A	T	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	2	0	0	**2**
	.	.	T	.	A	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	0	1	0	**1**
	.	.	T	.	.	T	.	.	.	.	.	.	.	.	A	.	-	.	.	.	.	.	.	.	.	T	.	0	0	9	**9**
	.	.	T	.	.	T	.	.	.	.	C	.	.	.	A	.	-	.	.	.	.	.	.	.	.	T	.	1	0	0	**1**
	.	.	T	.	.	T	.	.	G	.	C	.	.	.	A	.	-	.	.	.	.	.	.	.	.	T	.	1	0	0	**1**
	.	.	T	.	.	T	.	.	.	.	C	.	.	.	A	.	-	.	.	.	.	.	.	G	.	T	.	1	0	0	**1**
	.	A	T	.	.	T	.	.	.	.	.	.	.	.	A	.	-	.	.	.	.	.	.	.	.	T	.	0	0	1	**1**
	.	.	T	.	.	T	.	.	.	.	.	.	.	.	A	.	-	T	.	.	.	.	.	.	.	T	.	0	0	4	**4**
	.	.	T	.	A	T	.	.	.	.	.	.	A	.	A	.	.	.	.	.	T	.	.	.	.	T	.	1	0	0	**1**
																												31	9	15	**55**

Figure 4.3. Summary of the differences observed in 489 base pairs sequenced from the 5' end of the mitochondrial control region and the number of dolphin samples with each haplotype. These haplotypes are all characteristic of the offshore ecotype.

Stable Isotope Analysis

We analyzed stable isotopes of nitrogen and carbon in biopsy samples obtained from bottlenose dolphins, pilot whales and spotted dolphins collected during these surveys. Carbon stable isotopes are useful in tracing the foraging location of an animal by reflecting the source of its primary productivity, *e.g.* inshore versus offshore and benthic versus pelagic feeding. Nitrogen stable isotopes are useful in determining the trophic level at which an animal is feeding, since enrichment in ^{15}N from one trophic level to another is predictable, at approximately 3‰ per trophic level.

We analyzed stable isotopes on biopsy samples collected from coastal and offshore bottlenose dolphins to distinguish differences between the two populations (Figure 4.4). Offshore bottlenose dolphin had significantly lower nitrogen values and significantly higher carbon values than coastal bottlenose dolphins (Figure 4.5). These data suggest that the coastal bottlenose

dolphins occupy a slightly higher trophic level than the offshore stock. The $\delta^{13}C$ values are likely lower in the coastal animals because they forage closer to freshwater sources of carbon, such as rivers and estuaries.

We confirmed the sex of 41 female and 41 male short-finned pilot whales from molecular analysis of biopsy samples (P. Rosel, pers. comm.). Male short-finned pilot whales had significantly higher $\delta^{15}N$ values, but similar $\delta^{13}C$ values, when compared to female pilot whales. 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‰. Our findings do, however, indicate that the diets of the two sexes differ. We plan to explore differences in the foraging behavior of male and female pilot whales using data collected with further deployments of digital acoustic tags (D-Tags) that record depth, sound and orientation.

We also examined differences in isotopic values among adults, sub-adults and calves in 26 stranded pilot whales. We found no significant differences in isotopic signatures amongst three age classes of stranded pilot whales. This result was somewhat surprising because other studies have found that nursing marine mammals have lower $\delta^{13}C$ and higher $\delta^{15}N$ values than adults. It is possible that the stranded animals that were identified as calves were no longer nursing. 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.

Finally, we conducted an analysis of stable isotopes from samples of 82 pilot whales (including stranded and live animals) and compared them to offshore bottlenose dolphins (n=14) to distinguish differences in foraging habitat use. The pilot whales had significantly higher $\delta^{15}N$ values than the bottlenose dolphins, but the mean difference in $\delta^{15}N$ was only 0.5‰, indicating that they are feeding at a similar trophic level; however, pilot whales had significantly enriched $\delta^{13}C$ values compared to the bottlenose dolphins (Figure 4.5), suggesting that pilot whales are feeding on prey found at depth, where carbon is stored in higher concentrations. Interestingly, one Atlantic spotted dolphin sample obtained from the same area as the pilot whales and offshore bottlenose dolphins contained lower nitrogen and carbon levels than the other species (Figure 4.5).

In the future, we plan to conduct stable isotopes analysis on all of our biopsy samples to address questions of foraging strategies, habitat use and population structure among species found in these regions.

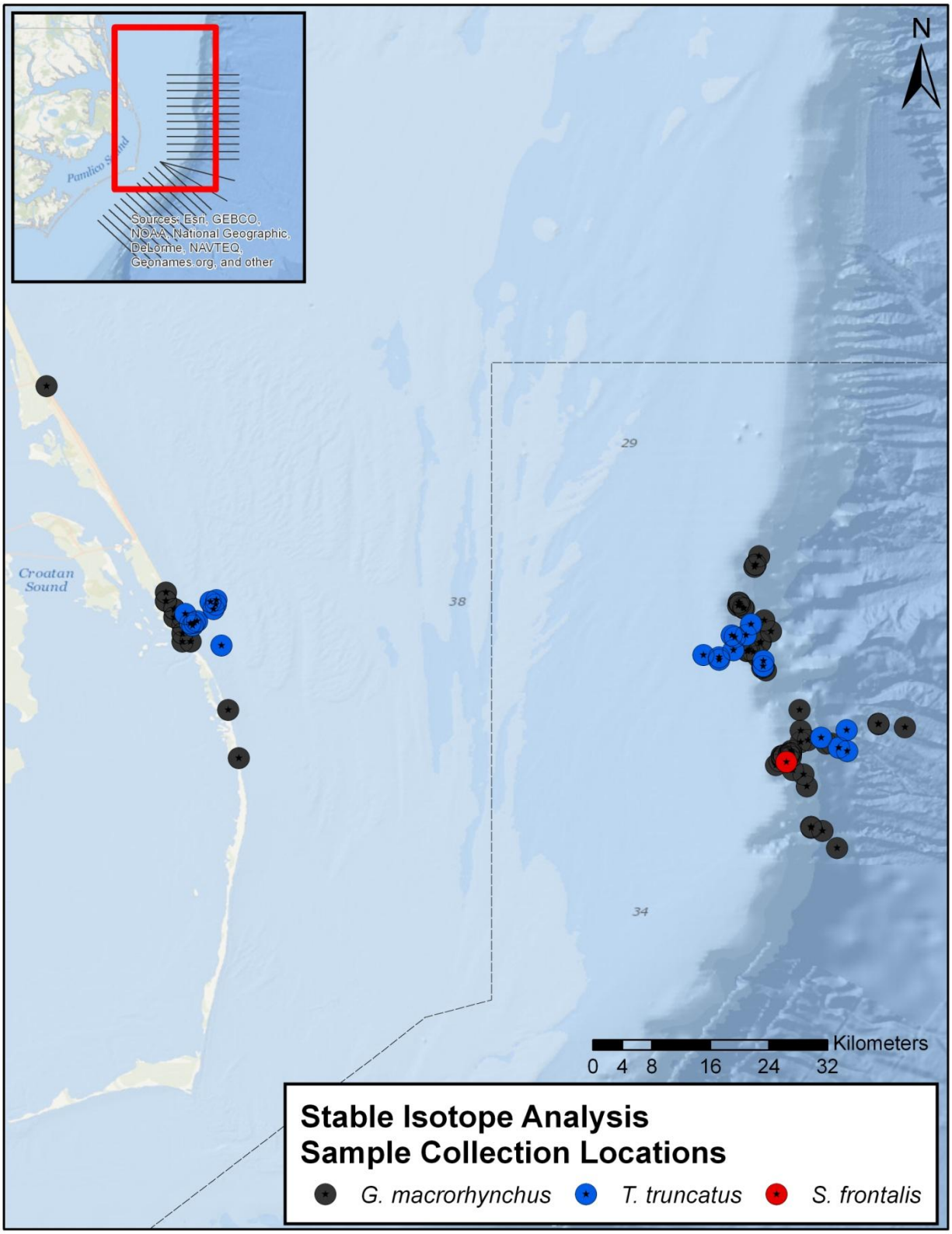

Figure 4.4. Locations of sampled Atlantic spotted dolphin (n = 1), coastal (n = 10) and offshore bottlenose dolphins (n = 14) and stranded (n = 55) and biopsied short-finned pilot whales (n = 27).

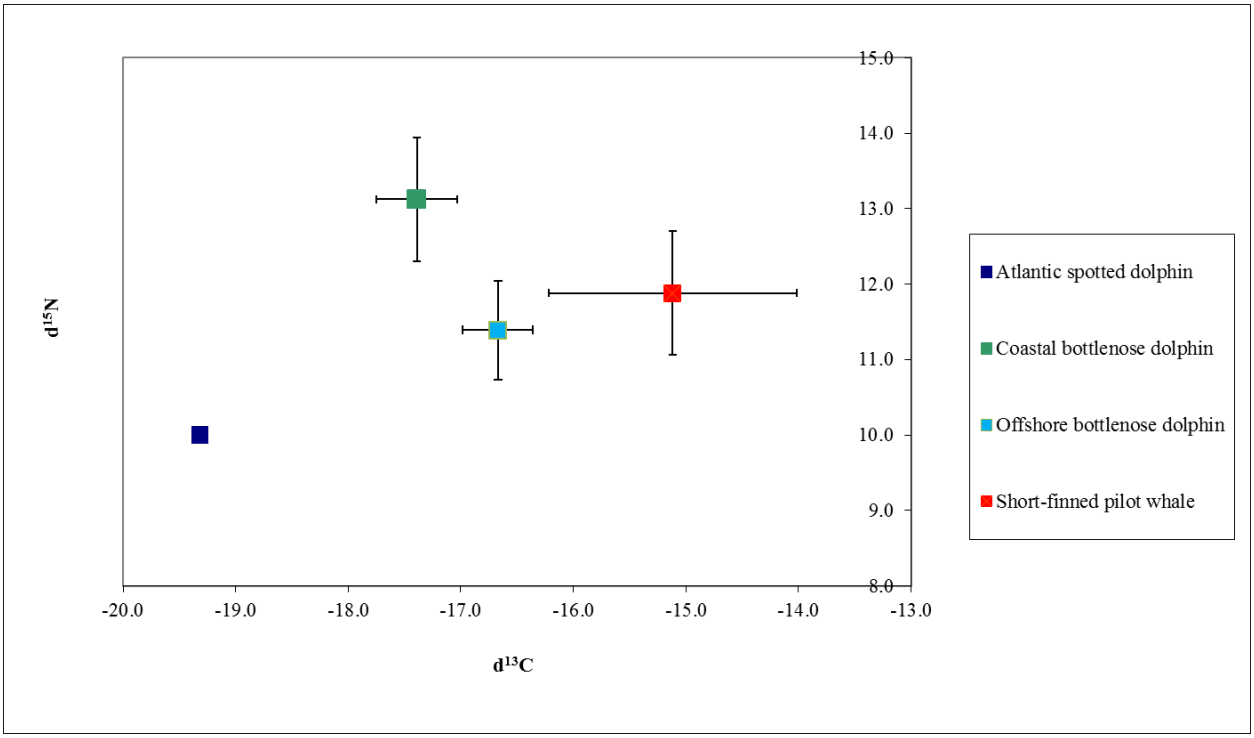

Figure 4.5. Stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ for Atlantic spotted dolphins (n = 1), coastal bottlenose dolphins (n = 10), offshore bottlenose dolphins (n = 14) and short-finned pilot whales (n = 82).

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