

Mid-Atlantic Baleen Whale Monitoring, Virginia Beach, Virginia: 2020/21 Annual Progress Report

Submitted to:

Naval Facilities Engineering Systems Command Atlantic
under Contract No. N62470-20-D-0016, Task Order
21F4005, issued to HDR Inc.



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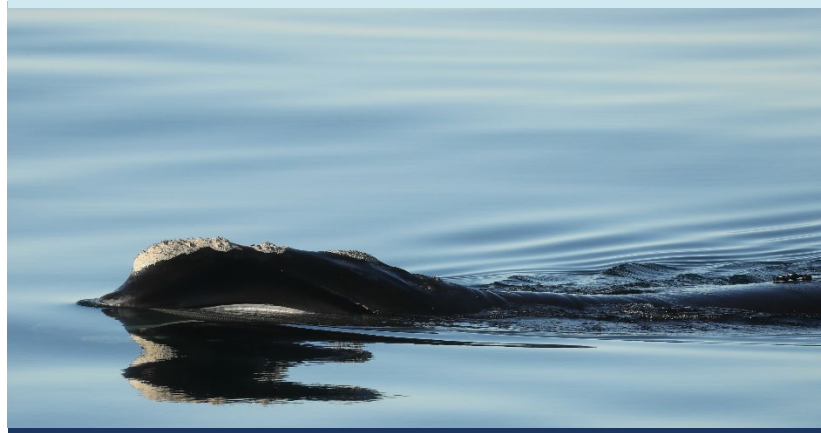
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June 2022



Suggested Citation:

Aschettino, J.M., D. Engelhaupt, A. Engelhaupt, M. Richlen, and M. Cotter. 2022. *Mid-Atlantic Baleen Whale Monitoring, Virginia Beach, Virginia: 2020/21 Annual Progress Report*. Prepared for U.S. Fleet Forces Command. Submitted to Naval Facilities Engineering Systems Command Atlantic, Norfolk, Virginia, under Contract N62470-20-0016, Task Order 21F4005, issued to HDR Inc., Virginia Beach, Virginia. June 2022.

Cover Photo Credit:

A North Atlantic right whale (*Eubalaena glacialis*), tagged with satellite and acoustic tags, surfaces off the coast of Virginia Beach, Virginia. Cover photograph by Mark Cotter, taken under National Marine Fisheries Service Scientific Research Permit No. 21482, issued to Dan Engelhaupt, HDR.

This project is funded by U.S. Fleet Forces Command and managed by Naval Facilities Engineering Systems Command Atlantic as part of the U.S. Navy's marine species monitoring program.

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Acronyms and Abbreviations

| | |
|----------|--|
| BSS | Beaufort sea state |
| CTD | conductivity, temperature, and depth |
| DTAG | Digital Acoustic Recording Tag |
| GPS | Global Positioning System |
| km | kilometer(s) |
| LiDAR | Light Detection and Ranging |
| LIMPET | Low Impact Minimally Percutaneous External-electronics Transmitter |
| m | meter(s) |
| MAHWC | Mid-Atlantic Humpback Whale Catalog |
| min | minute(s) |
| MINEX | Mine Neutralization Exercise |
| NAHWC | North Atlantic Humpback Whale Catalog |
| NAVFAC | Naval Facilities Engineering Command |
| OPAREA | Operating Area |
| photo-ID | photo-identification |
| SE | standard error |
| SMA | Seasonal Management Area |
| SPOT | Smart Position and Temperature |
| sUAS | small Unmanned Aerial System |
| UME | Unusual Mortality Event |
| U.S. | United States |
| VACAPES | Virginia Capes Operating Area |
| VHF | very-high frequency |

1. Introduction and Background

Norfolk, Virginia, is home to the world's largest United States (U.S.) Navy installation, Naval Station Norfolk, and it is also ranked the sixth busiest container port in the U.S ([U.S. Army Corps of Engineers, 2017](#)). These factors, combined with the presence of recreational, fishing, and other commercial vessels, result in a constant and often heavy flow of vessel traffic through the mouth of Chesapeake Bay and adjacent areas. During winter months, humpback whales (*Megaptera novaeangliae*) of the West Indies distinct population segment (Bettridge et al. 2015) migrate from six northern feeding grounds in the Gulf of Maine, the Gulf of St. Lawrence, Newfoundland/Labrador, western Greenland, Iceland, and Norway to waters of the West Indies (Katona and Beard 1990; [Christensen et al. 1992](#); [Palsbøll et al. 1997](#)). An unknown portion of the population does not migrate to Caribbean waters but rather uses the Mid-Atlantic region to overwinter (Swingle et al. 1993; Barco et al. 2002). Understanding the occurrence and behavior of humpback whales in this region is important in mitigating potentially harmful impacts on the species.

Until recent years, humpback whale sighting information off the Virginia Beach area had been collected via sporadic and varied field efforts. Shore-based counts in 1991, vessel-based photo-identification (photo-ID) efforts in 1992 (Swingle et al. 1993), and further cataloging efforts using photographs taken on whale-watching excursions and information from stranded whales (Wiley et al. 1995; [Barco et al. 2002](#)) have been the primary data sources. These studies have shown that some individuals return in subsequent years, and the area may act as a supplemental winter feeding ground for the returning whales ([Barco et al. 2002](#)). Photographs of whales sighted off the Virginia coast have been matched to cataloged whales from the Gulf of Maine, Newfoundland, and Gulf of St. Lawrence regions ([Barco et al. 2002](#); [Aschettino et al. 2018](#); [Malette and Barco 2019](#)). Until recently, information on the movements of individuals within this region has been limited. Results from the first 3 years of this study were recently published ([Aschettino et al. 2020a](#)) and have highlighted that humpback whales in this region spend a significant portion of their time in high-traffic areas, including the shipping lanes at the mouth of Chesapeake Bay. This growing body of data is important to assess the potential for disturbance to humpback whales found in U.S. Navy training ranges and high-traffic areas in the Chesapeake Bay and mid-Atlantic coastal waters.

This multi-year project was established under the U.S. Navy's Marine Species Monitoring Program to collect baseline information on occurrence and behavior of humpback whales near Naval Station Norfolk and within the Virginia Capes (VACAPES) Operating Area (OPAREA) by addressing the following questions:

- What age classes (juveniles, sub-adults, adults) are utilizing the waters within and adjacent to the mouth of Chesapeake Bay?
- Do humpback whales exhibit site fidelity over periods of days to years?
- Do humpback whales congregate in specific high-traffic and/or high-use U.S. Navy training areas?

- Do humpback whales spend significant time within or move through areas of U.S. Navy live-fire and mine neutralization exercise (MINEX) training?

Primary objectives of this project have included the following:

- Collect baseline occurrence data (location, sex, group size, behavior) of humpback whales (and other species of baleen whales, opportunistically)
- Obtain identification photographs of humpback whales for inclusion in regional and local catalogs
- Collect biopsy samples of humpback whales for sex determination, mitochondrial control region sequencing and microsatellite genotyping of tissue samples, and stable isotope analysis to assess foraging related to prey consumption
- Conduct satellite tagging to document seasonal humpback whale movement patterns in the nearshore waters off Virginia Beach, specifically whether the whales spend significant time in areas of high shipping traffic and/or areas of U.S. Navy training exercises

The humpback whale field season off Virginia Beach runs from approximately the end of October through March, typically concentrated between December and February, with a smaller number of sightings occurring outside this timeframe. Since this project's inception, there have been seven dedicated field seasons to address the above objectives (**Table 1**), starting with collection of basic baseline information using photo-ID, focal follow, and biopsy sampling methods. Subsequently the project has evolved to include deployment of satellite-linked telemetry and Digital Acoustic Recording Tags (DTAGs), collaboration with researchers from Duke University to examine behavioral response of humpbacks to large vessels ([Shearer et al. 2020](#)), photogrammetry using small Unmanned Aerial Systems (sUAS), and most recently an expansion into the mid-shelf region with addition of other baleen whale species including fin (*Balaenoptera physalus*) whales and North Atlantic right whales (*Eubalaena glacialis*). This report will therefore present details of both the nearshore and mid-shelf effort during the 2020/21 season.

Twenty-three surveys were completed during the 2020/21 field season. Thirteen of these surveys were considered nearshore surveys, nine surveys were defined as mid-shelf, and one extraneous survey was conducted off the Massachusetts coast as a follow-up to collect data from a tagged North Atlantic right whale. In total, there were 42 baleen whale sightings, including 29 of humpback whales composed of 42 individuals, 8 of fin whales composed of 13 individuals, and of 5 North Atlantic right whales composed of 8 individuals. Eleven satellite tags were deployed: seven tags were deployed on humpback whales, two tags were deployed on North Atlantic right whales, and two tags were deployed on fin whales. Six biopsy samples were collected from tagged humpback whales (**Table 1**).

Table 1. Summary of field season and objectives since initiation of project in 2014

| Season | Begin | End | Objectives | Biopsy samples | Tags deployed | Report |
|-----------------------------|-------------|-------------|---|----------------|-----------------|--|
| 1 – (2014/15 ^a) | 31-Dec-2014 | 15-May-2015 | Collect baseline information | 12 | N/A | Aschettino et al. 2015 ; Engelhaupt et al. 2015 |
| 2 – (2015/16) | 01-Dec-2015 | 09-May-2016 | Collect baseline information and deploy telemetry tags | 11 | 9 | Aschettino et al. 2016 |
| 3 – (2016/17) | 01-Nov-2016 | 21-Mar-2017 | Collect baseline information and deploy telemetry tags | 29 | 26 | Aschettino et al. 2017 |
| 4 – (2017/18) | 01-Oct-2017 | 01-Mar-2018 | Collect baseline information and deploy telemetry tags, expand spatial extent of coverage | 3 | 9 ^b | Aschettino et al. 2018 |
| 5 – (2018/19) | 12-Nov-2018 | 20-May-2019 | Collect baseline information and deploy telemetry tags, collaborate on behavioral response of humpbacks to large vessels (Shearer et al. 2019 ; Shearer et al. 2020) | 9 | 10 | Aschettino et al. 2019 Aschettino et al. 2020b |
| 6 – (2019/20) | 21-Dec-2019 | 27-Mar-2020 | Collect baseline information, deploy telemetry tags, photogrammetry using sUAS, collaborate on behavioral response of humpbacks to large vessels (Shearer et al. 2021) | 7 | 10 ^c | Aschettino et al. 2021 |
| 7 – (2020/21) | 19-Nov-2020 | 27-Mar-2021 | Collect baseline information, deploy telemetry and acoustic tags, photogrammetry using sUAS, expansion to mid-shelf region with addition of other baleen whale species, collaborate on behavioral response of humpbacks to large vessel project (Shearer et al. 2022) | 6 | 11 ^d | Current report |

Key: N/A = not applicable; sUAS = small Unmanned Aerial System

^a Additional humpback whale sighting information from coastal line-transect surveys for bottlenose dolphins (*Tursiops truncatus*) conducted from 2012 through 2015 (see [Engelhaupt et al. 2016](#)) was also incorporated into these analyses

^b Six tags on humpback whales, 3 tags on fin whales

^c Nine tags on humpback whales, 1 tag on a fin whale

^d Seven tags on humpback whales, 2 tags on fin whales, 2 tags on North Atlantic right whales

2. Methods

The study area for this project includes waters in and around the mouth of Chesapeake Bay as well as the W-50 MINEX region off Virginia Beach and, beginning the 2020/21 field season, the mid-shelf area (**Figure 1**). Two primary areas of interest in the nearshore study area are U.S. Navy training areas and commercial shipping lanes. Inbound and outbound shipping lanes are defined by the Traffic Separation Scheme. Initially, the “shipping lane study area” was defined by the Traffic Separation Scheme in the mouth of Chesapeake Bay (**Figure 1**). However, as tag locations showed movements outside the defined area but within shipping channels, the area was extended using multiple nautical charts and datasets, including using the following guidelines: the Traffic Separation Scheme; Coastal Maintained Channels in U.S. Waters (U.S. Army Corps of Engineers); and Shipping Fairways, Lanes, and Zones for U.S. Waters (National Oceanic and Atmospheric Administration). The U.S. Navy training areas include portions of the W-50 MINEX range. In the mid-shelf study area, the Dominion Wind Energy Lease Block, where there are currently two wind turbines installed, is also an area of interest (**Figure 1**).

Local availability of researchers allowed the survey effort to be flexible and take advantage of limited winter weather windows in order to maximize the ability to achieve project objectives. Optimal weather conditions included good visibility and a Beaufort sea state (BSS) of 3 or lower. Once a survey was underway, if BSS reached 4 or 5, or visibility was reduced to less than 1 nautical mile because of rain, fog, or snow, the survey was typically aborted, and the vessel returned to port. Efforts were coordinated with the W-50 MINEX range so the research vessel had clearance to operate when training was not being conducted. Because of frequent range closures and limited weather windows, it was not always possible to conduct surveys within the W-50 MINEX range.

The primary survey vessel for nearshore effort was the 8.8-meter (m) fiberglass hybrid-foam-collar boat *Whale Research* (**Figure 2**), owned and operated by HDR. Surveys using this vessel departed from Long Bay Pointe Marina, located in Lynnhaven Inlet, Virginia Beach. While working in the mid-shelf area, surveys used the 16-m fishing vessel *Top Notch* (**Figure 3**) or 16.5-m fishing vessel *Game On*. Surveys using these vessels departed from the Virginia Beach Fishing Center, located on Rudee Inlet.

The crew typically consisted of three or four qualified marine mammal scientists, with one also serving as the vessel operator when working from the *R/V Whale Research*. Once departed from the inlet, the vessel would transit to areas where baleen whales were previously seen or reported. If no whales were located in these areas, the vessel would expand the search into waters farther offshore, north, or south of the primary study area (see **Figure 1**). Sightings of non-target species in the survey area (i.e., bottlenose dolphins) were not always recorded and are not presented in this report.

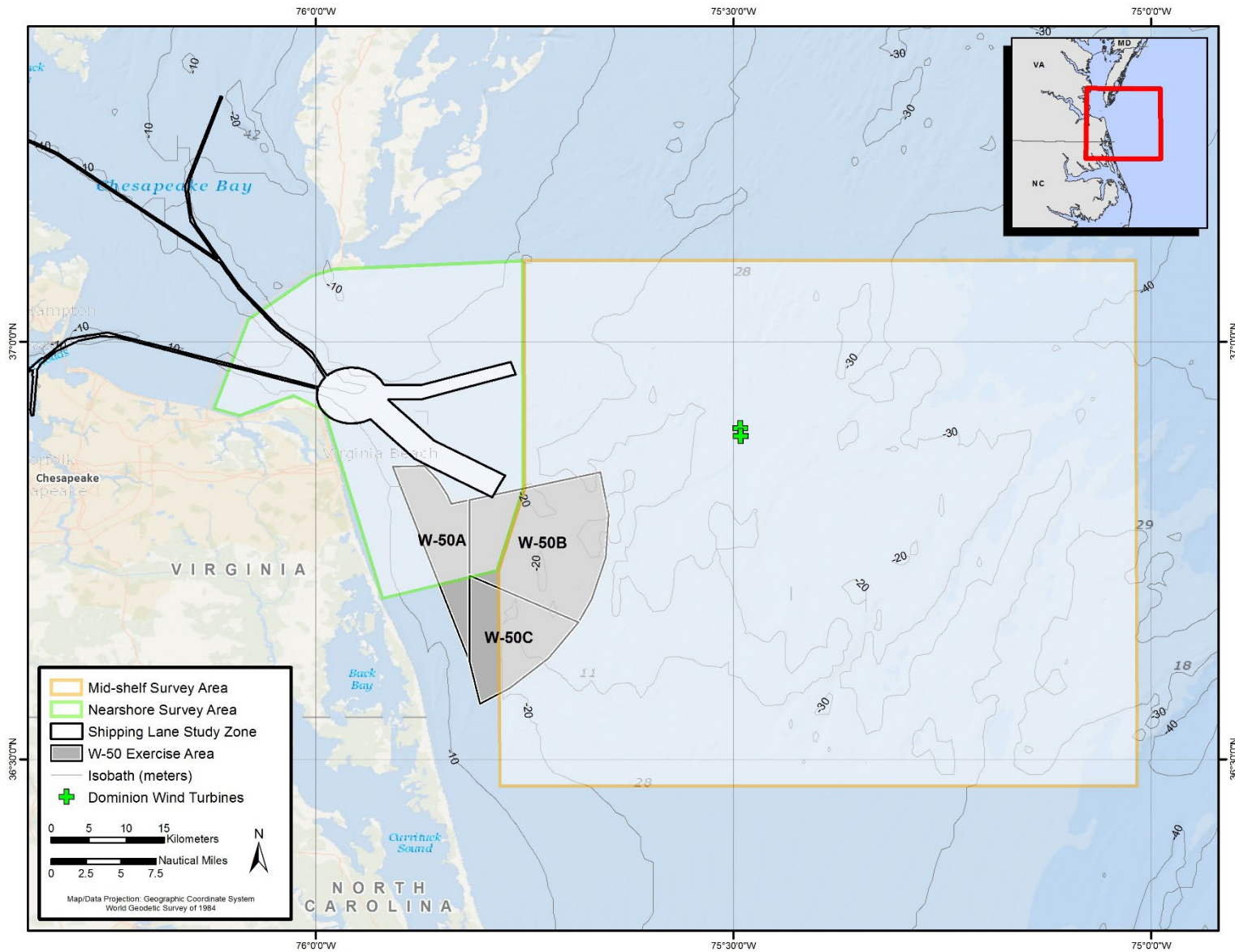


Figure 1. Map of the nearshore study area (outlined by the green boundary), which includes waters in and around the mouth of Chesapeake Bay as well as the W-50 MINEX region off Virginia Beach and the mid-shelf area (outlined by the orange boundary) and includes waters past the nearshore study area, including the Dominion Wind Turbines and farther out



Figure 2. Nearshore survey vessel *Whale Research* (photograph © Alexis Rabon, Rudee Flipper)



Figure 3. Mid-shore survey vessel *Top Notch* approaches a humpback whale to deploy a satellite tag and Digital Acoustic Recording Tag simultaneously (photograph © Kristin Rayfield, Rudee Flipper)

2.1 Photo-ID and Photogrammetry

Photographs of humpback, fin, right, and minke (*Balaenoptera acutorostrata*) whales were collected using a digital SLR camera (Canon 7D, 7D Mark II, or 1DX Mark II) with a zoom lens (Canon 100- to 400-millimeter). Photographs were post-processed using ACDSee (Versions 7–9) by cropping the best image of each individual whale’s dorsal fin (left and right) and tail flukes (when obtained).

Photographs were assembled into a project catalog managed by HDR in which each new whale was assigned a unique identifier using the naming mechanism “HDRVA,” followed by the two-letter abbreviation for the scientific name of the species, followed by a numerical sequence of three numbers (e.g., HDRVAMn001 or HDRVABa001). Each whale was then compared with the others. At the end of the 2014/15 field season, images of humpback whale flukes were submitted to Allied Whale for comparison to the North Atlantic Humpback Whale Catalog (NAHWC), and images of humpback whale dorsal fins and flukes were submitted to the Virginia Aquarium and Marine Science Center for comparison and integration with the Mid-Atlantic Humpback Whale Catalog (MAHWC). Images of fin whales were shared with Duke University as well as researchers from the Center for Coastal Studies in Provincetown, Massachusetts. Images of humpback whales from all subsequent seasons were submitted to the MAHWC (see [Malette and Barco 2019](#)) as well as compared with images from local whale watch operation Rudee Tours. Photographs of North Atlantic right whales were submitted to the New England Aquarium for incorporation into the North Atlantic Right Whale Catalog (<http://rwcatalog.neaq.org/>). Given the small living population of North Atlantic right whales (<500), the official catalog identifications were used rather than the conventional HDR naming mechanism for other species.

During the 2018/19 season, the use of a sUAS was incorporated into the field effort. The use of a sUAS was incorporated into the field effort beginning in the 2018/19 field season. A DJI Phantom 4 Pro V2.0 was used to collect morphometric data and assess overall body condition. In the field, live video was also used to assist the research team during tagging attempts to maximize successful deployments. Data were typically collected at flight heights between 15 and 30 m, depending on the behavior of the focal animal during the time of the encounter. The sUAS collected 4K ultra-high-definition video at 30 frames per second. Initial measurements were made from data using altitude values from the drone’s stock barometer, and some error is expected with this method. HDR used open-source software developed by researchers at Duke University (Torres and Bierlich 2020) to calculate lengths. Following the methodology described in [Dawson et al. \(2017\)](#), HDR retrofitted the DJI Phantom 4 Pro V2.0 and installed a custom Light Detection and Ranging (LiDAR) altimeter in 2020. This upgrade increases precision (to within 5 centimeters) and consistency of the sUAS altimetry measurements to minimize possible error in measured animal lengths. The photogrammetry techniques remain the same; however, this technique has greater accuracy than the stock DJI barometer.

2.1 Biopsy Sampling

Biopsy samples were collected, when possible, from whales of interest using either a crossbow or biopsy rifle. Finn Larsen designed crossbow bolts outfitted with 25-millimeter, ethanol sterilized, stainless steel tips were projected by a 68-kilogram pull Barnett recurve crossbow (Barnett Outdoors, LLC, Tarpon Springs, Florida). Alternatively, a Paxarms biopsy rifle (Paxarms New Zealand Ltd., Cheviot, New Zealand) fired 6- by 20-millimeter sterilized dart tips propelled by .22 caliber blank cartridges.

Samples were post-processed by sectioning the skin into three equal-sized pieces. One-third of the skin was placed in a cryovial and frozen (-40 degrees Celsius) for stable isotope analysis by Duke University, one-third was placed in a cryovial with a dimethylsulfate and sodium chloride

solution in preparation for analysis by University of Groningen, and one-third was frozen (-40 degrees Celsius) for archival storage for Southeast Fisheries Science Center. Blubber from the samples was wrapped in foil and frozen for archiving for Southeast Fisheries Science Center. Stable isotope analysis and gender determination was performed on a portion of samples at the end of the 2016/17 season (see [Waples 2017](#)). At the end of the 2018/19 field season, all humpback whale samples were sent to the University of Groningen for processing. In 2021, Bérubé and Palsbøll (provided in **Appendix B**) analyzed HDR's 63 humpback whale and 8 fin whale samples, collected since the project's inception, and matched them to the larger archive of more than 9,200 North Atlantic humpback whale and more than 1,700 fin whale samples.

2.2 Satellite Tagging

Satellite tagging has been a primary component of the project since the 2015/16 field season. Initially, Wildlife Computers (Redmond, Washington) Smart Position and Temperature (SPOT6) Argos satellite-linked tags in the Type-A ([Andrews et al. 2019](#)) Low Impact Minimally Percutaneous External-electronics Transmitter (LIMPET) configuration (Andrews et al. 2008) were used. SPLASH10-F-333 tags and SPLASH10-292B tags, which in addition to collecting location data also collect depth data, were incorporated into the project in subsequent seasons. The SPLASH10-F tags use Fastloc® GPS technology and were intended to be deployed during windows of opportunity during which researchers from Duke University might also be in the area and could potentially “double-tag” whales using DTAGs (see [Shearer et al. 2020, 2021](#)). Tags were remotely deployed using a DAN-INJECT JM25 pneumatic projector (www.dan-inject.com). The LIMPET tags use two 6.8-centimeter, surgical-grade, titanium darts with six backwards-facing petals to attach tags to the dorsal fin or just below the dorsal fin (**Figure 4**).

Given existing information on attachment durations of LIMPET tags on humpback whales, maximum tag attachment duration was expected to be on the order of days to weeks. Therefore, tags were programmed to maximize the number of transmissions and locations received during attachment rather than to extend battery life. Based on satellite availability in the area, tags were programmed to transmit for between 18 and 22 hours per day with an unlimited number of transmissions for SPOT6 tags and 800 to 1,200 transmissions per day for SPLASH10-292B and SPLASH10-F tags.

In order to constitute a “dive” for the Wildlife Computers generated behavior and time-series data outputs of the SPLASH10-292B and SPLASH10-F tags, a definition was established for humpback whales in which a submergence needed to be both deeper than 2 m and longer than 120 seconds in order to be classified as a dive. Locations of tagged individuals were approximated by the Argos system using the Kalman filtering location algorithm (Argos User's Manual © 2007–2015 CLS), and unrealistic locations (i.e., those on land) were manually removed using tools provided within Movebank (www.movebank.org).

Biopsy samples were collected from most tagged whales using the same protocol described previously, and conductivity, temperature, and depth (CTD) casts were typically taken following a tag deployment.



Figure 4. LIMPET SPLASH10-F tag on a humpback whale immediately after deployment

2.3 DTAG Tagging

In the 2020/21 field season, HDR added a suction-cup tagging component to the project with the goal of deploying DTAGs (Johnson and Tyack 2003) on baleen whales in the mid-shelf and/or MINEX region of the study area. Version 3 DTAGs were deployed using a hand-held carbon fiber pole. DTAGs are equipped with hydrophones and pressure sensors as well as a three-axis accelerometer and magnetometer. The audio sampling rate was set to 120 kilohertz for baleen whales. Programmed release time was set according to conditions and logistics to facilitate best opportunity for tag retrieval. Each tag also contained a very-high frequency (VHF) transmitter that, following release, allows recovery using Communications Specialists, Inc. R-1000 VHF receivers with hand-held Yagi antennas (www.com-spec.com) to direct the vessel to the tag location after release from the animal.

Tag calibration and data visualization following recovery of the tag was completed using a suite of tools found on animaltags.org using MATLAB R2021b (www.mathworks.com).

3. Results

3.1 Nearshore and Mid-shelf Survey Results: 2020/21 Field Season

Survey efforts typically begin once humpbacks sightings are reported by the local whale watch-operations and other mariners. For the 2020/21 season, this first survey took place on 19 November 2020, and the last survey occurred on 27 March 2021. In total, we conducted 23 surveys, covering 3,213 kilometers (km) of trackline with more than 193 hours of effort (**Table 2, Figure 5**). Thirteen of these surveys were considered nearshore (**Figure 6**), nine surveys were defined as mid-shelf (**Figure 7**), and one additional survey was conducted off Massachusetts as a follow-up to collect data from a satellite-tagged North Atlantic right whale (**Figure 8**). Excluding the Massachusetts survey, there were 40 baleen whale sightings, including 28 humpback whale sightings composed of 41 individuals, 8 fin whale sightings composed of 13 individuals, and 4 North Atlantic right whale sightings composed of 7 individuals (**Figure 5, Table 2, and Table A-1 in Appendix A**).

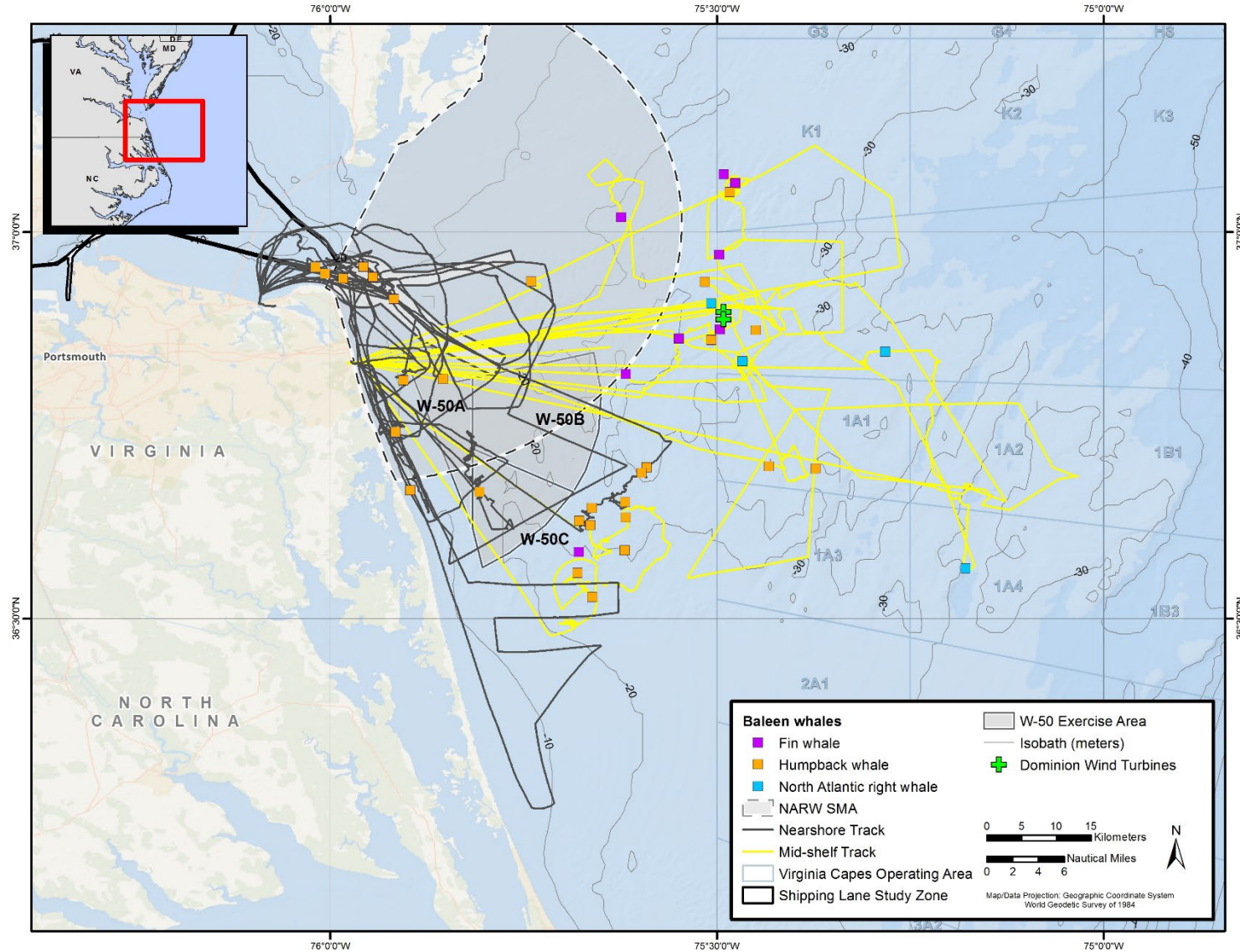


Figure 5. Nearshore (gray) and mid-shelf (yellow) survey tracks, with locations of all humpback (n=28), fin (n=8), and North Atlantic right (n=4) whale sightings for the 2020/21 field season

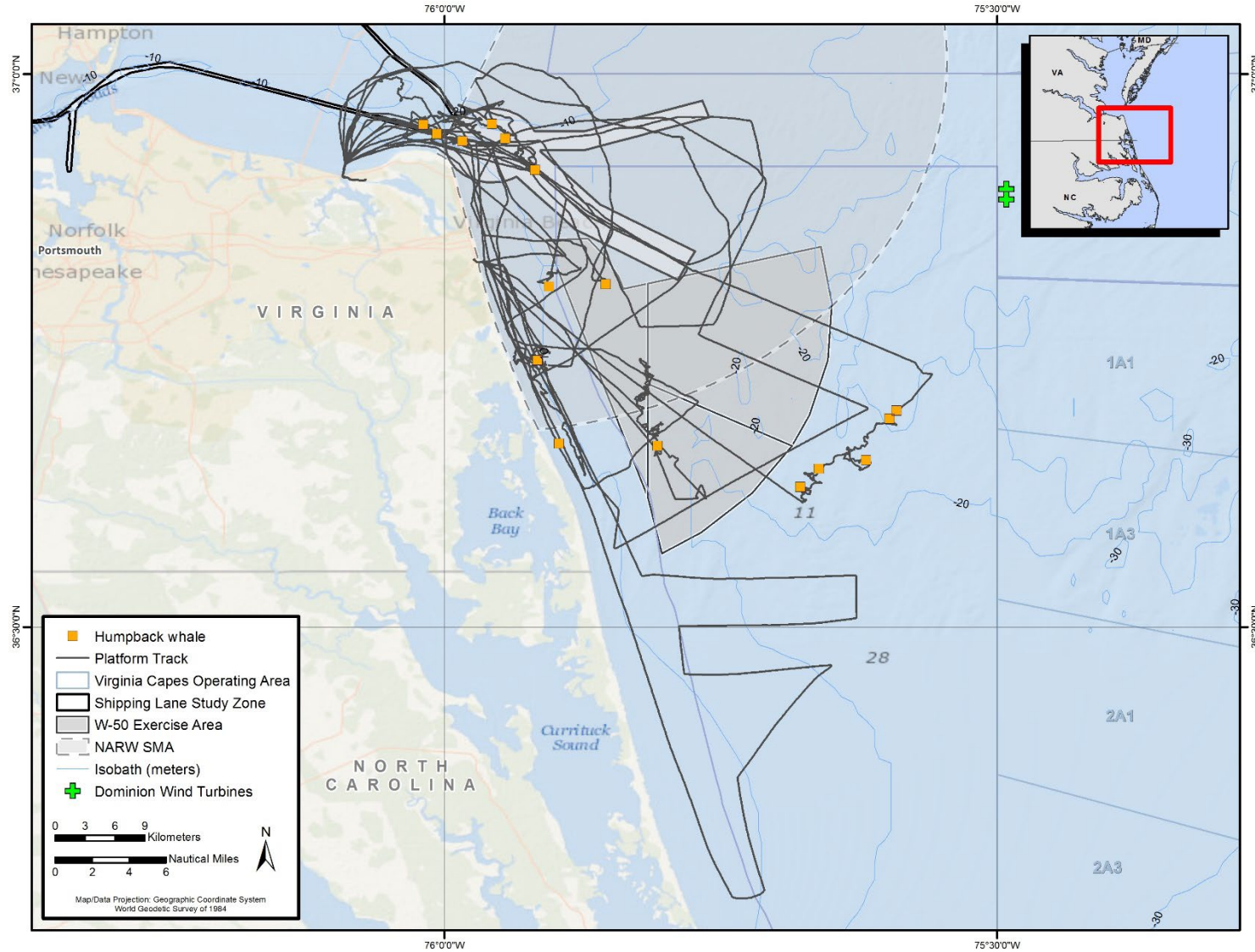


Figure 6. Nearshore survey tracks (gray), with locations of all humpback whale sightings (n=16) for the 2020/21 field season

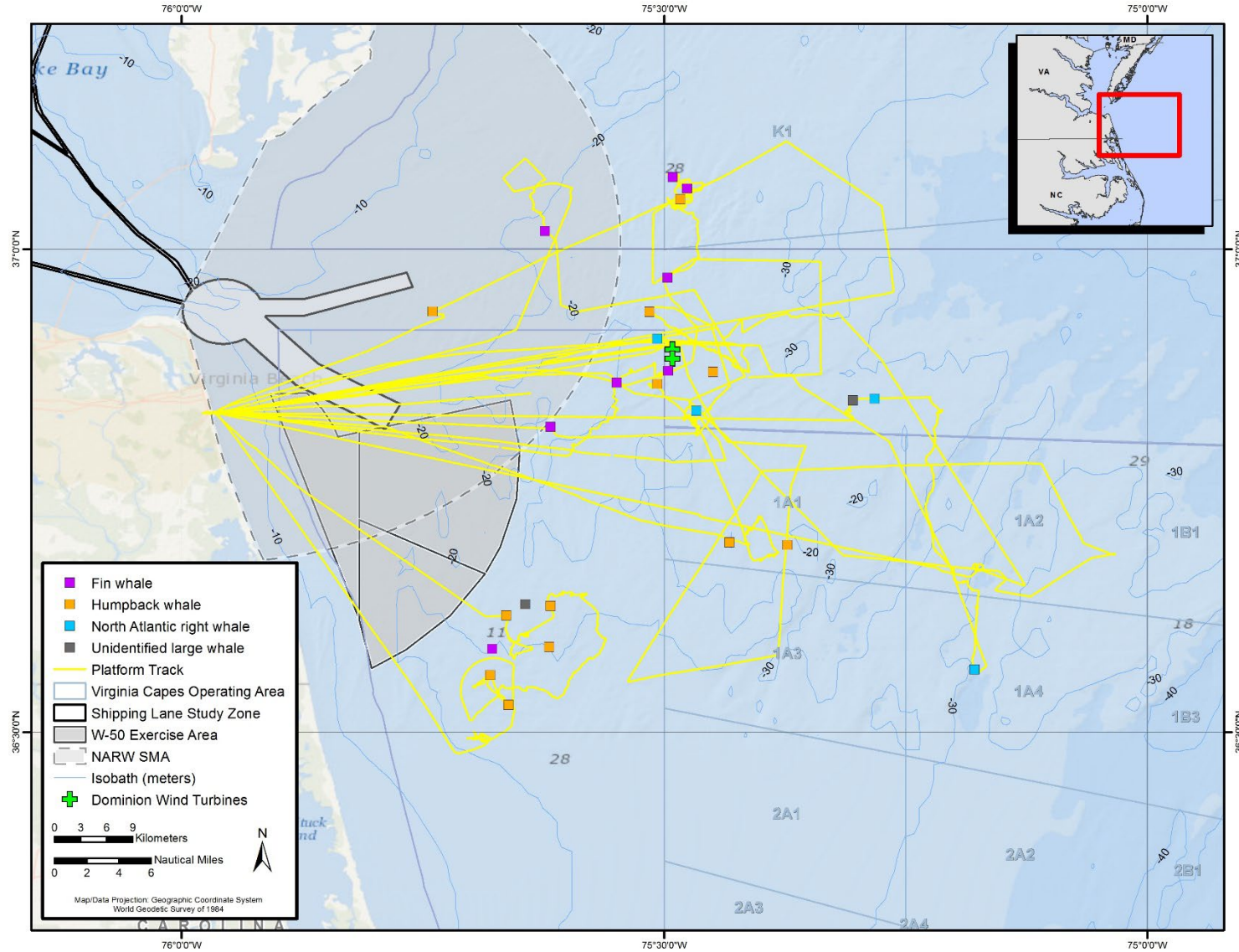


Figure 7. Mid-shelf survey tracks (yellow), with locations of all humpback (n=12), fin (n=8), and North Atlantic right (n=4) whale sightings for the 2020/21 field season

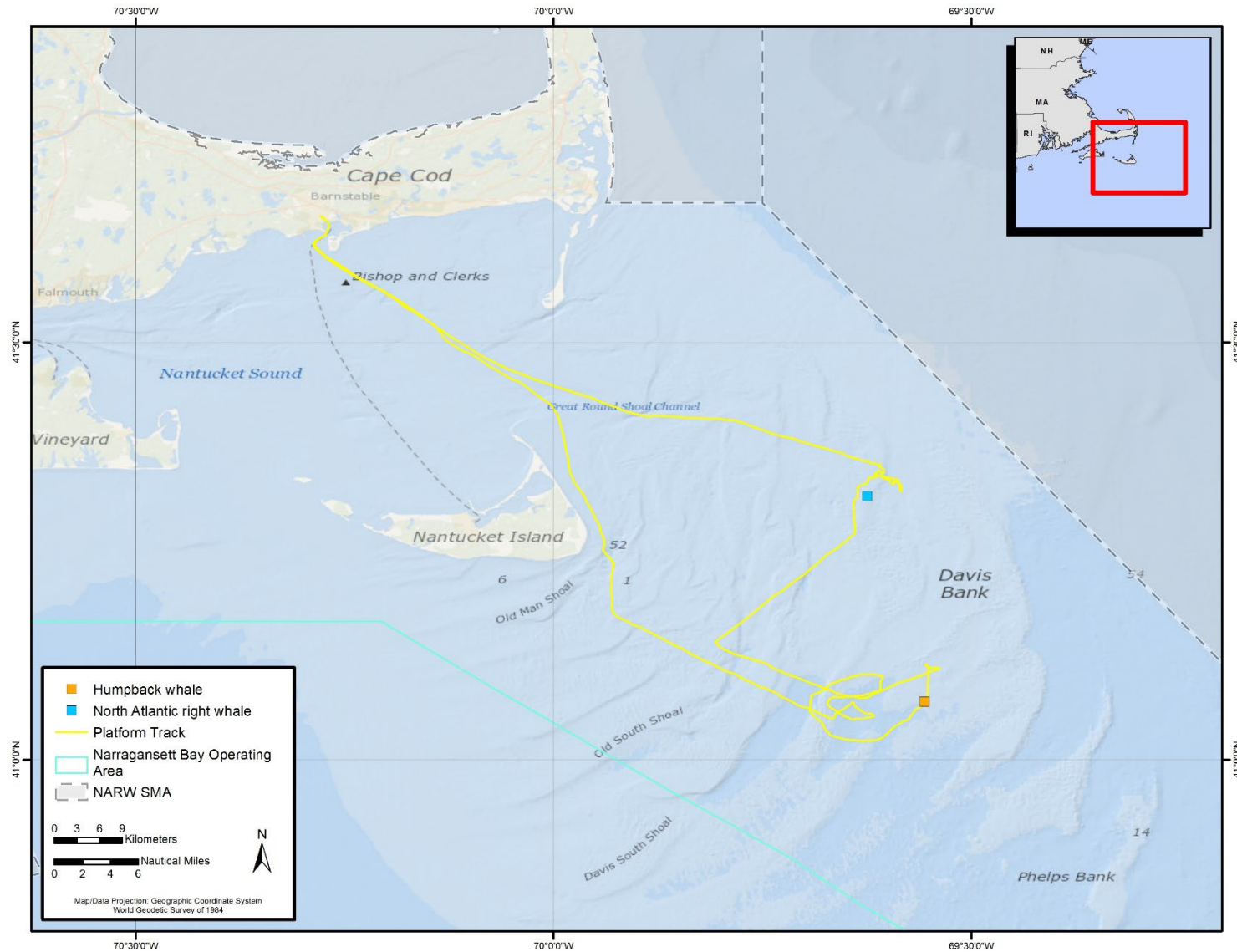


Figure 8. Survey tracks (yellow) off the Massachusetts coast, with locations of humpback (n=1) and North Atlantic right (n=1) whale sightings for the 2020/21 field season

Table 2. Summary of nearshore and mid-shelf survey efforts off Virginia Beach, Virginia, for the 2020/21 field season

| Date | Survey Type | Survey Time (min) | Distance surveyed (km) | # Sightings Mn | # Individual Mn | # Sightings Bp | # Individual Bp | # Sightings Eg | # Individual Eg |
|--------------|----------------------------|-------------------|------------------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|
| 19-Nov-20 | Nearshore | 579 | 72 | 2 | 3 | 0 | 0 | 0 | 0 |
| 21-Nov-20 | Nearshore | 338 | 127 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25-Nov-20 | Nearshore | 409 | 129 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-Dec-20 | Nearshore | 484 | 112 | 1 | 1 | 0 | 0 | 0 | 0 |
| 19-Dec-20 | Nearshore | 501 | 111 | 1 | 1 | 0 | 0 | 0 | 0 |
| 21-Dec-20 | Nearshore | 161 | 57 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27-Dec-20 | Nearshore | 528 | 137 | 5 | 12 | 0 | 0 | 0 | 0 |
| 5-Jan-21 | Nearshore | 417 | 111 | 1 | 1 | 0 | 0 | 0 | 0 |
| 11-Jan-21 | Mid-shelf | 633 | 186 | 3 | 6 | 2 | 3 | 0 | 0 |
| 13-Jan-21 | Mid-shelf | 653 | 168 | 2 | 4 | 0 | 0 | 1 | 3 |
| 14-Jan-21 | Mid-shelf | 485 | 138 | 0 | 0 | 2 | 3 | 0 | 0 |
| 24-Jan-21 | Nearshore | 316 | 72 | 3 | 3 | 0 | 0 | 0 | 0 |
| 25-Jan-21 | Mid-shelf | 579 | 163 | 2 | 2 | 3 | 5 | 0 | 0 |
| 30-Jan-21 | Nearshore | 225 | 85 | 1 | 1 | 0 | 0 | 0 | 0 |
| 4-Feb-21 | Nearshore | 363 | 108 | 1 | 1 | 0 | 0 | 0 | 0 |
| 6-Feb-21 | Mid-shelf | 894 | 176 | 5 | 5 | 1 | 2 | 0 | 0 |
| 8-Feb-21 | Nearshore | 453 | 81 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21-Feb-21 | Nearshore | 315 | 120 | 1 | 1 | 0 | 0 | 0 | 0 |
| 3-Mar-21 | Mid-shelf | 852 | 207 | 0 | 0 | 0 | 0 | 2 | 3 |
| 8-Mar-21 | Mid-shelf | 854 | 300 | 0 | 0 | 0 | 0 | 1 | 1 |
| 9-Mar-21 | Mid-shelf | 412 | 181 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-Mar-21 | Massachusetts ^a | 626 | 199 | 1 | 1 | 0 | 0 | 1 | 1 |
| 27-Mar-21 | Mid-shelf | 508 | 173 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | -- | 11,585 | 3,213 | 29 | 42 | 8 | 13 | 5 | 8 |

Key: Bp = *Balaenoptera physalus* (fin whale); Eg = *Eubalaena glacialis* (right whale); km = kilometer(s); min = minute(s); Mn = *Megaptera novaeangliae* (humpback whale)

^a Survey conducted off the Massachusetts coast as a follow-up to collect data from a tagged North Atlantic right whale

3.2 Photo-ID and Photogrammetry Results

The 28 sightings of humpback whales during the 2020/21 season included 41 total individuals and resulted in 26 unique humpback whales identified using dorsal fin and fluke images for the season. An additional six humpback whales were also seen during the Outer Continental Shelf Cetacean Study surveys in April and June 2021 and are included in catalog results ([Engelhaupt et al. 2022](#)) (**Figure 9, Table A-1 in Appendix A**). Of the 32 unique humpback whales seen during the 2020/21 season, 16 (50.0 percent) were categorized as juveniles based on their estimated size; 10 (31.3 percent) were classified as sub-adults/adults; 5 (15.6) were classified as adults; and for the first time since the project’s inception, a single calf was observed (3.1 percent). Six (18.8 percent) of the 32 individuals were re-sights to our catalog; 2 individuals had not been seen since the 2014/15 season (HDRVAMn003 and HDRVAMn025), 1 individual had not been seen since an out-of-season observation in July 2018 (HDRVAMn123), and the other 3 individuals had been seen during the previous 2019/20 season (HDRVAMn172, HDRVAMn174, and HDRVAMn179). The remaining 26 whales were new individuals added to our growing catalog. To date, the catalog includes 208 unique humpback whales (inclusive of identifications added from the Outer Continental Shelf Cetacean Study [Engelhaupt et al. 2022]) (**Figure 9, Table A-1 in Appendix A**). Only four of the 32 (12.5 percent) humpback whales were seen on more than one occasion during the 2020/21 field season, which is a lower proportion than in all previous seasons (42.9 percent during 2019/20, 44.7 percent during 2018/19, 21.9 percent during 2017/18, and 69.5 percent during 2016/17).

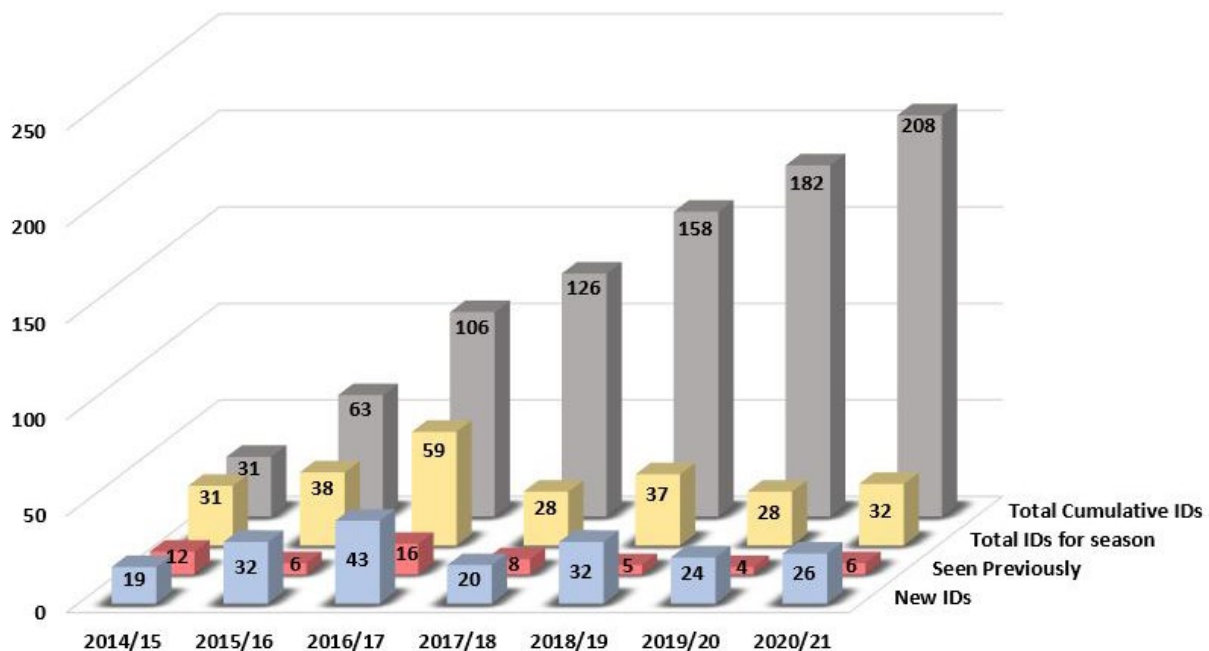


Figure 9. Humpback whale identifications over seven seasons in the Virginia study area (yellow bars = total number of IDs for season; red bars = number of those IDs seen in previous seasons; blue bars = number of new IDs added to catalog; gray bars = total number of cumulative unique IDs)

Evidence of human interaction, either presumed line-entanglement scars or propeller scars, was apparent on at least 19 of the 207 (9.2 percent) cataloged humpback whales (**Table A-1** in **Appendix A**).

Drone video was collected on numerous humpback whales, and the lengths of 30 individuals were calculated (data from December 2018 through June 2020) (**Table 3, Figure 10**). Each of these whales has a unique identifier in the catalog and had previously been assigned an age-class based on subjective size assessments from the research vessel. The length of the HDR research vessel was often used in making these subjective assessments. For example, individuals that were estimated to be approximately the length of the vessel (8.8 m) or smaller were typically classified as juveniles, whereas individuals that appeared longer than the research vessel were typically classified as sub-adults or adults. From the calculations based on the sUAS video, the measured humpback whales ranged in size from 6.8 to 14.0 m in total length, with a mean length of 9.5 m and a median length of 9.6 m. All whales that measured 10.5 m or greater (n=9) had been classified as sub-adults or adults in the field. All but two of the whales that measured 8.8 m or less (n=13) had been classified as juveniles in the field. Whales that ranged from 9.0 to 10.1 m (n=15) were classified as either juvenile (n=8), juvenile/sub-adult (n=1), or sub-adult (n=6) in the field, highlighting the difficulty of assigning an age-class for whales that may be of intermediate length and/or transitioning from one age-class to another.

Table 3. Overall lengths of all humpback whales measured to-date using drone photogrammetry

| Humpback Whale ID | Overall Length Based on Photogrammetry (m) | Total Unique Measurement Days | Age-class Assigned Based on Initial Visual Assessment |
|-------------------|--|-------------------------------|---|
| HDRVAMn166 | 6.8 | 2 | Juvenile |
| HDRVAMn163 | 7.0 | 1 | Juvenile |
| HDRVAMn165 | 7.3 | 1 | Juvenile |
| HDRVAMn152 | 7.4 | 2 | Juvenile |
| HDRVAMn189 | 7.5 | 1 | Juvenile |
| HDRVAMn177 | 7.6 | 3 | Juvenile |
| HDRVAMn175 | 7.9 | 1 | Juvenile |
| HDRVAMn183 | 8.2 | 1 | Sub-adult |
| HDRVAMn093 | 8.2 | 2 | Juvenile |
| HDRVAMn164 | 8.2 | 1 | Juvenile |
| HDRVAMn187 | 8.5 | 2 | Sub-adult |
| HDRVAMn173 | 8.7 | 3 | Juvenile |
| HDRVAMn147 | 8.8 | 1 | Juvenile |
| HDRVAMn170 | 9.0 | 1 | Sub-adult |
| HDRVAMn186 | 9.1 | 4 | Sub-adult |
| HDRVAMn208 | 9.5 | 1 | Juvenile |
| HDRVAMn184 | 9.6 | 1 | Juvenile |
| HDRVAMn196 | 9.6 | 1 | Sub-adult |
| HDRVAMn153 | 9.6 | 1 | Juvenile |
| HDRVAMn174 | 9.7 | 3 | Juvenile/Sub-adult ^a |
| HDRVAMn193 | 9.7 | 2 | Juvenile |
| HDRVAMn191 | 9.8 | 1 | Juvenile |

| Humpback Whale ID | Overall Length Based on Photogrammetry (m) | Total Unique Measurement Days | Age-class Assigned Based on Initial Visual Assessment |
|-------------------|--|-------------------------------|---|
| HDRVAMn021 | 10.0 | 2 | Sub-adult |
| HDRVAMn012 | 10.0 | 2 | Sub-adult |
| HDRVAMn199 | 10.0 | 1 | Juvenile |
| HDRVAMn149 | 10.1 | 1 | Juvenile |
| HDRVAMn190 | 10.1 | 1 | Juvenile |
| HDRVAMn215 | 10.1 | 1 | Sub-adult ^b |
| HDRVAMn200 | 10.5 | 1 | Sub-adult ^b |
| HDRVAMn181 | 10.8 | 1 | Sub-adult or adult |
| HDRVAMn097 | 10.8 | 1 | Sub-adult or adult |
| HDRVAMn172 | 11.1 | 5 | Sub-adult or adult |
| HDRVAMn132 | 11.3 | 3 | Sub-adult or adult |
| HDRVAMn223 | 11.4 | 1 | Sub-adult or adult |
| HDRVAMn202 | 13.0 | 1 | Sub-adult or adult |
| HDRVAMn003 | 13.9 | 2 | Adult |
| HDRVAMn197 | 14.0 | 1 | Sub-adult or adult |

Key: m = meter(s)

^a Assessed as two different age classes within same season

^b Most recent assessment; assigned a different age class in previous years



Figure 10. Total length measurement being taken in MorphoMetriX using a still video grab from sUAS-collected data for humpback whale HDRVAMn208

3.3 Biopsy Results

Six biopsy samples were collected from humpback whales during the 2020/21 season (**Table A-1** in **Appendix A**) and are awaiting analyses along with samples collected during the 2019/20 field season. Thirty-one samples (29 humpback and 2 fin whale samples) from 2014 to 2016 were processed for stable-isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The stable-isotope signatures for all samples were comparable to those reported for other regions of the North Atlantic ([Waples 2017](#)). There were significant differences in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the humpback and fin whales in the study area. The humpback whales were slightly more depleted in carbon and had significantly higher $\delta^{15}\text{N}$ signatures than the fin whales. The humpback whales had a mean $\delta^{15}\text{N}$ value of 14.6 (standard error [SE]=0.9) compared to the fin whales' value of 10.5 (SE=0.0).

Given a difference in $\delta^{15}\text{N}$ values between the two species, it is likely that the humpback whales in this area are feeding at a higher trophic level than the fin whales ([Waples 2017](#)). Genetic analyses identified 14 female and 15 male humpback whales from these samples. There were no significant differences in $\delta^{13}\text{C}$ values between male and female humpback whales, but females did have significantly lower $\delta^{15}\text{N}$ values than males, indicating that the diets of the two sexes may differ in this area ([Waples 2017](#)).

These biopsy samples were provided to the University of Groningen for genetic analysis and integration into a larger North Atlantic humpback whale population study. In addition to the 29 humpback and 2 fin whale samples collected between 2014 and 2016, an additional 34 humpback and 6 fin whale samples collected between 2016 and 2019 (totals n=63 humpbacks; n=8 fin whales) were also sent to the University of Groningen. Gender results with the larger sample size also show roughly equal sex ratios of humpback whales (32 males and 31 females) and a skewed gender ratio of 6 to 1 (males versus female) for fin whales (Bérubé and Palsbøll 2022; see **Appendix B**). Genetic matching to the larger NAHWC, which contains more than 9,200 individuals, showed that 18 HDR samples matched to samples collected elsewhere along the eastern U.S. There were no duplicate humpback whale samples in the HDR dataset. All samples matched 100 percent on all loci genotyped in both samples in each pair (i.e., no mismatching genotypes were detected). A single pair of duplicate samples was detected between two HDR fin whale samples; however, none of the HDR fin whale samples matched to the 1,789 samples contained in the North Atlantic fin whale genetic archive (Bérubé and Palsbøll 2022; see **Appendix B**).

3.4 Satellite-Tagging Results

In total, 11 Argos-linked satellite tags were deployed on baleen whales during the 2020/21 field season. Seven tags were deployed on humpback whales: 3 SPOT-6, 2 SPLASH10-292, and 2 SPLASH10-F (**Figures 11** through **20**, **Table 4**). Two SPLASH10-F tags were deployed on fin whales (**Figures 21** through **23**, **Table 5**) and 2 SPLASH10-F tags were deployed on North Atlantic right whales (**Figures 24** through **26**, **Table 6**). Humpback tags transmitted for between 6.7 and 45.9 days (mean = 16.1 days), fin whale tags transmitted for between 1.3 and 8.1 days (mean = 4.7 days) and North Atlantic right whale tags transmitted for between 1.8 and 16.7 days (mean = 9.3 days).

Whales tagged during the 2020/21 field season showed varied movement patterns, with some exclusively spending time in the primary study area and others moving outside the study area and farther offshore or to the north or south (**Figures 12 through 18**). Humpback HDRVAMn202, which was tagged in the mid-shelf region, traveled more than 1,300 km in the 11-day deployment, moving primarily southward in an apparent migration to breeding grounds (**Figure 16**). Humpback HDVAMn174, whose tag lasted for the longest deployment of any humpback whale to date, spent approximately 40 days in the primary nearshore study area before moving south (**Figure 15**). Humpback HDRVAMn174 was satellited tagged for the second time during the 2020/21 season. A comparison of tracklines from the previous tag deployment in January 2020 is shown in **Figure 19**. During the first tag deployment, HDRVAMn174 stayed within the primary nearshore study zone, spending time in the shipping channels as well as the W-50 MINEX region. During the second tag deployment, HDRVAMn174 spent time in VACAPES OPAREAs and between the Virginia and North Carolina border. Tag deployments were 39 kms apart.

Figure 20 shows a zoomed in view of all humpback whale tag locations during the 2020/21 field season, focused on the nearshore study zone. As in previous study years, a high number of locations occurred in and around the shipping channels and at the mouth of Chesapeake Bay. Unlike in previous seasons (e.g., [Aschettino et al. 2018, 2021](#)), Argos locations west of the Chesapeake Bay Bridge Tunnel were not common. Although the fin whale tag durations were relatively brief, both satellite-tagged individuals stayed close to their initial tagging locations, and 90 percent of their filtered Argos locations occurred within the VACAPES OPAREA (**Figure 19**). The two yearling North Atlantic right whales showed different movement patterns, with one individual moving southward over the short tag duration and the other traveling more than 1,600 km northward along the coast, eventually ending up off the Massachusetts coast, inside Cape Cod Bay, with the last location occurring outside of Boston Harbor (**Figure 22**).

Table 4. Satellite-tag deployments on humpback whales during the 2020/2021 field season

| Animal ID | Estimated Age Class | Tag Type | Argos ID | Deployment Latitude (°N) | Deployment Longitude (°W) | Deployment Date | Last Transmission Date | Tag Duration (Days) |
|------------|---------------------|--------------|----------|--------------------------|---------------------------|-----------------|------------------------|---------------------|
| HDRVAMn193 | Juvenile | SPOT-6 | 174075 | 36.9193 | -75.9204 | 13-Dec-20 | 28-Dec-20 | 15.1 |
| HDRVAMn172 | Sub-Adult/Adult | SPLASH10-292 | 183913 | 36.6309 | -75.6602 | 27-Dec-20 | 8-Jan-21 | 11.3 |
| HDRVAMn196 | Sub-Adult/Adult | SPOT-6 | 94797 | 36.6208 | -75.6757 | 27-Dec-20 | 9-Jan-21 | 13.1 |
| HDRVAMn174 | Juvenile | SPOT-6 | 174746 | 36.9580 | -76.0276 | 05-Jan-21 | 20-Feb-21 | 45.9 |
| HDRVAMn202 | Adult | SPLASH10-292 | 179198 | 36.9192 | -75.6269 | 13-Jan-21 | 24-Jan-21 | 11.1 |
| HDRVAMn204 | Juvenile | SPLASH10-F | 183929 | 36.9390 | -76.0872 | 24-Jan-21 | 31-Jan-21 | 6.7 |
| HDRVAMn003 | Adult | SPLASH10-F | 208686 | 37.0618 | -75.4623 | 25-Jan-21 | 04-Feb-21 | 9.7 |

Key: °N = degrees North; °W = degrees West

Table 5. Satellite-tag deployments on fin whales during the 2020/2021 field season

| Animal ID | Estimated Age Class | Tag Type | Argos ID | Deployment Latitude (°N) | Deployment Longitude (°W) | Deployment Date | Last Transmission Date | Tag Duration (Days) |
|------------|---------------------|------------|----------|--------------------------|---------------------------|-----------------|------------------------|---------------------|
| HDRVABp030 | Adult | SPLASH10-F | 178209 | 36.7892 | -75.5578 | 11-Jan-21 | 13-Jan-21 | 1.3 |
| HDRVABp097 | Adult | SPLASH10-F | 183932 | 36.5948 | -75.6498 | 06-Feb-21 | 14-Feb-21 | 8.1 |

Key: °N = degrees North; °W = degrees West

Table 6. Satellite-tag deployments on North Atlantic right whales during the 2020/2021 field season

| Animal ID | Estimated Age Class | Tag Type | Argos ID | Deployment Latitude (°N) | Deployment Longitude (°W) | Deployment Date | Last Transmission Date | Tag Duration (Days) |
|--------------|---------------------|------------|----------|--------------------------|---------------------------|-----------------|------------------------|---------------------|
| Calf of 2642 | Yearling | SPLASH10-F | 183933 | 36.6546 | -75.1956 | 03-Mar-21 | 20-Mar-21 | 16.7 |
| Calf of 1612 | Yearling | SPLASH10-F | 183930 | 36.9061 | -75.4993 | 08-Mar-21 | 10-Mar-21 | 1.8 |

Key: °N = degrees North; °W = degrees West

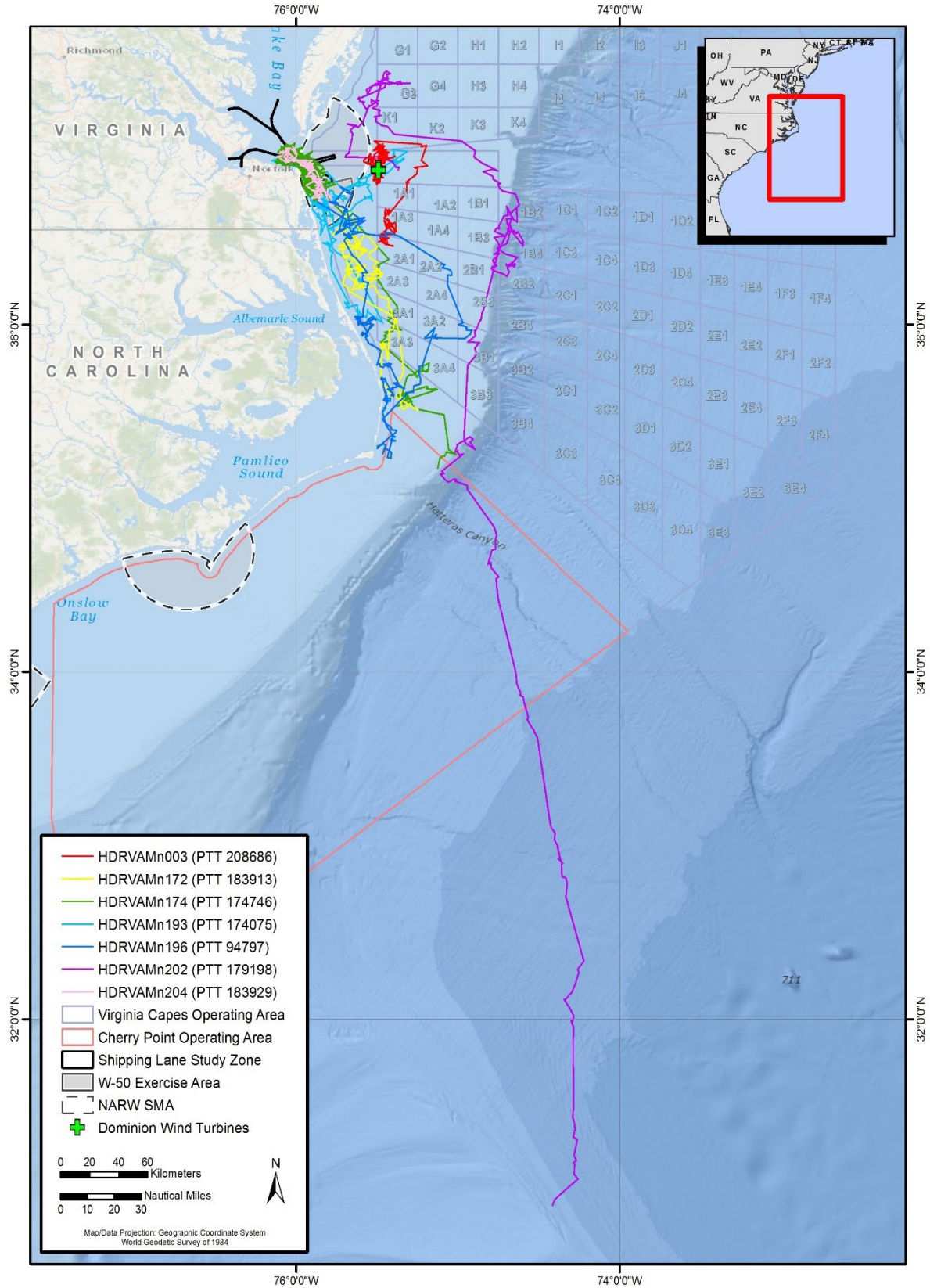


Figure 11. Argos tracks for all humpback whales tagged (n=7) during the 2020/21 field season

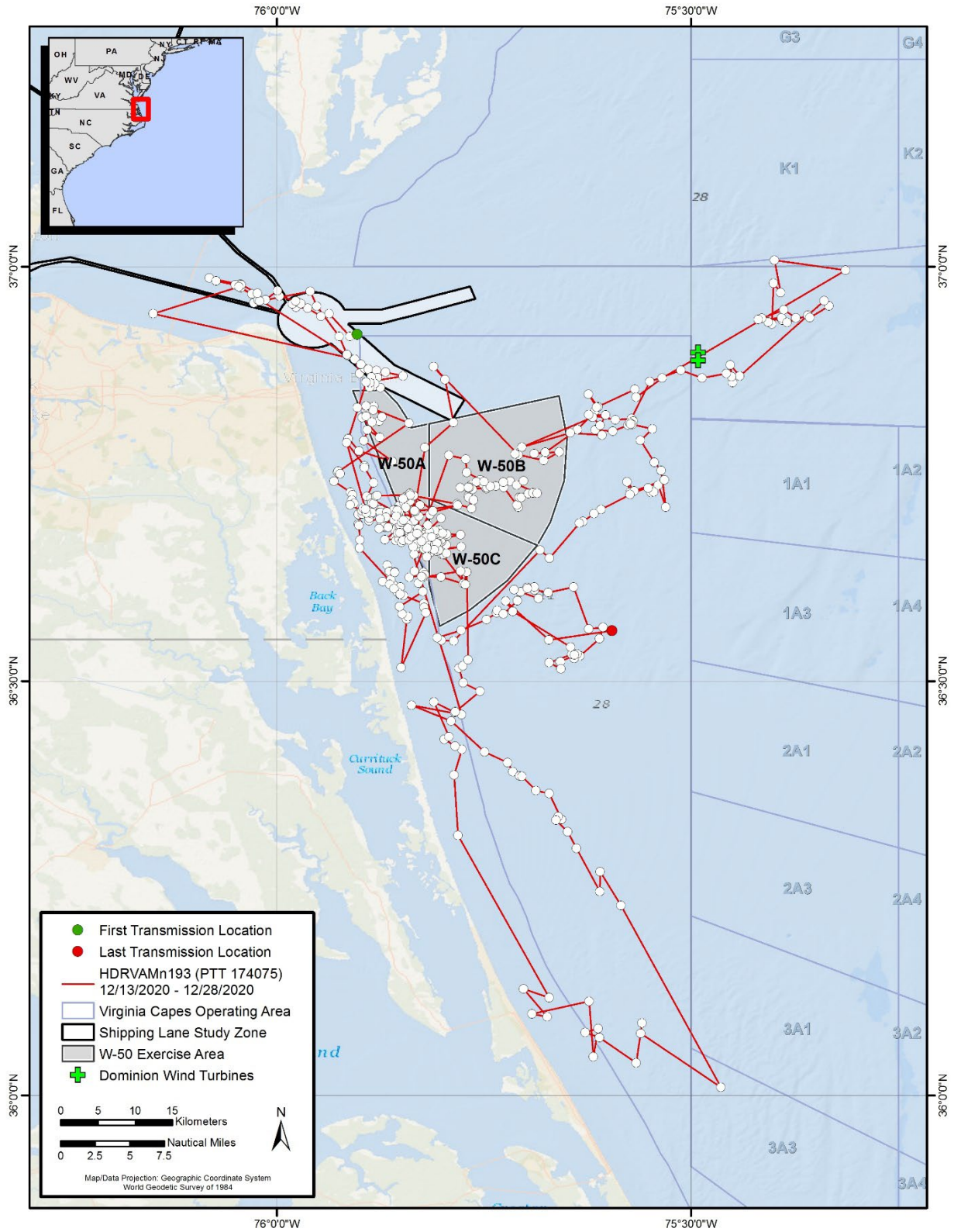


Figure 12. Filtered locations (white dots) and trackline (red line) of humpback whale HDRVAMn193, tagged on 13 December 2020 over 15.1 days of tag-attachment duration

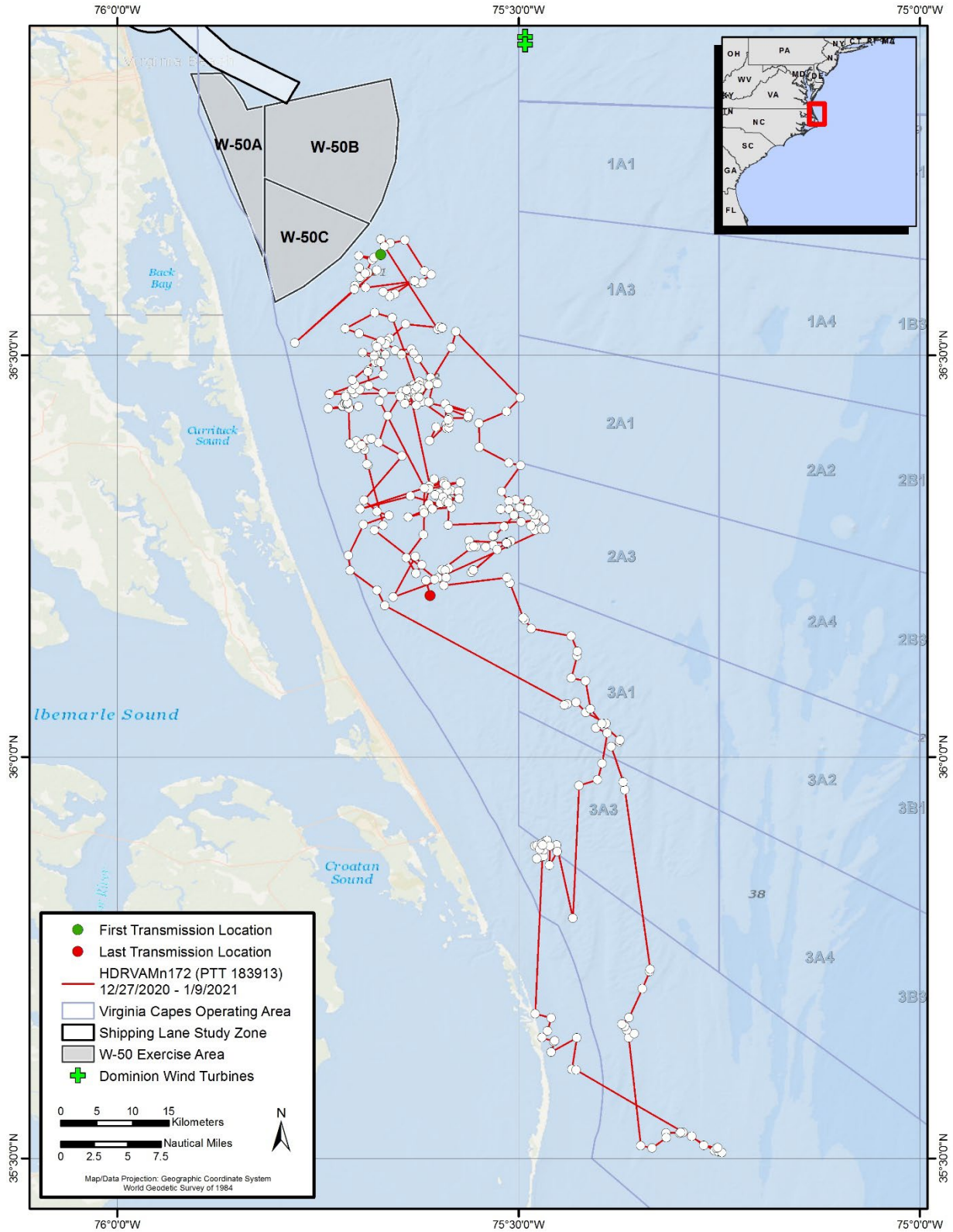


Figure 13. Filtered locations (white dots) and trackline (red line) of humpback whale HDRVAMn172, tagged on 27 December 2020 over 11.3 days of tag-attachment duration

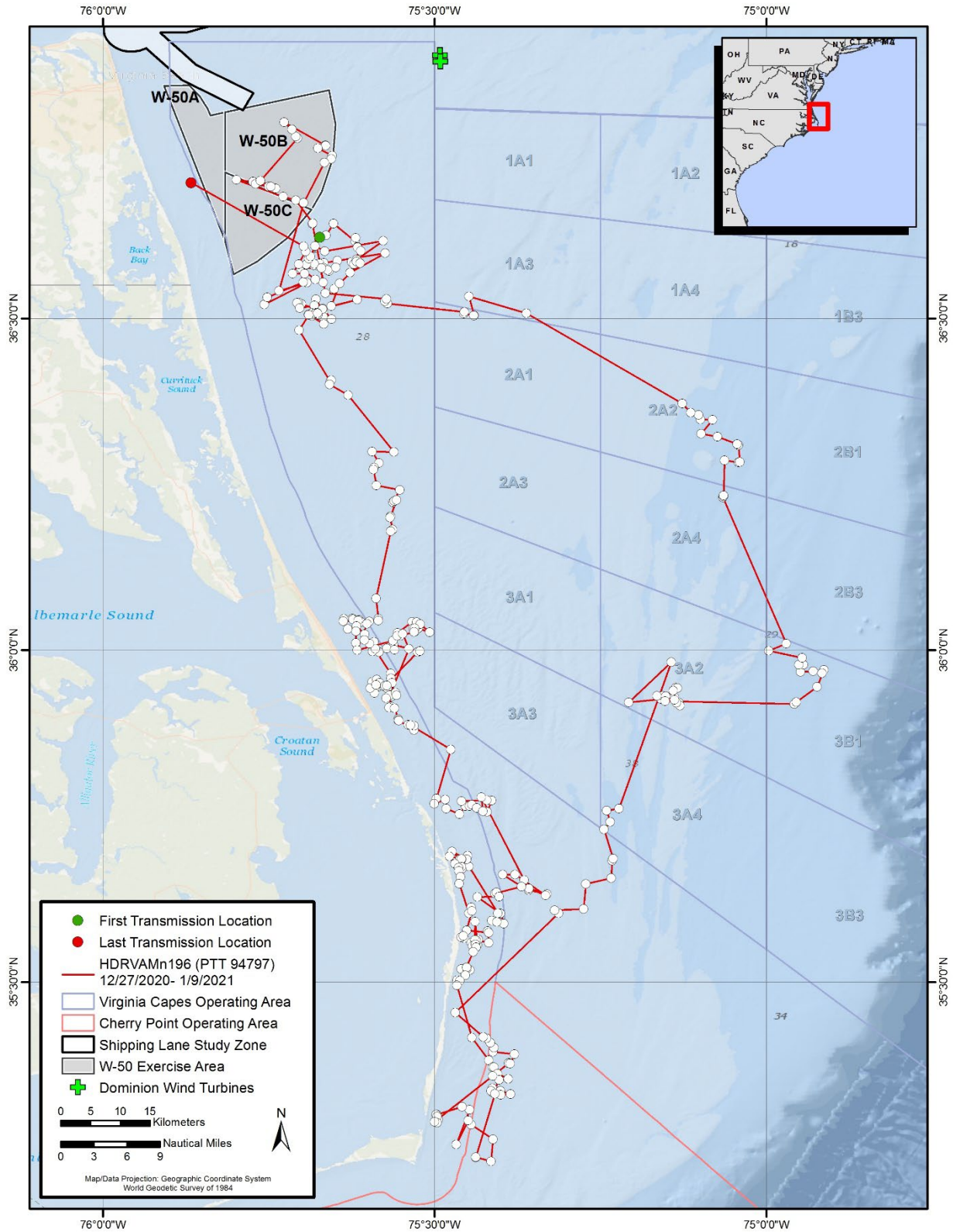


Figure 14. Filtered locations (white dots) and trackline (red line) of humpback whale HDRVAMn196, tagged on 27 December 2020 over 13.1 days of tag-attachment duration

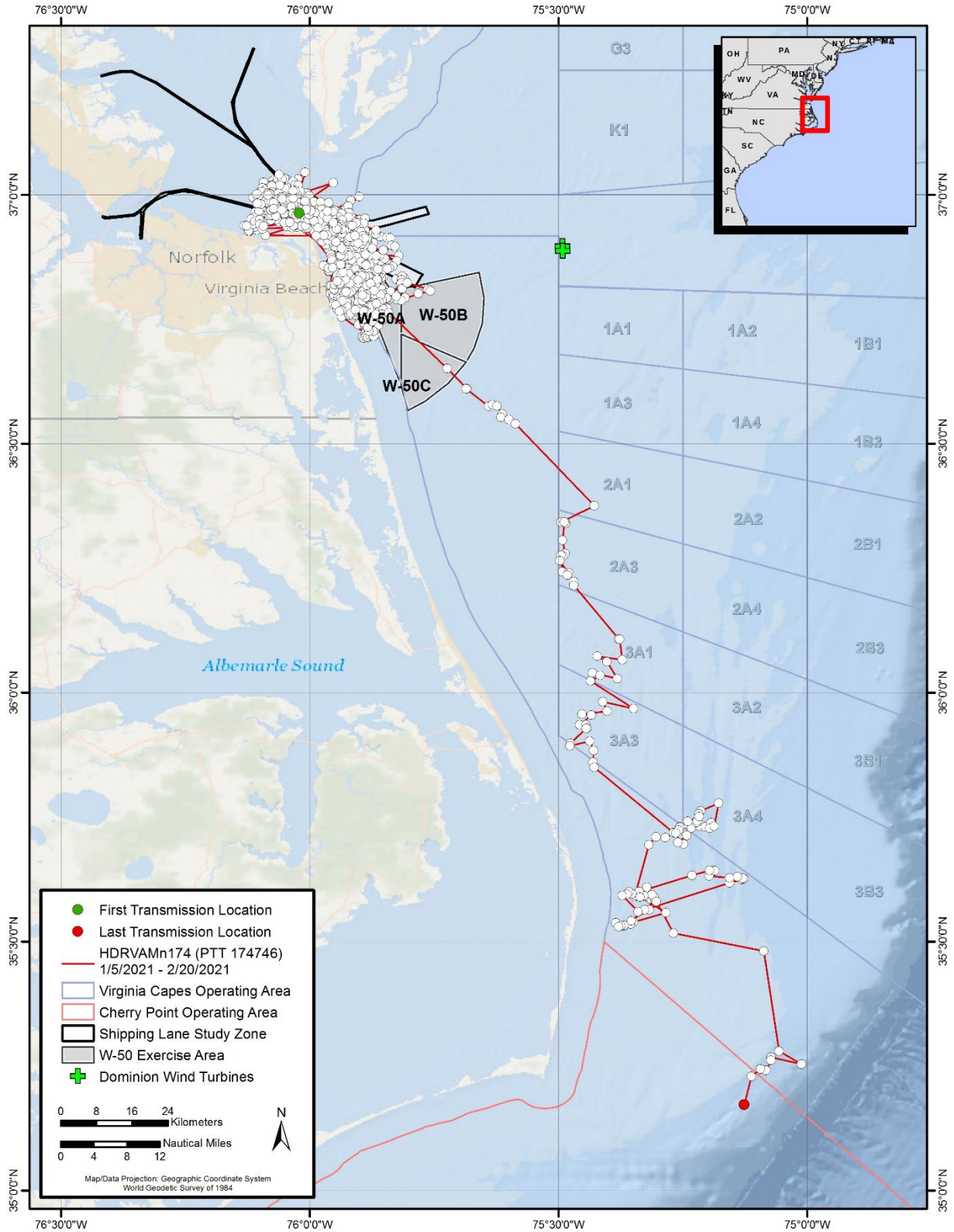


Figure 15. Filtered locations (white dots) and trackline (red line) of humpback whale HDRVAMn174, tagged on 05 January 2021 over 45.9 days of tag-attachment duration

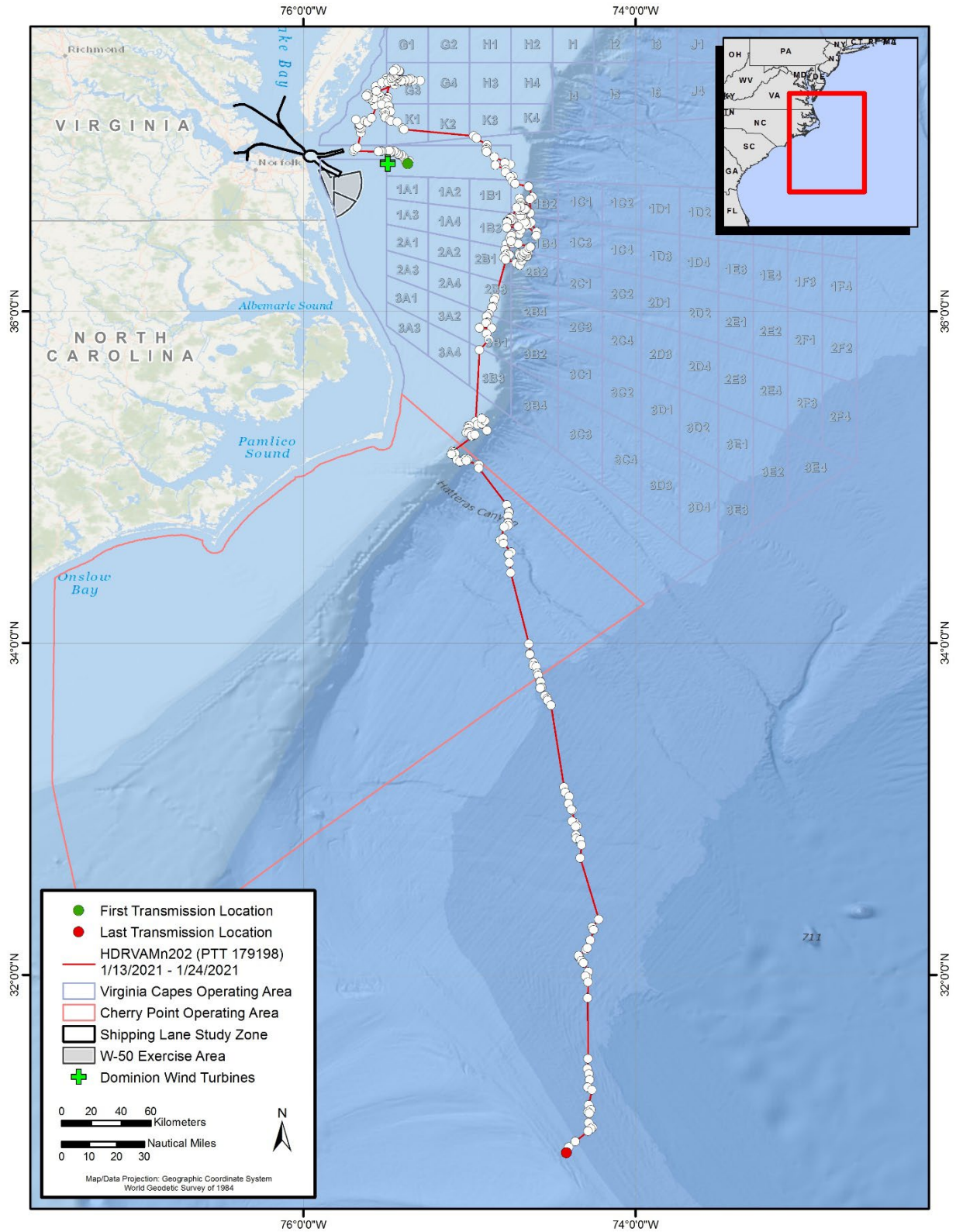


Figure 16. Filtered locations (white dots) and trackline (red line) of humpback whale HDRVAMn202, tagged on 13 January 2021 over 11.1 days of tag-attachment duration

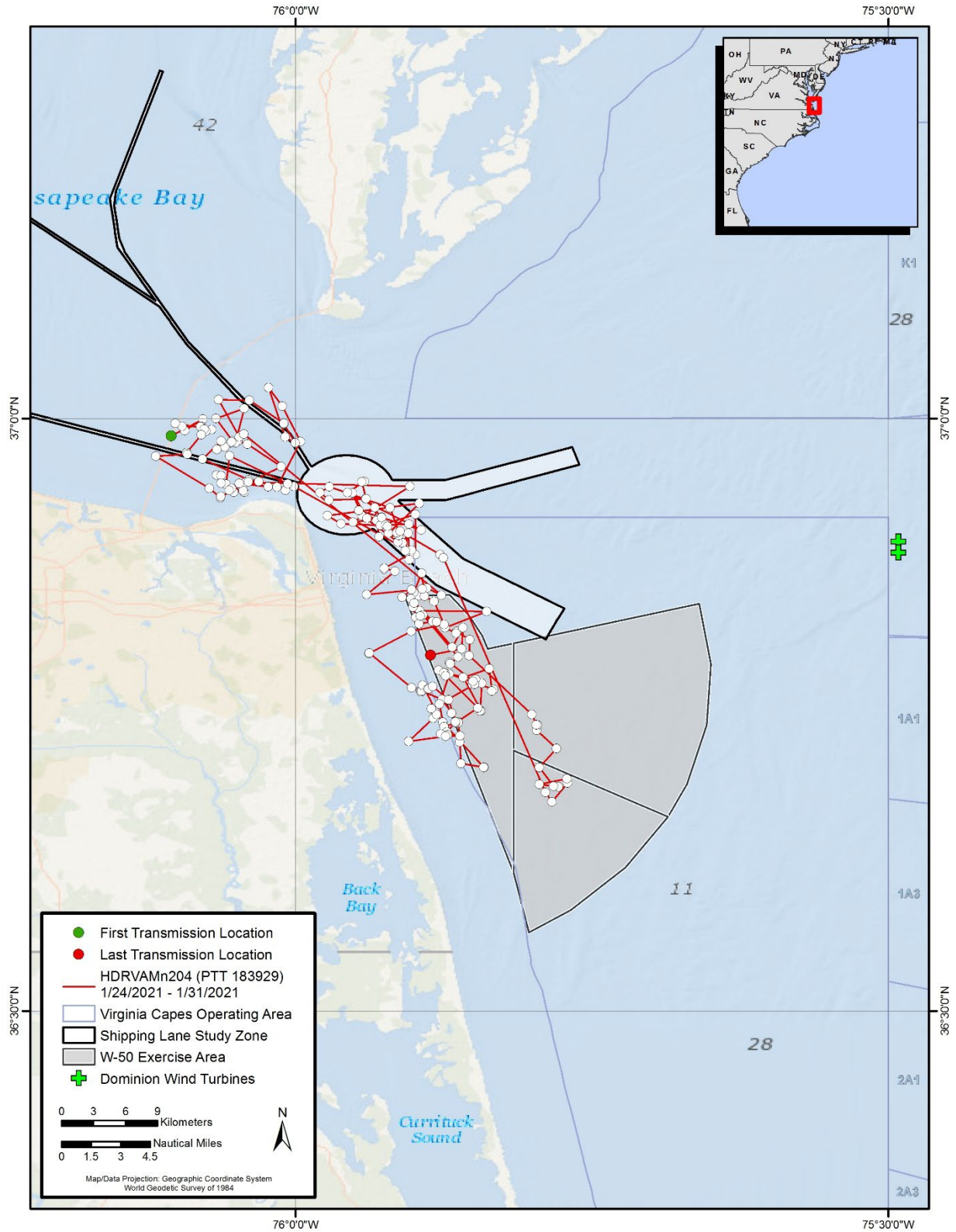


Figure 17. Filtered locations (white dots) and track line (red line) of humpback whale HDRVAMn204, tagged on 24 January 2020 over 6.7 days of tag-attachment duration

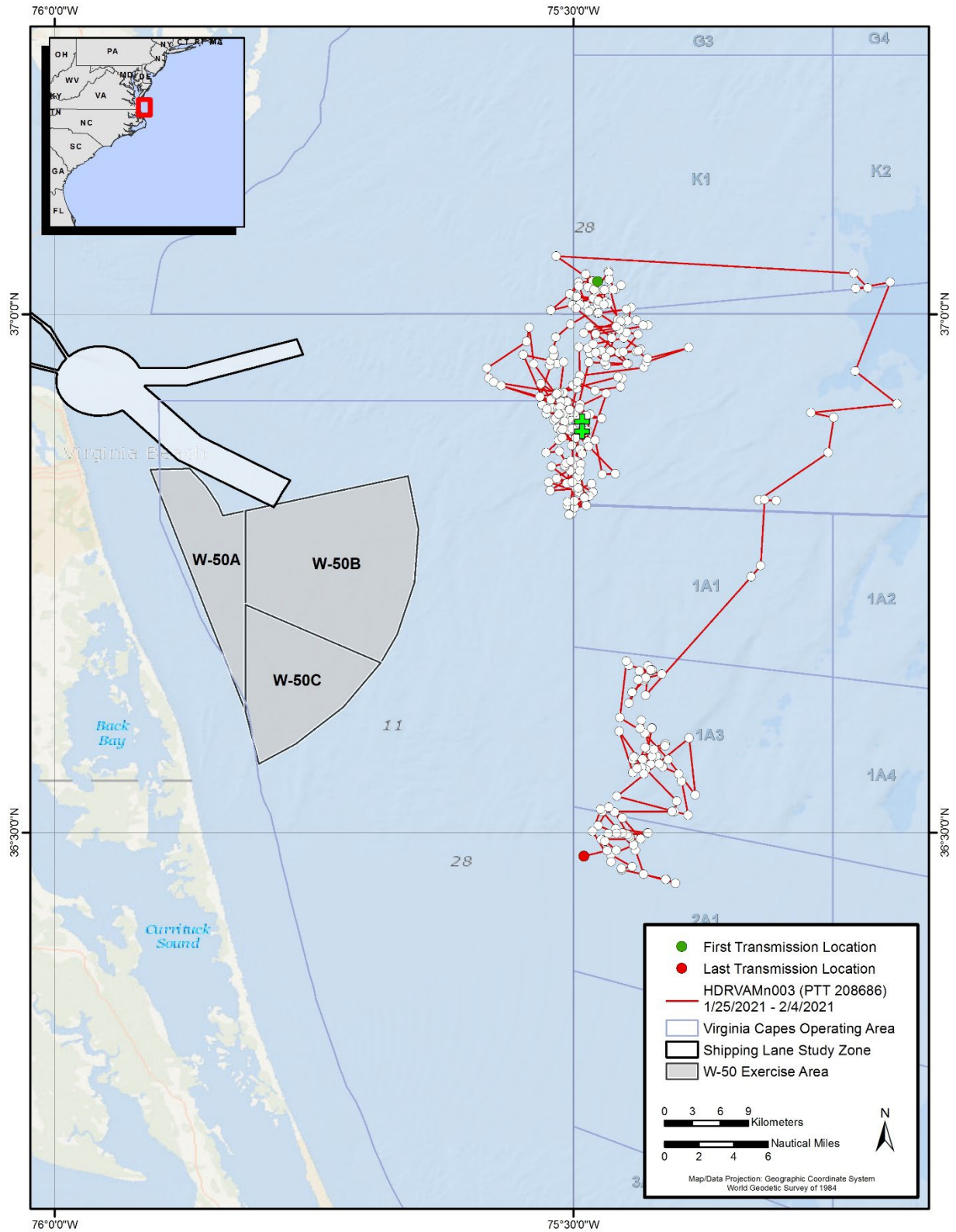


Figure 18. Filtered locations (white dots) and track line (red line) of humpback whale HDRVAMn003, tagged on 25 January 2021 over 9.7 days of tag-attachment duration

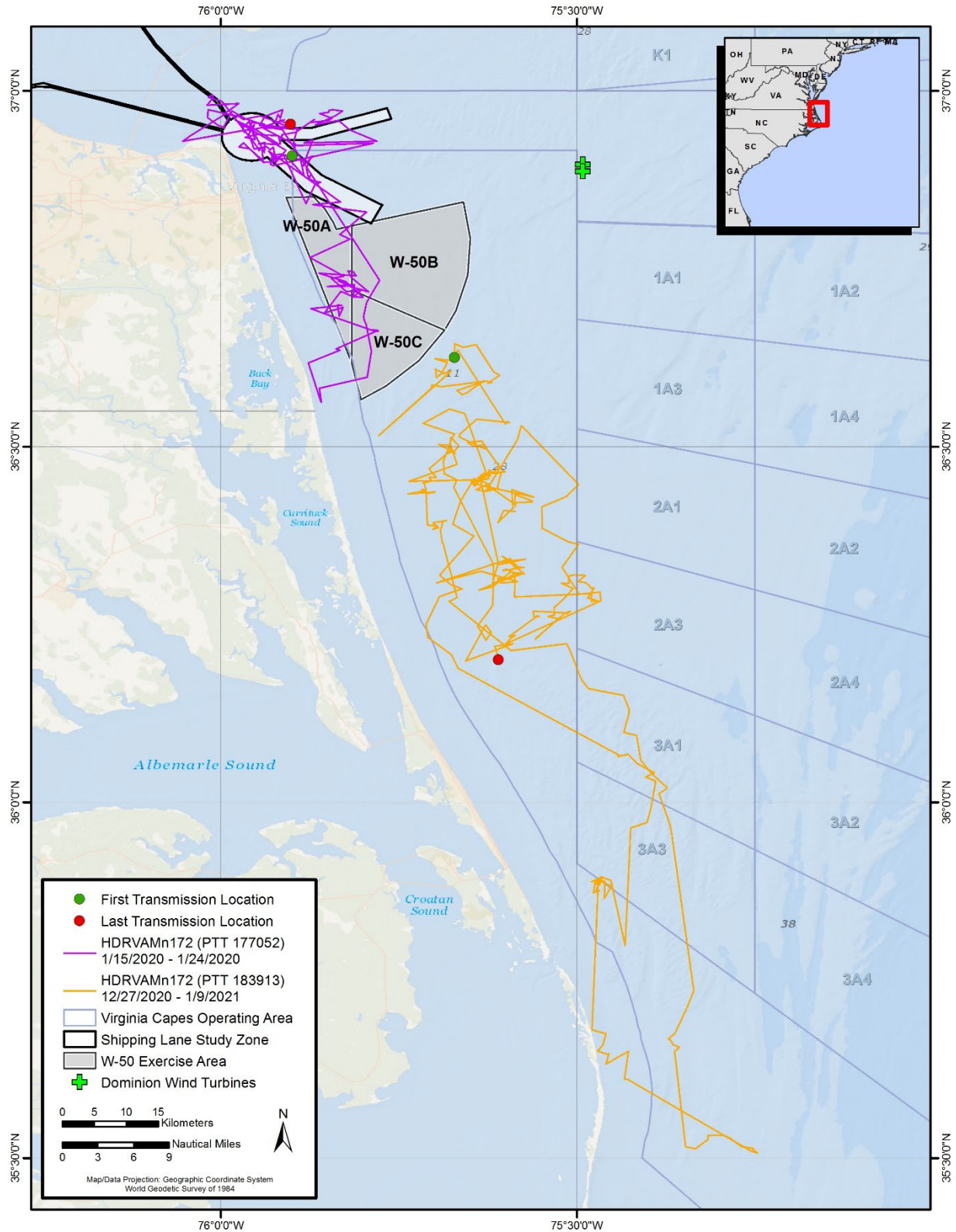


Figure 19. Comparison of tracks for HDRVAMn172 tagged in January 2020 (purple trackline, 8.4 days) and December 2020 (orange trackline, 11.2 days)

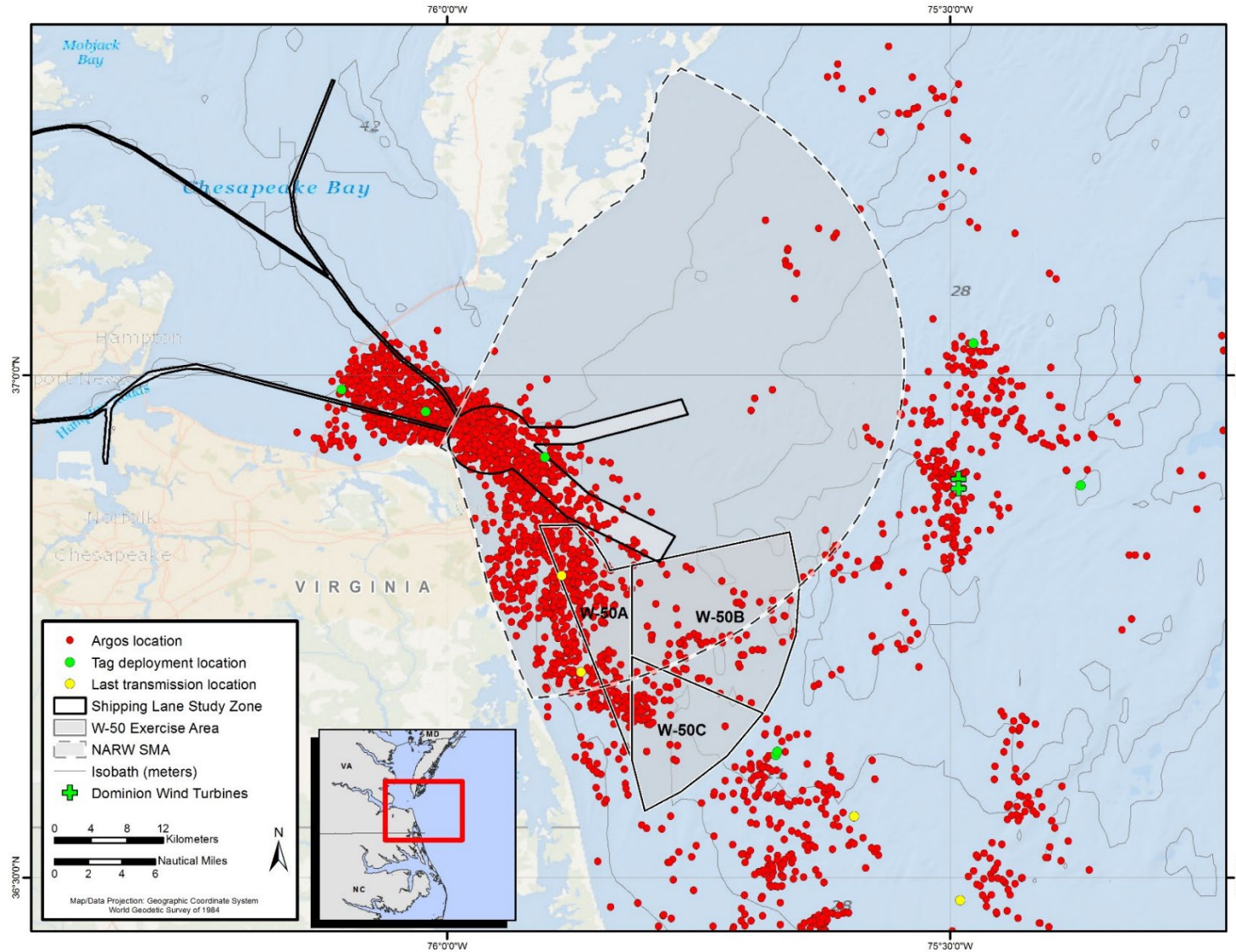


Figure 20. Filtered locations (red dots) of all humpback whale Argos locations in the immediate vicinity of shipping channels at the mouth of Chesapeake Bay from tag deployments (n=7) during the 2020/21 field season

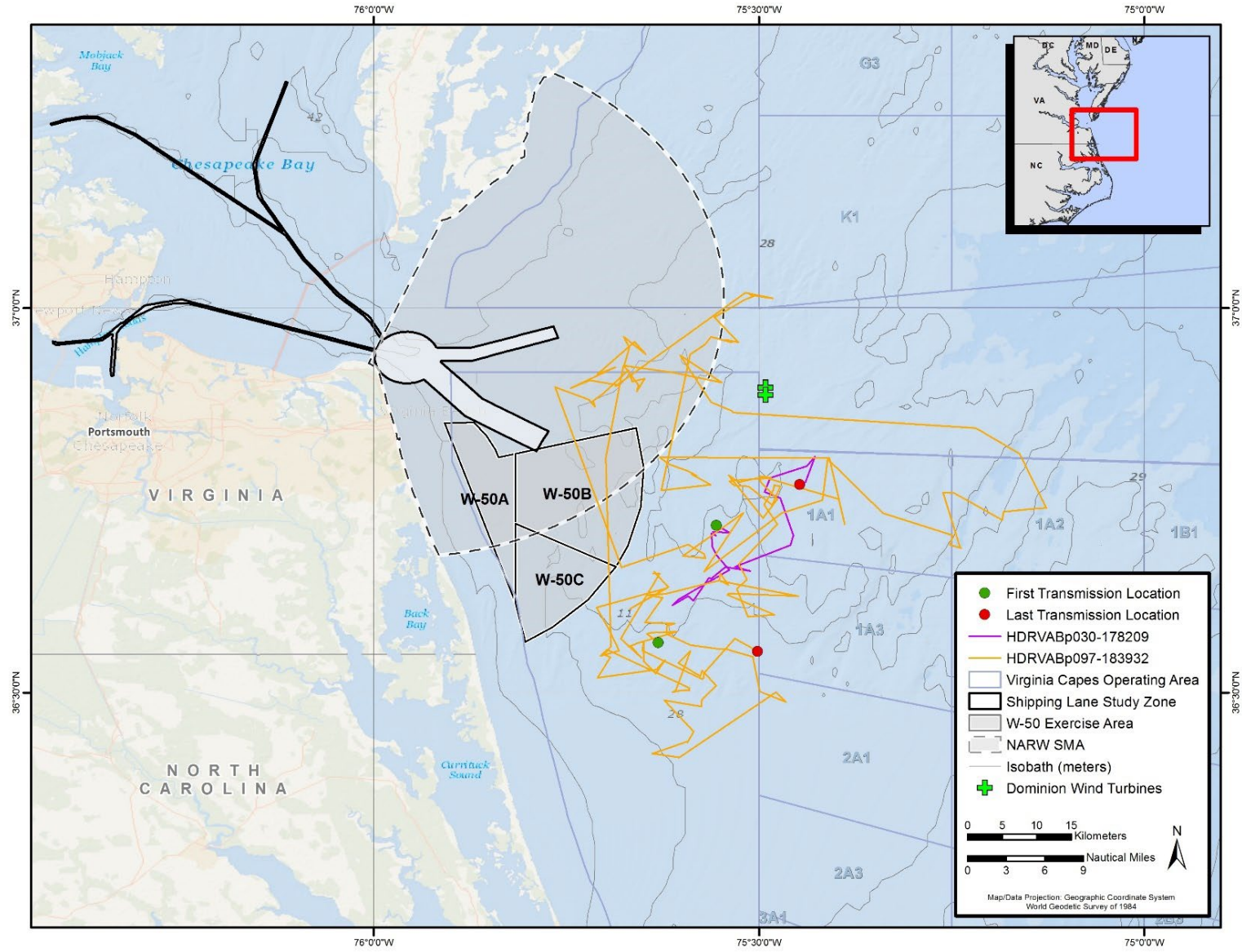


Figure 21. Argos tracks for all fin whales tagged (n=2) during the 2020/21 field season

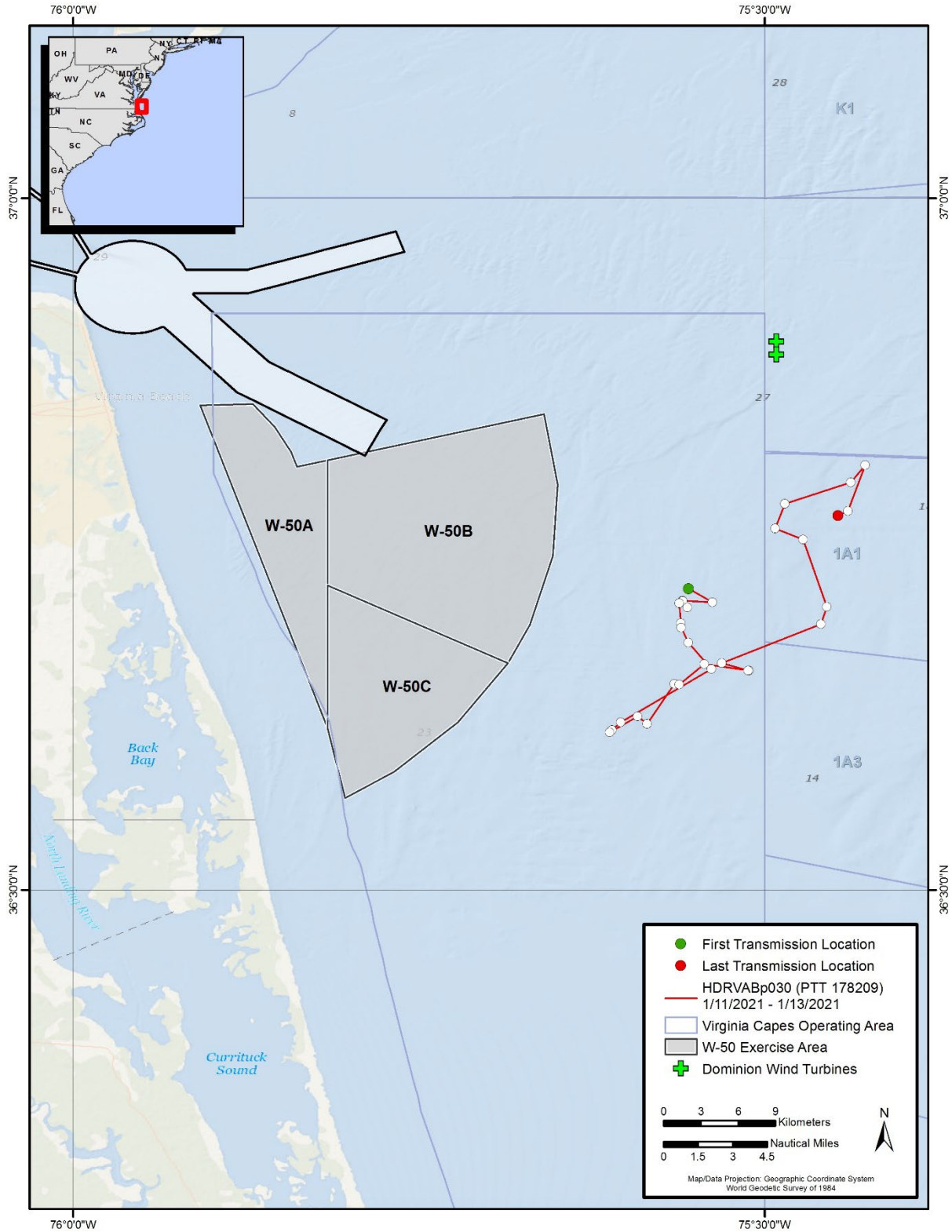


Figure 22. Filtered locations (white dots) and trackline (red line) of fin whale HDRVABp030, tagged on 11 January 2021 over 1.3 days of tag-attachment duration

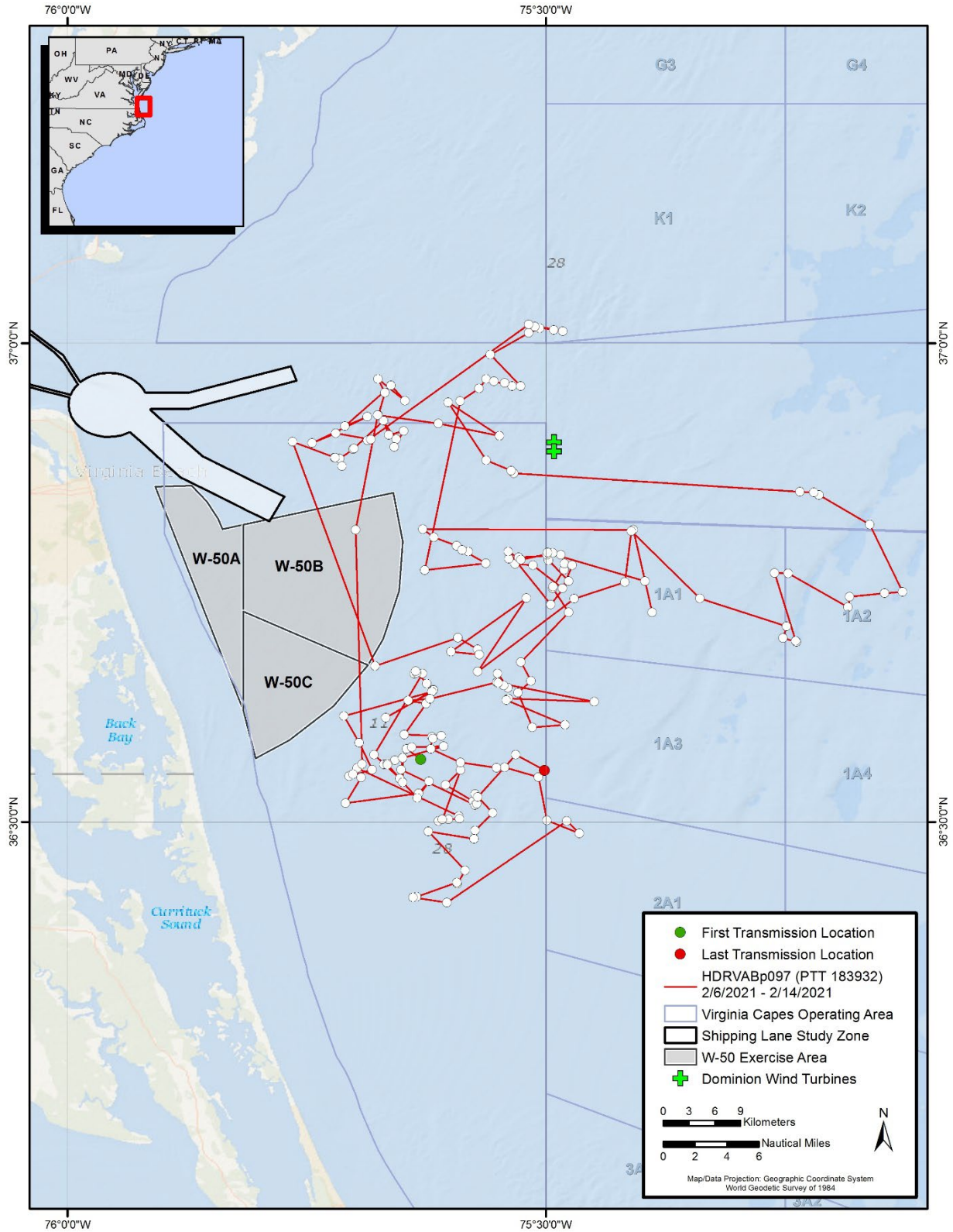


Figure 23. Filtered locations (white dots) and trackline (red line) of fin whale HDRVABp097, tagged on 6 February 2021 over 8.1 days of tag-attachment duration

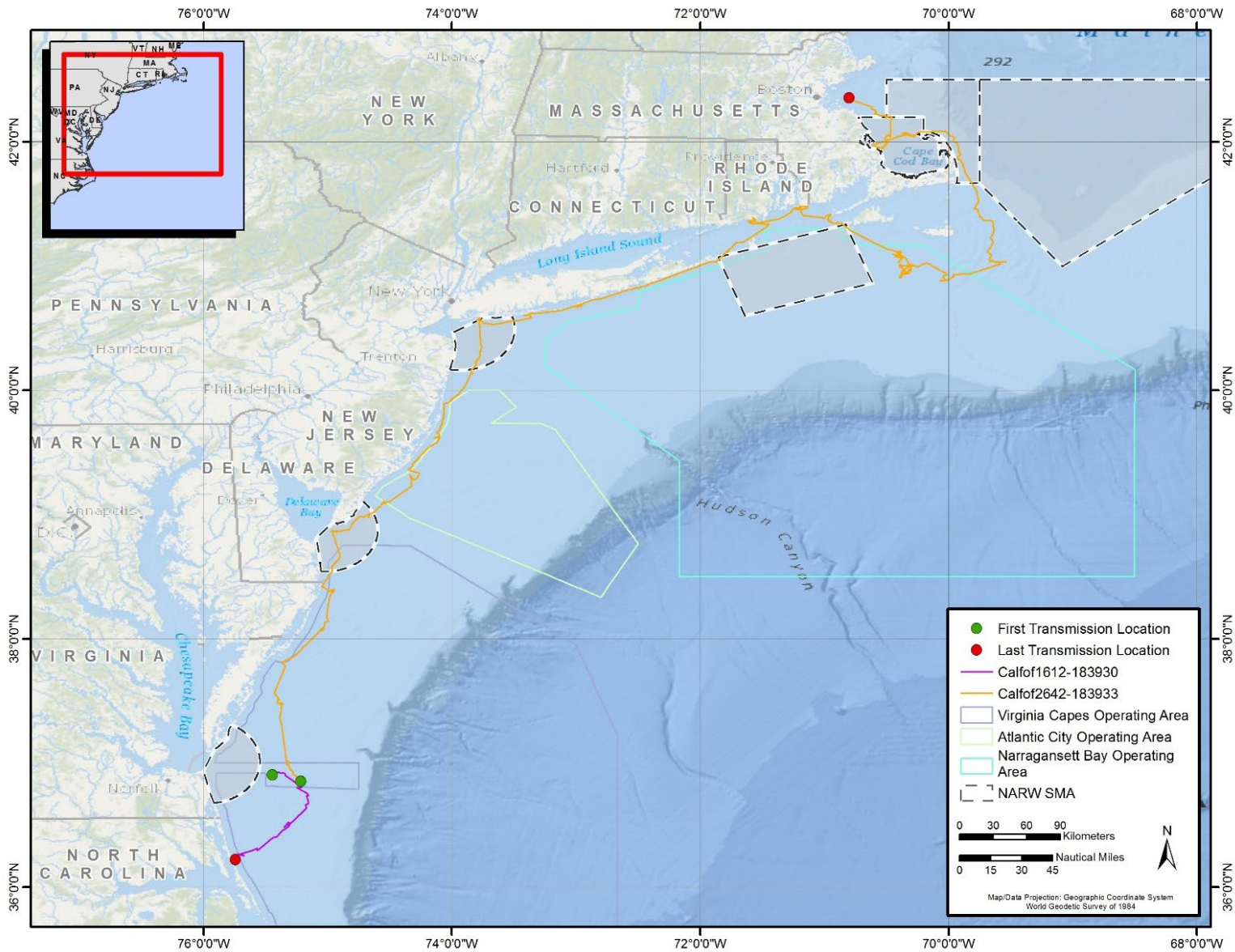


Figure 24. Argos tracks for all North Atlantic right whales tagged (n=2) during the 2020/21 field season

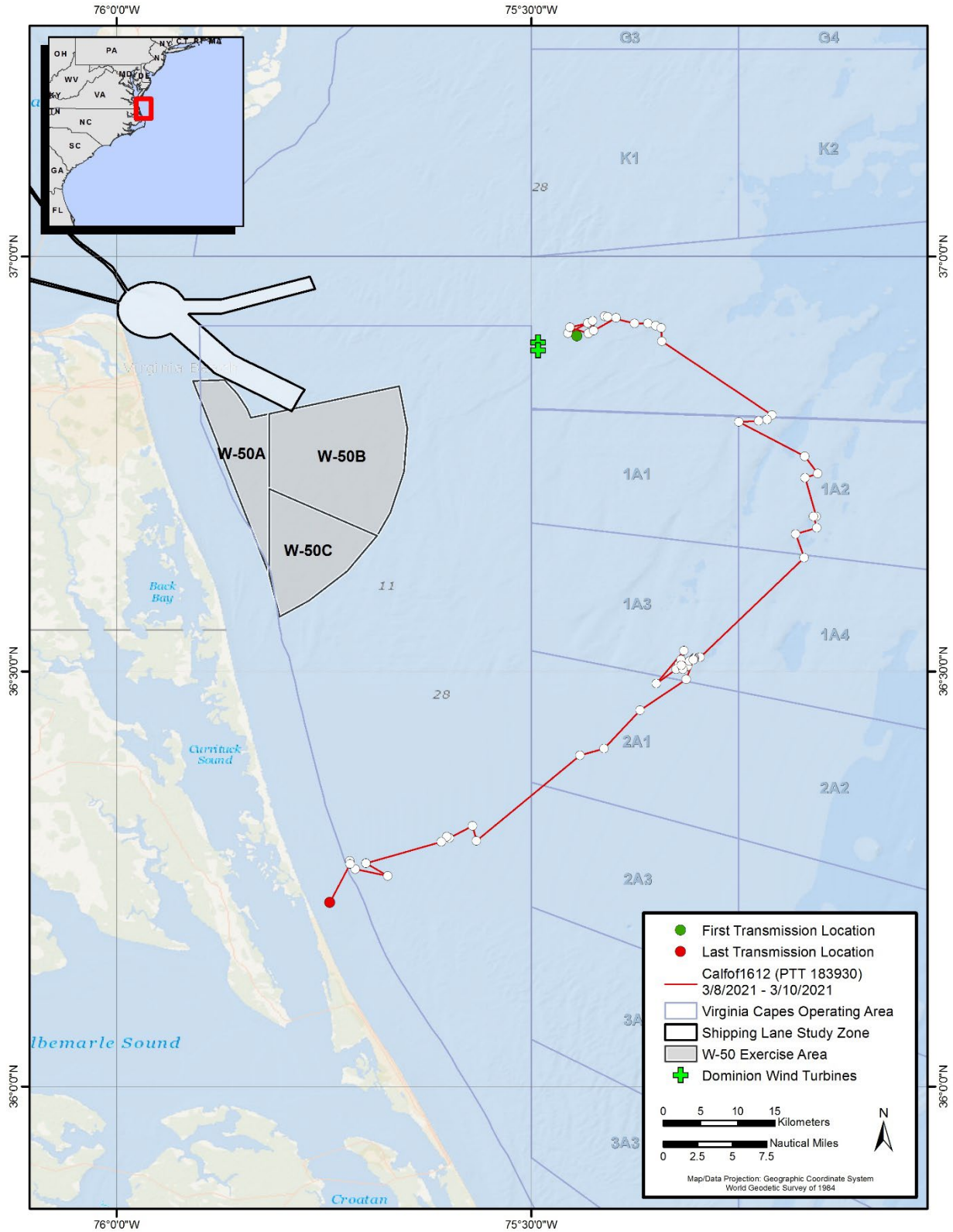


Figure 26. Filtered locations (white dots) and trackline (red line) of North Atlantic right whale Calf of 1612, tagged on 8 March 2021 over 1.8 days of tag-attachment duration

Maximum straight-line distance from the initial tagging location ranged from 15 to 667 km (mean = 205.8 km) for all species (**Table 7**). The percentage of locations occurring within the shipping channel study area ranged from 0 to 28.6 percent (mean = 5.0 percent), with four humpback whales spending no time in the shipping channels and neither the fin whales nor North Atlantic right whales spending any time in them (**Table 7**). The percentage of locations within the W-50 MINEX area ranged from 0 to 34.1 percent (mean = 7.2 percent), whereas the percentage of locations with the OPAREA ranged from 22.8 to 100 percent (mean = 60.1 percent) (**Table 7**).

Table 7. Summary of results from satellite-tag data for all baleen whales tagged during the 2020/21 season

| Animal ID | Species | No. Locations Post Filtering | Percent Within Shipping Channels | Percent Within VACAPES | Max Distance from Initial Location (km) | Mean Distance from Initial Location (km) |
|--------------|----------------------------|------------------------------|----------------------------------|------------------------|---|--|
| HDRVAMn193 | Humpback whale | 495 | 4.7 | 62.4 | 108.2 | 31.2 |
| HDRVAMn172 | Humpback whale | 1,489 | 28.6 | 22.8 | 214.3 | 21.9 |
| HDRVAMn196 | Humpback whale | 347 | 0.0 | 65.4 | 666.8 | 159.3 |
| HDRVAMn174 | Humpback whale | 208 | 21.2 | 36.1 | 44.7 | 20.9 |
| HDRVAMn202 | Humpback whale | 303 | 0.0 | 59.4 | 64.7 | 24.1 |
| HDRVAMn204 | Humpback whale | 381 | 0.0 | 57.2 | 156.2 | 71.6 |
| HDRVAMn003 | Humpback whale | 326 | 0.0 | 96.6 | 129.8 | 40.9 |
| HDRVABp030 | Fin whale | 30 | 0.0 | 100.0 | 15.1 | 7.6 |
| HDRVABp097 | Fin whale | 186 | 0.0 | 88.7 | 51.4 | 22.2 |
| Calf of 2642 | North Atlantic right whale | 56 | 0.0 | 69.6 | 80.3 | 37.6 |
| Calf of 1612 | North Atlantic right whale | 568 | 0.0 | 11.8 | 732.9 | 476.8 |

Key: km = kilometer(s); Max = Maximum

Eight of the 11 satellite tags were capable of recording data on dive depth and duration in addition to the Argos capabilities (**Table 8**). Four humpback whale tags recorded a total of 4,119 dives. Mean dive depths ranged from 15.7 to 30.5 m with a maximum dive depth of 243 m by one individual. Mean dive durations ranged from 2.47 to 3.8 minutes (min). Two fin whale tags recorded a total of 515 dives. Mean dive depth ranged from 14.2 to 16.3 m, and mean dive durations ranged from 3.7 to 5.5 min. Two North Atlantic right whale tags recorded a total of 2,360 dives. Mean dive depths ranged from 19.3 to 23.1 m, and mean dive durations ranged from 6.02 to 6.58 min.

Dive durations of humpback whales tagged this season were similar to humpbacks tagged during the 2019/20 field season (n = 11,708 dives) where the mean dive durations ranged from 2.4 to 3.9 min whereas dive depths tended to be deeper than the 2019/20 humpbacks whose dive depth ranged from 12.6 to 16.3 m ([Aschettino et al. 2021](#)). Dives for humpback whales tagged during the 2018/19 field season were shorter, ranging from 1.8 to 3.0 min ([Aschettino et al. 2020b](#)), and shallower, ranging from 8.6 to 14.6 m, which may be a result of a smaller

dataset (n = 230 dives) or different foraging strategies. HDRVAMn202, the humpback whale that appeared to be migrating south based on its trackline (**Figure 16**) had the greatest mean dive depth (30.5 m) and the deepest maximum dive depth (243 m) recorded during this study to-date. Given that this was the only individual to show an apparent migration south it is not surprising that dive behavior would differ from those whales staying within the primary study areas.

Table 8. Summary of dive depth and duration data collected from tagged baleen whales during the 2020/21 season

| Animal ID | Species | No. Dives Logged | Mean Dive Depth (m) | Max Dive Depth (m) | Mean Dive Duration (mm:ss) | Max Dive Duration (mm:ss) |
|--------------|----------------------------|------------------|---------------------|--------------------|----------------------------|---------------------------|
| HDRVAMn172 | Humpback whale | 1,535 | 17.6 | 31.0 | 03:31 | 14:01 |
| HDRVAMn202 | Humpback whale | 1,045 | 30.5 | 243 | 03:48 | 13:47 |
| HDRVAMn204 | Humpback whale | 244 | 15.7 | 28.0 | 02:28 | 04:23 |
| HDRVAMn003 | Humpback whale | 1,295 | 20.2 | 31.0 | 02:57 | 07:43 |
| HDRVABp030 | Fin whale | 140 | 16.3 | 24.0 | 05:32 | 12:15 |
| HDRVABp097 | Fin whale | 375 | 14.2 | 37.0 | 03:39 | 08:33 |
| Calf of 2642 | North Atlantic right whale | 2,250 | 19.3 | 66.0 | 06:01 | 20:35 |
| Calf of 1612 | North Atlantic right whale | 110 | 23.1 | 39.0 | 06:42 | 13:55 |

Key: m = meter(s); Max = Maximum; mm:ss = minutes:seconds

3.5 DTAG Results

In January 2019, Duke University researchers initiated a concurrent tagging project on humpback whales around the shipping lanes in the Chesapeake Bay study area. This study continued into the 2020/21 field season. High-resolution DTAGs were deployed on overwintering humpback whales to better understand the factors that influence their responses to approaching vessels. More information about this project can be found in [Shearer et al. \(2022\)](#).

In November 2020, HDR also incorporated the use of DTAGs into their existing project. The goal was to deploy tags on individuals in the mid-shelf region to learn more about their foraging and fine-scale dive behavior in these areas. In total, four DTAGs were deployed on baleen whales during the 2020/21 season (**Table 9**). Two tags were deployed on humpback whales, which generated 313 and 583 min of recordings, respectively. Neither of these individuals was satellite tagged. Two DTAGs were also deployed on the satellite-tagged North Atlantic right whales, which produced 255 and 960 (estimated) min of recordings. These data are still being analyzed; however, dive-depth profiles for all individuals are shown below (**Figures 27 and 28**).

Table 9. DTAG deployments on humpback and North Atlantic right whales during the 2020/2021 field season

| Animal ID | Species | DTAG #/Deployment ID | Deployment (GMT) | Depth at Tagging (m) | Tag off animal (GMT) | Tag Duration (min) |
|------------------|----------------------------|-----------------------------|-------------------------|-----------------------------|---------------------------------|---------------------------|
| HDRVAMn190 | Humpback whale | 321/mn20_324a | 2020-Nov-19, 15:29 | 14 | 2020-Nov-19, 20:42 | 313 |
| HDRVAMn208 | Humpback whale | 321/mn21_037a | 2021-Feb-06, 16:15 | 19 | 2021-Feb-07, 01:58 | 583 |
| Calf of 2642 | North Atlantic right whale | 313/eg21_062a | 2021-Mar-03, 17:02 | 30 | 2021-Mar-03, 21:15 | 255 |
| Calf of 1612 | North Atlantic right whale | 313/eg21_067a | 2021-Mar-08, 21:56 | 23 | 2021-Mar-09, 20:30 ^a | 960 ^a |

Key: GMT = Greenwich Mean Time; m = meter(s); min = minute(s)

^a Research team was not present during tag release; the tag-off time and tag duration are estimated

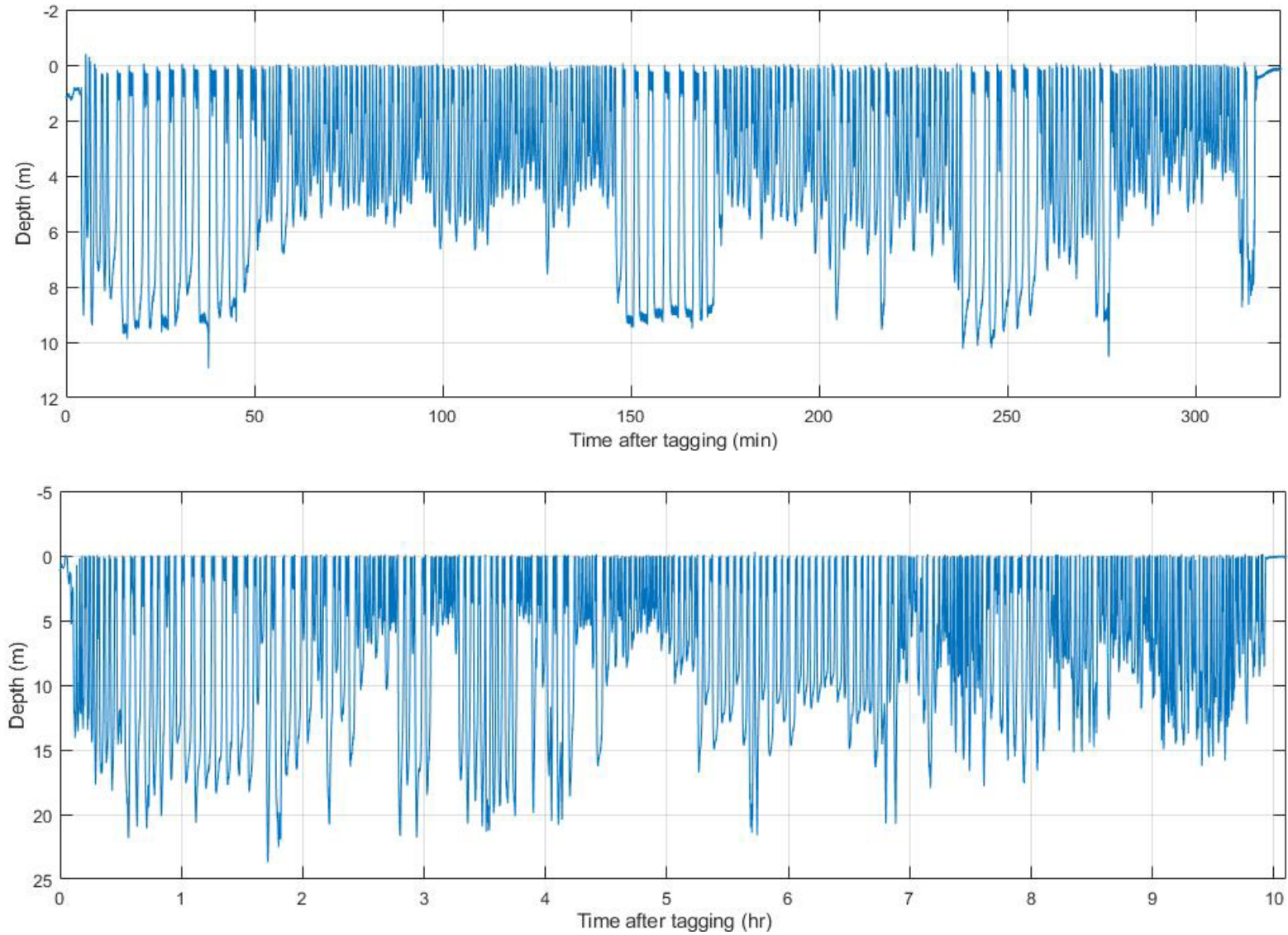


Figure 1. Dive depth profile (in meters) for humpback whales, DTAG *mn20_324a* (top) and DTAG *mn21_037a* (bottom)

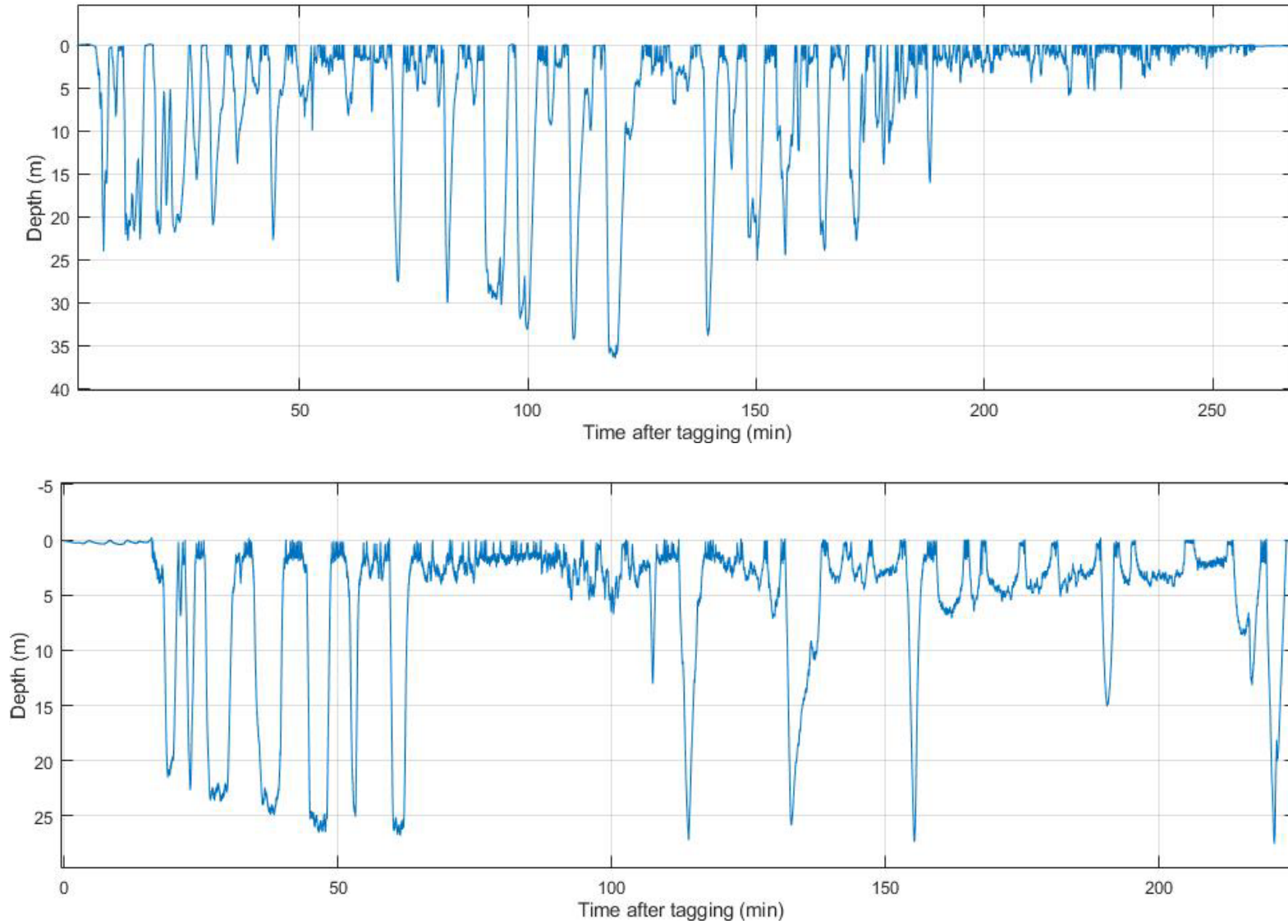


Figure 2. Dive depth profile (in meters) for North Atlantic right whales, DTAG eg21_062a (top) and DTAG eg21_067a (bottom)

4. Discussion

Analyses of data from this multi-year project are ongoing; however, each season of data helps build a more complete picture of how baleen whales utilize the waters in and around the mouth of Chesapeake Bay and surrounding area. Shipping channels, W-50 MINEX, and U.S. Navy OPAREAS all occur within the habitat that these whales use seasonally. Results continue to show site fidelity in the study area for many individuals and a high level of occurrence in areas that are heavily utilized by the U.S. Navy, commercial shipping, and recreational and commercial fishing vessels. These findings are supported by information collected during the past 7 years of this study, including photo-IDs, focal follows, and satellite-tagging results.

Interactions with vessels, both large and small, are a significant cause for concern for both humpback and endangered fin whales in the study area. In April 2017, the National Marine Fisheries Service declared an Unusual Mortality Event (UME) for humpback whales in the Atlantic Ocean from Maine to North Carolina based on elevated mortalities of this species since January 2016. At the time of this report, 157 humpback whales are included in this UME and 47 (29.9 percent) of those have occurred in Virginia or North Carolina (NOAA 2022). Given this UME designation, a group of subject matter experts will further investigate what is causing or contributing to the increased number of deaths of humpback whales in this area. While the UME team will look at humpback whales of all age classes, approximately three-quarters of the humpback whales identified during the 7 years of survey effort on this project appear to be juveniles that are spending more time in the study area than larger animals, presumed to be adults, and may be at greater risk for injury. Sightings of sub-adult-sized humpback whales are highest early in the field seasons or farther from shore in the mid-shelf region, and those individuals are typically not re-sighted, suggesting that sightings early in the season may be whales passing through the area rather than whales remaining in the nearshore study area for longer durations. The large percentage of juveniles observed in this study matches both historic stranding (e.g., Wiley et al. 1995) and observational (e.g., Swingle et al. 1993) data for the area.

The number of humpback whale identifications per season grew steadily over the course of the first 3 years of this project, decreased during the 2017/18 field season, increased slightly for the 2018/19 season before dropping slightly in 2019/20 season, and increased slightly in the 2020/21 season (**Figure 9**). Because these surveys are not intended to support density or abundance estimates, trends in sightings across study years cannot be evaluated statistically; however, some subjective inferences may be made.

Part of the increase in the number of identifications over the first three seasons is likely due to effort—the 2016/17 and 2017/18 field seasons began 2 months earlier than the 2014/15 season and 1 month earlier than the 2015/16 field season. Also, during the 2014/15 season, effort was focused on collecting focal follows of individual whales, so priority was given to staying with one whale over a longer period of time rather than collecting as many photo-IDs of animals in the surrounding areas. Overall effort on the water, both in terms of survey days and hours, also increased during the first three field seasons, partially accounting for the increase in sighting information during the 2016/17 field season.

The 2017/18 field season was also somewhat anomalous in terms of temperature, with multiple cold-weather systems significantly impacting water temperature in and around Chesapeake Bay and the surrounding areas. Based on HDR's experience, when water temperatures drop, the whales seem to be less numerous or altogether absent despite continued survey effort. It is presumed that the colder water temperatures likely affected prey distribution in the area and may have forced animals to look elsewhere for food—either farther south, toward the Outer Banks of North Carolina, or farther offshore, as was observed in some of the tag data and evidenced by the need to survey farther offshore to locate whales. The decrease in the number of overall sightings and overall individuals identified during the 2017/18 field season may be related to the low water temperatures that began in early January 2018.

Less time was spent on the water (both in terms of number of days and overall effort) during the 2019/20 field season compared to the previous 2018/19 field season, which may also account for the smaller number of identifications. Additional sightings in during the 2020/21 field season correspond with increased survey effort.

Integration of the drone component to the study, beginning in 2018, has allowed for additional opportunities to examine body condition and estimate length. The drone also proves valuable in assisting with tag deployments, observing unique behavior (such as bubble-net feeding), and collecting follow-up images from tagged whales (**Figure 29**). We recently retrofitted the drone and installed a custom LiDAR altimeter, which increases the precision and consistency of the sUAS altimetry measurements to minimize possible error in measured animal lengths. To transition to a Department of Defense-compliant platform following recent 2020 National Defense Authorization Act restrictions, we are acquiring a new American-made sUAS with improved capabilities such as a LiDAR sensors, longer flight times, and a higher-resolution camera.



Figure 29. Still image of North Atlantic right whale from low altitude to collect follow-up imagery from the satellite tag and DTAG

With six seasons of satellite tag deployments completed, trends are emerging, as is the variability both between individuals and between seasons. The mouth of Chesapeake Bay, and shipping lanes in particular, continues to be an area heavily used by humpback whales seasonally. From November through April, there is a ship-speed reduction rule in effect at the mouth of Chesapeake Bay as part of the Seasonal Management Area (SMA) set up to protect Endangered Species Act-listed North Atlantic right whales. These speed restrictions require all vessels 65 feet (19.8 m) or longer to travel at 10 knots (18.5 km/hour) or less.

The SMA in this study area begins at the mouth of Chesapeake Bay and extends outwards to 37 km; however, as Argos locations from tagged humpback whales have shown, these boundaries do not necessarily protect all large whales using the area (**Figure 17**). Portions of the Chesapeake Bay, west of the Chesapeake Bay Bridge Tunnel, were not used by any tagged humpback whales during the 2015/16, 2017/18, or 2020/21 field seasons; only sparsely used during the 2018/19 field season; and heavily used during the 2016/17 and 2019/20 field seasons.

Short-term distributional shifts related to oceanographic conditions may have caused prey to become concentrated farther into the bay during the 2016/17 and 2019/20 field seasons, resulting in an increased presence of humpback whales in that area. The presence of humpback whales west of the Chesapeake Bay Bridge Tunnel raises additional concerns given the high traffic flow in that area, increased vessel speeds allowed, and extent of marine-based training occurring out of Joint Expeditionary Base Little Creek.

While the percentage of tag locations occurring within the shipping lanes decreased this 2020/21 season, there was an increase in the percentage of locations within the VACAPES OPAREA. While this shift may cause certain individuals to be less at risk for ship strikes, there may be different risk factors in the offshore areas. Increased survey and tagging effort into the offshore waters during the 2020/21 field season have shown this to also be an important habitat for humpback, fin, and North Atlantic right whales. Further analysis of water-temperature data collected from CTD measurements, buoy data, and tag data may provide a better understanding of thresholds that result in humpback whales (and presumably their prey) remaining in or moving out of the nearshore area. Efforts for the 2021/22 field season will continue to focus on pushing farther into the mid-shelf waters when not supporting the nearshore collaborative efforts with Duke University. During the 2021/22 field season, we will continue to deploy DTAGs on baleen whales using the W-50 MINEX and mid-shelf areas. This addition will allow us to continue to better detail fine-scale movement, dive patterns, foraging behavior, and record acoustic measurements to add to the existing medium-duration dataset.

State-Space Modeling and home range analyses were previously performed on a subset of data (see [Aschettino et al. 2018](#)), and results provided inference on animal behavior for all but the shortest (or sparsely reporting) tags. Animals showed varied movement strategies, the most common of which was area-restricted search centered around the mouth of Chesapeake Bay, where most tags were deployed. It may be that tags were lost before significant movement was undertaken, but it still highlights the lower Chesapeake Bay as an important foraging area. Other strategies included looping down the Outer Banks to feed and then returning north, foraging deeper into the Chesapeake Bay, and directing movements northwards along the coast and the shelf break before recruiting to additional locations where area-restricted search

behavior was performed. Updating these analyses following the 2021/22 field season, with the inclusion of additional tags, will provide a more robust picture of humpback whale habitat use in Chesapeake Bay. This population has been shown to engage in diverse feeding and movement strategies, which need to be considered when mitigating impacts and making management decisions.

In addition to integrating additional tag data into the switching state-space model, further tag analyses will continue. As additional SPLASH10-F tags are deployed, dive depths and durations will be looked at more closely and in association with the concurrent DTAG efforts being conducted by Duke University (see Shearer et al. 2022).

The numbers of sightings of humpback, fin, and North Atlantic right whales, as well as the level of interaction between whales and vessel traffic detailed to date, support previous recommendations to continue this study using the same techniques in order to better understand movement patterns and habitat use. Continued photo-ID effort will build a more complete picture of inter-annual site fidelity to this region. The inclusion of SPLASH10-F tags with Fastloc® GPS technology, capable of providing high-resolution data logging, will provide superior quality with respect to accuracy of locations. Coupled with the DTAG collaboration effort with Duke University, which will examine the three-dimensional movements of humpback whales within and around high-traffic shipping channels, the entirety of these data will provide a better understanding of the occurrence and behavior of large whales in this area and further support future mid-Atlantic behavioral response studies.

5. Acknowledgements

This project is funded by U.S. Fleet Forces Command and managed by Naval Facilities Engineering Systems Command (NAVFAC) Atlantic as part of the U.S. Navy's Marine Species Monitoring Program. HDR would like to acknowledge the Contracting Officer's Technical Representative Joel Bell for his continued oversight and support for this project as well as his time assisting in the field. HDR thanks those who assisted in the field, including Todd Pusser and the NAVFAC EV53 team. HDR would like to acknowledge Kristin Rayfield and Alexis Rabon as well as the captains and crew of Rudee Flipper Tours for coordination of real-time humpback whale sightings. Alexis and Kristin were also especially helpful in sharing photograph and sighting information from tagged and/or biopsied humpback whales. HDR also thanks the skilled captains, Russ and Gene Kostinas, of the fishing vessel *Top Notch* and Chris Howlin, of the fishing vessel *Game On*. Andrew DiMatteo performed the State-Space Modeling analyses, which are available in an earlier report ([Aschettino et al. 2018](#)). HDR thanks Martine Bérubé and Per Palsbøll from the University of Groningen for the genetic matching of humpback and fin whale samples (**Appendix B**) and Danielle Waples from Duke University for performing stable isotope analyses which are available in an earlier report ([Waples 2017](#)). Will Cioffi from Duke University also provided valuable guidance with his goniometer program. HDR would also like to thank Claire Webster and the staff at Blue Pearl Pet Hospital for dart sterilization. All surveys were conducted under National Marine Fisheries Service Scientific Permit Number 21482, issued to Dan Engelhaupt, HDR.

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Appendix A Sighting History Table

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Table A-1. Sighting history (number of days seen per season) and additional information for all photo-identified baleen whales off Virginia Beach, Virginia: December 2014–April 2021

| HDR Catalog ID | Season 1 Dec 2014–Apr 2015 | Season 2 Oct 2015–May 2016 | Season 3 Nov 2016–Mar 2017 | Season 4 Oct 2017–Mar 2018 | Season 5 July 2018–May 2019 | Season 6 Dec 2019–Mar 2020 | Season 7 Nov 2020–Apr 2021 | Total No. Days Seen | Total No. Seasons Seen | Biopsied? (Y/N) Gender (M/F/U) | Satellite Tagged? (Y/N) | D-Tagged? (Y/N) | Estimated Age Class (A/SA/J/Ye) | Propeller Scars or Injuries? (Y/N/P) | Total No. Focal Follows | Total No. Focal Follow Minutes |
|-------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales: | | | | | | | | | | | | | | | | |
| HDRVAMn003 | 1 | | | | | | 1 | 2 | 2 | N/U | N | N | A | N | 0 | - |
| HDRVAMn004 | 1 | | | | | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVAMn005 | 2 | | 5 | | | | | 7 | 2 | Y/F | Y | N | A | N | 1 | 64 |
| HDRVAMn006 | 2 | | | | | | | 2 | 1 | N/U | N | N | J | N | 1 | 69 |
| HDRVAMn007 | 4 | 1 | 7 | | 8 | 1 | | 21 | 5 | Y/F | Y | N | J | N | 1 | 60 |
| HDRVAMn008 | 5 | | | | | | | 5 | 1 | N/U | N | N | J | N | 3 | 215 |
| HDRVAMn009 | 4 | | | | | | | 4 | 1 | Y/F | N | N | J | N | 2 | 112 |
| HDRVAMn010 | 1 | 2 | | 1 | | | | 4 | 3 | Y/M | Y (2) | N | J | N | 1 | 76 |
| HDRVAMn011 | 4 | | 1 | | | | | 5 | 2 | Y/F | N | N | J | N | 1 | 60 |
| HDRVAMn012 | 3 | 2 | 7 | 3 | 3 | | | 17 | 5 | Y/F | Y (2) | N | J | N | 2 | 25 |
| HDRVAMn013 | 10 | | | | | | | 10 | 1 | Y/F | N | N | J | N | 4 | 357 |
| HDRVAMn014 | 5 | 1 | 1 | 1 | | | | 8 | 4 | Y/F | N | N | J | N | 1 | 60 |
| HDRVAMn015 | 2 | | 1 | | | | | 3 | 2 | Y/F | N | N | J | N | 1 | 58 |
| HDRVAMn016 | 1 | | | | | | | 1 | 1 | N/U | N | N | U | N | 0 | - |
| HDRVAMn017 | 1 | | | | | | | 1 | 1 | N/U | N | N | U | N | 0 | - |
| HDRVAMn018 | 1 | | | | | | | 1 | 1 | N/U | N | N | U | Y | 0 | - |
| HDRVAMn019 | 1 | | | | | | | 1 | 1 | N/U | N | N | U | N | 0 | - |
| HDRVAMn020 | 1 | | | | | | | 1 | 1 | N/U | N | N | U | N | 0 | - |
| HDRVAMn021 | 2 | | 3 | 2 | 6 | 1 | | 14 | 5 | N/U | N | N | SA | N | 1 | 78 |

Key: Y = yes; N = no; P = possible; A = adult; SA = sub-adult; J = juvenile; C = calf; Ye = yearling; U = unknown

^a Deceased

^b Sighting occurred on offshore survey

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn022 | 2 | | | | | | | 2 | 1 | N/U | N | N | J | N | 1 | 85 |
| HDRVAMn023 | 1 | | 3 ^a | - | - | - | | 4 | 2 | Y/M | Y | N | J | N | 1 | 80 |
| HDRVAMn024 | 2 | | | | | | | 2 | 1 | Y/M | N | N | A | P | 1 | 60 |
| HDRVAMn025 | 1 | | | | | | 1 | 2 | 2 | Y/M | N | N | SA/A | N | 1 | 62 |
| HDRVAMn027 | 2 | 3 | 1 | 1 ^b | | | | 7 | 4 | Y/F | N | N | J | N | 1 | 61 |
| HDRVAMn028 | 1 | | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn029 | 1 | | | | | | | 1 | 1 | Y/M | N | N | J | P | 1 | 63 |
| HDRVAMn030 | 1 | 1 | | | | | | 2 | 2 | N/U | N | N | A | N | 1 | 62 |
| HDRVAMn031 | 1 | | 1 | 1 | | | | 3 | 3 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn032 | 1 | | | | | | | 1 | 1 | N/U | N | N | SA | N | 1 | - |
| HDRVAMn033 | 1 | | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | 63 |
| HDRVAMn034 | 1 | | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn035 | | 2 | | | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn036 | | 2 | | | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn037 | | 3 | | | | | | 3 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn039 | | 1 | | | | | | 1 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn041 | | 1 | | | | | | 1 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn042 | | 6 | | | | | | 6 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn043 | | 1 | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn044 | | 1 | | | | | | 1 | 1 | Y/F | Y | N | J | Y | 0 | - |
| HDRVAMn045 | | 6 | | | | | | 6 | 1 | Y/M | Y | N | J | Y | 0 | - |

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn046 | | 4 | | | | | | 4 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn047 | | 1 | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn048 | | 2 | 1 | | | | | 3 | 2 | Y/M | Y | N | SA/A | N | 0 | - |
| HDRVAMn049 | | 2 | 6 | 1, 1 ^b | | | | 10 | 3 | Y/F | Y (2) | N | SA/A | N | 0 | - |
| HDRVAMn050 | | 4 | | | | | | 4 | 1 | Y/M | N | N | J | N | 0 | - |
| HDRVAMn051 | | 9 | | | | | | 9 | 1 | Y/M | N | N | J | Y | 0 | - |
| HDRVAMn052 | | 3 | | | | | | 3 | 1 | Y/F | N | N | J | N | 0 | - |
| HDRVAMn053 | | 2 | | | | | | 2 | 1 | N/U | N | N | J | Y | 0 | - |
| HDRVAMn054 | | 7 | | | | | | 7 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn055 | | 2 | | | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn056 | | 2 | | | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn057 | | 1 | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn058 | | 1 | | | | | | 1 | 1 | N/U | N | N | J/SA | Y | 0 | - |
| HDRVAMn059 | | 1 | 2 | | | | | 3 | 2 | Y/M | Y | N | SA | N | 0 | - |
| HDRVAMn060 | | 1 | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn061 | | 3 | | | | | | 3 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn062 | | 3 | | | | | | 3 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn063 | | 4 | | | | | | 4 | 1 | Y/M | Y | N | J | N | 1 | 120 |
| HDRVAMn064 | | 2 | 12 | 2 | 3 | 2 | | 21 | 5 | Y/F | Y (2) | N | J | N | 0 | - |
| HDRVAMn065 | | 1 | 3 | | | | | 4 | 2 | N/U | N | N | J | N | 0 | - |
| HDRVAMn066 | | 2 | 2 | | | | | 4 | 2 | Y/M | Y | N | J | N | 0 | - |

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn067 | | 1 ^b | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn068 | | 1 | | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn069 | | | 1 | | | | | 1 | 1 | Y/F | Y | N | SA | N | 0 | - |
| HDRVAMn071 | | | 2 | | | | | 2 | 1 | Y/M | Y | N | SA | N | 0 | - |
| HDRVAMn072 | | | 1 | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn073 | | | 1 | | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVAMn074 | | | 1 | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn075 | | | 1 | | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVAMn076 | | | 1 | | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVAMn077 | | | 1 | | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVAMn078 | | | 1 ^a | - | - | - | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn079 | | | 3 | | | | | 3 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn080 | | | 1 | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn081 | | | 9 | | | | | 9 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn082 | | | 4 | | - | - | | 3 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn083 | | | 2 | | | | | 2 | 1 | N/U | Y | N | J | N | 0 | - |
| HDRVAMn084 | | | 11 | | | | | 11 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn085 | | | 8 | | | | | 8 | 1 | N/U | N | N | J | Y | 0 | - |
| HDRVAMn086 | | | 1 | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn087 | | | 3 | | | | | 3 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn088 | | | 6 | | | | | 6 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn089 | | | 1 | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn090 | | | 4 ^a | - | - | - | | 4 | 1 | Y/M | Y | N | J | Y | 0 | - |
| HDRVAMn091 | | | 5 ^a | - | - | - | | 5 | 1 | Y/M | N | N | SA | Y | 0 | - |
| HDRVAMn092 | | | 6 | | | | | 6 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn093 | | | 6 | | 4 | 1 | | 11 | 3 | Y/F | Y (3) | N | J | N | 0 | - |
| HDRVAMn094 | | | 1 | | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn095 | | | 2 | | | | | 2 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn096 | | | 5 | | | | | 5 | 1 | Y/M | N | N | J | N | 0 | - |
| HDRVAMn097 | | | 1 | | | | | 1 | 1 | N/U | Y | N | J | N | 0 | - |
| HDRVAMn098 | | | 8 | | | | | 8 | 1 | Y/F | N | N | J | N | 0 | - |
| HDRVAMn099 | | | 6 | | | | | 6 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn100 | | | 1 ^a | - | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn101 | | | 1 | | | | | 1 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn102 | | | 7 | | | | | 7 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn103 | | | 4 | | | | | 4 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn104 | | | 4 | | | | | 4 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn105 | | | 3 | | | | | 3 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn106 | | | 3 | | | | | 3 | 1 | N/U | N | N | J | P | 0 | - |
| HDRVAMn107 | | | 2 | | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn108 | | | 2 | | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn109 | | | 3 | | | | | 3 | 1 | Y/M | N | N | J | N | 0 | - |
| HDRVAMn110 | | | 2 | | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn111 | | | 1 | | | | | 1 | 1 | Y/F | N | N | SA/A | N | 0 | - |
| HDRVAMn112 | | | 1 | | | | | 1 | 1 | Y/M | N | N | J | P | 0 | - |
| HDRVAMn113 | | | | 1 | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn114 | | | | 2 | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn115 | | | | 2 | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn116 | | | | 1 | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn117 | | | | 1 | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn118 | | | | 1 | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn119 | | | | 2 | | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn120 | | | | 1 | | | | 1 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn121 | | | | 1 | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn122 | | | | 3 | | | | 3 | 1 | Y/M | N | N | J | N | 0 | - |
| HDRVAMn123 | | | | 1 | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn124 | | | | 1 | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn125 | | | | 2 | | | | 2 | 1 | N/U | N | N | J | Y | 0 | - |
| HDRVAMn126 | | | | 1 ^b | | | | 1 | 1 | Y/M | Y | N | SA/A | N | 0 | - |
| HDRVAMn127 | | | | 1 ^b | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn128 | | | | 1 ^b | | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVAMn129 | | | | 1 ^b | | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVAMn130 | | | | 1 ^b | | | | 1 | 1 | N/U | N | N | J/SA | N | 0 | - |
| HDRVAMn131 | | | | 1 ^b | | | | 1 | 1 | N/U | N | N | U | N | 0 | - |
| HDRVAMn132 | | | | | 1 | | 1 | 2 | 2 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn133 | | | | | 1 ^b | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn134 | | | | | 1 ^b | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn135 | | | | | 2 | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn136 | | | | | 1 | | | 1 | 1 | N/U | Y | N | J | N | 0 | - |
| HDRVAMn137 | | | | | 2 | | | 2 | 1 | N/U | N | N | J | N | 0 | - |

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn138 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn139 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn140 | | | | | 1 | | | 1 | 1 | N/U | N | N | U | N | 0 | - |
| HDRVAMn142 | | | | | 4 | | | 4 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn143 | | | | | 2 | | | 2 | 1 | N/U | N | N | J/SA | N | 0 | - |
| HDRVAMn144 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn145 | | | | | 2 | | | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn146 | | | | | 1 ^b | | | 1 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn147 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | Y | 0 | - |
| HDRVAMn148 | | | | | 3 | | | 3 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn149 | | | | | 4 | | | 4 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn150 | | | | | 1 | | | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn151 | | | | | 2 ^a | - | | 2 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn152 | | | | | 3 | | | 3 | 1 | Y/M | Y | N | J | N | 0 | - |
| HDRVAMn153 | | | | | 2 | | | 2 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn154 | | | | | 1 | | | 1 | 1 | Y/F | Y | N | J | N | 0 | - |
| HDRVAMn155 | | | | 1 ^b | | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn156 | | | | | 2 | | | 2 | 1 | Y/F | N | N | SA/A | N | 0 | - |
| HDRVAMn157 | | | | | 1 | | | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn158 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn159 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn160 | | | | | 1 | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVAMn161 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn162 | | | | | 1 | | | 1 | 1 | N/U | Y | N | J | N | 0 | - |

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn163 | | | | | 2 | | | 2 | 1 | Y/U | Y | N | J | N | 0 | - |
| HDRVAMn164 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn165 | | | | | 1 | | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn166 | | | | | | 3 | | 3 | 1 | Y/U | Y | N | J | N | 0 | - |
| HDRVAMn167 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | Y | 0 | - |
| HDRVAMn168 | | | | | | 1 | | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn169 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn170 | | | | | | 2 | | 2 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn171 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn172 | | | | | | 3 | 1 | 4 | 2 | Y/U | Y (2) | N | SA/A | N | 0 | - |
| HDRVAMn173 | | | | | | 4 | | 4 | 1 | Y/U | Y | N | J | N | 0 | - |
| HDRVAMn174 | | | | | | 3 | 3 | 6 | 2 | N/U | Y | N | J | Y | 0 | - |
| HDRVAMn175 | | | | | | 1 | | 1 | 1 | Y/U | Y | N | J | N | 0 | - |
| HDRVAMn176 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn177 | | | | | | 4 | | 4 | 1 | N/U | Y | N | J | N | 0 | - |
| HDRVAMn178 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn179 | | | | | | 1 | 1 | 2 | 2 | N/U | N | N | J | Y | 0 | - |
| HDRVAMn180 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn181 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn182 | | | | | | 2 | | 2 | 1 | Y/U | N | N | SA | N | 0 | - |
| HDRVAMn183 | | | | | | 1 | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVAMn184 | | | | | | 4 | | 4 | 1 | Y/U | Y | N | J | N | 0 | - |
| HDRVAMn185 | | | | | | 1 | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVAMn186 | | | | | | 2 | | 2 | 1 | Y/U | Y | N | SA | N | 0 | - |

Key: Y = yes; N = no; P = possible; A = adult; SA = sub-adult; J = juvenile; C = calf; Ye = yearling; U = unknown

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn187 | | | | | | 2 | | 2 | 1 | N/U | Y | N | SA | N | 0 | - |
| HDRVAMn188 | | | | | | 1 | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVAMn189 | | | | | | 1 | | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn190 | | | | | | | 1 | 1 | 1 | Y/U | N | Y | J | N | 1 | 313 |
| HDRVAMn191 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn192 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn193 | | | | | | | 2 | 2 | 1 | Y/U | Y | N | J | N | 0 | - |
| HDRVAMn194 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn195 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn196 | | | | | | | 1 | 1 | 1 | Y/U | Y | N | SA/A | N | 0 | - |
| HDRVAMn197 | | | | | | | 2 | 2 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn198 | | | | | | | 1 | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVAMn199 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn200 | | | | | | | 2 | 2 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn201 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn202 | | | | | | | 1 | 1 | 1 | Y/U | Y | N | SA/A | N | 0 | - |
| HDRVAMn203 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVAMn204 | | | | | | | 2 | 2 | 1 | Y/U | Y | N | J | N | 0 | - |
| HDRVAMn205 | | | | | | | 2 | 2 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn206 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn207 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn208 | | | | | | | 1 | 1 | 1 | N/U | N | Y | J | N | 1 | |

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|-------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Humpback Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVAMn209 | | | | | | | 1 | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVAMn210 | | | | | | | 1 ^b | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVAMn211 | | | | | | | 1 ^b | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn212 | | | | | | | 1 ^b | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVAMn213 | | | | | | | 1 ^b | 1 | 1 | N/F | N | N | A | N | 0 | - |
| HDRVAMn214 | | | | | | | 1 ^b | 1 | 1 | N/U | N | N | C | N | 0 | - |
| Total | 66 | 91 | 168 | 38 | 69 | 48 | 38 | | | 69 | 60 / 67 | 2 | | 15 | 31 | 2264 |
| Fin Whales: | | | | | | | | | | | | | | | | |
| HDRVABp001 | 1 | | | | | | | 1 | 1 | N/U | N | N | A | N | 1 | 61 |
| HDRVABp002 | 1 | | | | | | | 1 | 1 | N/U | N | N | C | N | 0 | - |
| HDRVABp003 | 1 | | | | | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABp009 | | 2 | | | | | | 2 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVABp010 | | 2 | | | | | 1 | 3 | 2 | N/U | N | N | SA/A | N | 0 | - |
| HDRVABp011 | | 1 | | | | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABp016 | | | 1 ^b | 1 | | | | 2 | 2 | N/U | Y | N | SA | N | 0 | - |
| HDRVABp020 | | | 1 ^b | 1 | | | | 2 | 2 | N/U | Y | N | SA | N | 0 | - |
| HDRVABp027 | | | | | | | 1 | 2 | | Y/M | Y | N | SA/A | N | 0 | - |
| HDRVABp030 | | | 1 ^b | 1 ^b | | | 1 | 3 | 3 | N/U | Y | N | SA/A | P | 0 | - |
| HDRVABp035 | | | | 1 ^a | - | - | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVABp037 | | | | 1 | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVABp038 | | | | 1 | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVABp040 | | | | 1 | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVABp041 | | | | 1 | | | | 1 | 1 | N/U | Y | N | SA | N | 0 | - |

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|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| Fin Whales (continued): | | | | | | | | | | | | | | | | |
| HDRVABp042 | | | | 1 | | | | 1 | 1 | N/U | N | N | SA | N | 0 | - |
| HDRVABp060 | | | | | | 1 | | 1 | 1 | Y/U | Y | N | SA/A | N | 0 | - |
| HDRVABp092 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVABp093 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVABp094 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVABp095 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVABp096 | | | | | | | 1 | 1 | 1 | N/U | N | N | SA/A | N | 0 | - |
| HDRVABp097 | | | | | | | 1 | 1 | 1 | N/U | Y | N | J | N | 0 | - |
| HDRVABp098 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| HDRVABp099 | | | | | | | 1 | 1 | 1 | N/U | N | N | J | N | 0 | - |
| Total | 3 | 5 | 3^b | 7 | 0 | 1 | 11 | | | 0 | 7 | 0 | | 1 | 1 | 61 |
| Minke Whales: | | | | | | | | | | | | | | | | |
| HDRVABa003 | | | 1 | | | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABa004 | | | 1 | | | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABa009 | | | | | 1 | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABa010 | | | | | 1 | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABa011 | | | | | 1 | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABa012 | | | | | 1 | | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| HDRVABa013 | | | | | | 1 | | 1 | 1 | N/U | N | N | A | N | 0 | - |
| Total | | 0 | 2 | 0 | 4 | 2 | 0 | | | 0 | 0 | 0 | | 0 | 0 | 0 |

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|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------|------------------------|-----------------------------------|----------------------------|--------------------|------------------------------------|--|-------------------------|-----------------------------------|
| North Atlantic Whales: | | | | | | | | | | | | | | | | |
| 3360/'Horton' | | | | 1 ^b | | | 1 | 2 | 1 | N/F | N | N | A | N | 0 | - |
| 3821/'ZigZag' | | | | | | | 2 | 2 | 1 | N/M | N | N | A | N | 0 | - |
| 2142/'Rhino' | | | | | | | 1 | 1 | 1 | N/M | N | N | A | N | 0 | - |
| 4523 | | | | | | | 1 | 1 | 1 | N/M | N | N | A | N | 0 | - |
| 2020Calfof2642 | | | | | | | 1 | 1 | 1 | N/U | Y | Y | Ye | Y | 1 | |
| 2020Calfof1612 | | | | | | | 2 | 2 | 1 | N/U | Y | Y | Ye | Y | 1 | |
| Total | | 0 | 0 | 1 | 0 | 0 | 6 | | | 0 | 2 | 2 | | 0 | 0 | |

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^a Deceased

^b Sighting occurred on offshore survey



Appendix B
Matching of humpback,
***Megaptera novaeangliae*,**
and fin whale, *Balaenoptera*
***physalus*, biopsies**
collected by HDR

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Matching of humpback, *Megaptera novaeangliae*, and fin whale, *Balaenoptera physalus*, biopsies collected by HDR.

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Contact information: p.j.palsboll@rug.nl (phone +31 6 57779495), m.berube@rug.nl

Introduction

This report outlines the molecular genotyping, sexing and DNA sequencing of skin biopsies collected from free-ranging humpback (*Megaptera novaeangliae*) and fin (*Balaenoptera physalus*) whales by HDR. The purpose of the contracted work was to ascertain if any whales sampled by HDR had been sampled elsewhere by means of so-called "genetic tagging", which consists of identifying samples with identical genetic "fingerprints" (Palsbøll, Allen, et al., 1997). Two aspects are key to reliable genetic tagging; (a) employing sufficient genetic markers that each unique genetic fingerprint is distinct for each individual, and (b) low laboratory error rates, which can result in otherwise identical genetic fingerprints becoming non-identical due to random errors. Aspect (a) can be evaluated statistically by estimating and employing a sufficiently low probability of identity (i.e., the probability of two different individuals having identical genetic fingerprints). Aspect (b) is assessed by (i) employing more genetic markers than the bare minimum, and (ii) by conducting regular, internal assessments of the genotype and laboratory error rates. In our laboratory past assessments have yielded an error rate of one incorrect genotype for every 1,000 genotypes.

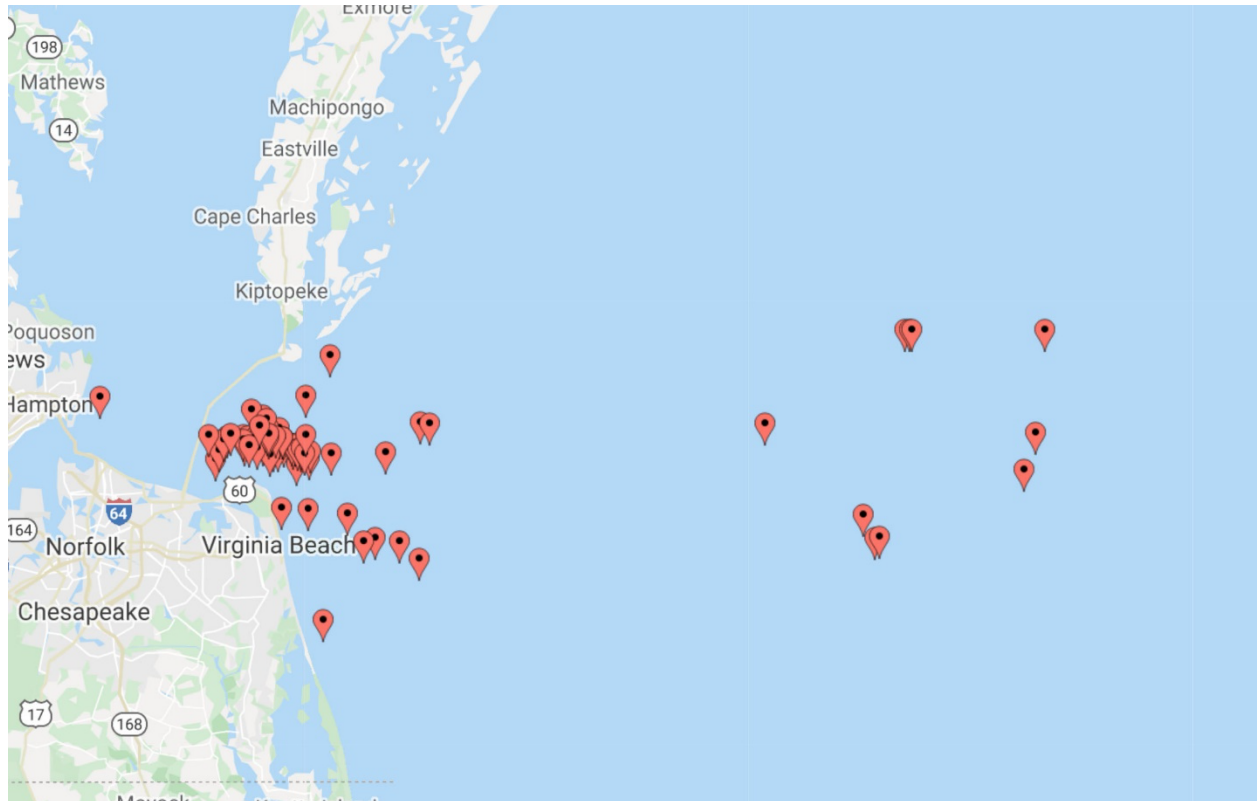
Materials and methods

HDR biopsy samples

With the exception of a single sample collected with a satellite dart, the remainder 71 samples were collected as skin biopsies from free-ranging whales, using either a compound crossbow or a

Paxarms™ biopsy gun. In total eight samples were collected from fin whales and 63 samples from humpback whales. Most humpback whale samples were collected along the coast line, except for two samples; VA180001 (HDR sample no. 2018Feb09_DTE_Mn_001) and VA190001 (HDR sample no. 2019Jan04_DTE_Mn_001). These two latter humpback whale samples were collected in the same offshore area where the fin whale samples were obtained (Figure1).

Figure 1. Location of biopsy samples.



Laboratory methods

Total-cell DNA was extracted from the supplied tissue samples using Puregene Gentra™ DNA extraction columns (Qiagen Inc.) according to the manufacturer’s instructions.

The sex of each sample was determined by the presence of a Y-chromosome specific fragment (a small region of the SRY gene on the Y chromosome), which was co-amplified during the microsatellite multiplex genotyping.

The first 400 nucleotides of the mitochondrial control region (the most variable part) were PCR-amplified (polymerase chain reaction, Mullis & Faloona, 1987), using the primers MT4F

(Arnason, Gullberg, & Widegren, 1993) and BP16071R (Drouot et al., 2004). The initial PCR amplifications were performed in a 10 μ L volume comprising 0.2 μ M of each dNTP, 67mM Tris-HCl (pH 8.8), 2mM MgCl₂, 17mM NH₃SO₄, 10mM β -mercaptoethanol, 0.1 μ M of each primer, 0.4 units of *Taq* DNA polymerase and approximately 10 - 20 ng of DNA extraction. The thermo-cycling conditions were: 2 min at 94° C, followed by 25 cycles each consisting of 15 sec. at 94°C, 30 sec. at 54°C and 120 sec. at 72°C. After amplification, unincorporated nucleotides and excess primers were enzymatically removed using *shrimp alkaline phosphatase* and *exonuclease* I as described by Werle *et al.* (1994). The cleaned PCR amplification products were sequenced using fluorescently labelled ddNTPs according to the manufacturer's instructions (Big Dye™ v3.1 Terminator Ready Reaction Mix, Life Technologies Inc.), using either the primers MT4F or BP16071R. Excess dideoxy-terminator nucleotides were removed by ethanol/EDTA precipitation and re-suspended in 10 μ L deionized formamide (Calbiochem Inc.). The order of sequencing products was resolved by capillary electrophoresis using an ABI Prism™ 3730 sequencer (Applied Biosystems Inc.).

Humpback whale samples were genotyped at 20 microsatellite loci: AC087 (Bérubé et al., 2005), EV001, EV037, EV094, EV096 (Valsecchi & Amos, 1996), 1996, GATA028, GATA098, GATA053, GATA417, TAA031 (Palsbøll, Bérubé, Larsen, & Jørgensen, 1997), GT011 (Bérubé et al., 1998), GT015, GT023, GT101, GT195, GT211, GT271, GT575 (Bérubé, Jørgensen, McEwing, & Palsbøll, 2000), GATA43950, GATA97408 and the Y-chromosome specific marker SRY (Bérubé, unpublished, and Palsbøll, Vader, & Bakke, 1992).

Fin whale samples were genotyped at 21 microsatellite loci: EV037 and EV094 (Valsecchi & Amos, 1996), GATA028, GATA098, GATA417, and TAA023 (Palsbøll, Bérubé, et al., 1997), GT011 (Bérubé et al., 1998), GT023, GT211, GT271, GT310, and GT575 (Bérubé et al., 2000), AC087 and CA234 (Bérubé et al., 2005), GATA25072, GATA43950, GATA5947654, GATA6063318, GATA91083 (Bérubé, unpublished), and EV001 (Valsecchi & Amos, 1996).

Samples were genotyped in multiplex PCR reactions (between six to eight microsatellite loci per amplification), using the MM2X™ Multiplex kit Plus (Qiagen Inc.) in 5 μ L reaction volumes. The thermocycling conditions were: 2 min. at 94°C, followed by 35 cycles each of 30 sec. at 94°C, 90 sec. at 57°C and 30 sec. at 72°C followed by a final cycle of 10 min. at 68°C. The PCR

products were separated by capillary electrophoresis using an ABI Prism™ 3730 (Applied Biosystems Inc.). The size of the amplification products was estimated against a Genescan™ ROX-500 size standard (Applied Biosystems Inc.) in the software GENEMAPPER™ (version 4.0; Applied Biosystems Inc.).

Data analyses

The goodness of fit of the observed sex ratio with parity was assessed using a log-likelihood ratio test.

The multi-locus genotypes obtained from all HDR samples were matched against the collection of individual multi-locus genotypes from North Atlantic humpback and fin whales curated by our group, in the following manner.

The minimum number of identical microsatellite locus genotypes necessary to rigorously identify samples from the same individual was determined from the probability of identity (denoted I , Paetkau & Strobeck, 1994) for unrelated individuals, estimated from the entire sample. The parameter I denotes the probability that two different individuals have an identical multi-locus genotype, and is simply the product of each locus' I . While full-siblings and parent-offspring pairs have the highest probabilities of genotype identity, they constitute a very small fraction of the total number of possible pairs (see Rew, Robbins, Mattila, Palsbøll, & Bérubé, 2011). Therefore, we employed I for unrelated individuals as guide for determining the minimum required genotypes to discern among individuals by adding loci (starting with the locus with the highest estimate of I) until the total number of expected chance matches (assuming all samples were unrelated) was below 0.001.

All samples genotyped at less than the minimum loci required to discern among different individuals (above) were removed from the data set prior to matching.

Results

The sex ratio among the seven fin whale samples were heavily male biased (6 males:1 female). Such male-bias among fin whales on foraging grounds was reported earlier by Berube and co-workers and may represent pre-mating behavior (Bérubé, Berchok, & Sears, 2001). We detected

32 males and 31 females among the 63 individual humpback whales (Table 1), a ratio that did not differ significantly from parity ($\chi^2_{1df} = 0.016, P = 0.90$).

The North Atlantic genetic humpback whale catalogue comprised a total of 9,265 genotyped at 20+ microsatellite loci. Among the 9,265 samples were 4,121, and 5,049 females and males, respectively. Sex was undetermined in 95 samples. The total number of expected single-locus genotypes (i.e., 9,265 times 20 loci) was 185,300 among which were 4,680 missing single-locus genotypes yielding an overall genotype rate at 0.975. Locus GATA43950 was only typed in 72 % of all samples. The reason for the lower genotyping rate was the identification of a so-called null-allele (and allele that is undetected), which implied that we needed to redesign the PCR amplification oligos and retype all homozygote individuals genotyped with the original PCR amplification oligos. This work update is yet to be completed and consequently, locus GATA43950 was removed from all samples resulting in a genotype rate at 0.988 for 19 loci.

The expected number of pairs of different individuals matching at all 19 loci among a total of 579,726 possible pairs (63 HDR samples compared to 9,202 non-HDR samples) were estimated at $4.2 \cdot 10^{-3}$, $5.52 \cdot 10^{-7}$ and $1.82 \cdot 10^{-15}$ for full-siblings, parent-offspring pairs and unrelated individuals, respectively (Table 1). Since a chance match at 11 loci between unrelated individuals (the vast majority of comparisons) was estimated at less than 0.001 pairs (of 579,726); a match at 11 loci was set as the threshold for identifying unique individuals (Table 1). Accordingly, all specimens with identical microsatellite genotypes at minimum 11 loci were inferred as duplicate specimens collected from the same individual.

Table 1. Probability of identical multi-locus genotypes between different individuals

| # Loci | Locus | <i>Full siblings</i> | | | <i>Parent-offspring</i> | | | <i>Unrelated</i> | | |
|-----------|--------------|----------------------|------------------|-------------------------|-------------------------|------------------|-------------------------|------------------|------------------|-------------------------|
| | | I_{locus} | $I_{cumulative}$ | $N_{expected\ matches}$ | I_{locus} | $I_{cumulative}$ | $N_{expected\ matches}$ | I_{locus} | $I_{cumulative}$ | $N_{expected\ matches}$ |
| 1 | GATA028 | 0.516 | 5.16E-01 | 2.99E+05 | 0.531 | 5.32E-01 | 3.08E+05 | 0.304 | 3.05E-01 | 1.77E+05 |
| 2 | EV001 | 0.462 | 2.38E-01 | 1.38E+05 | 0.424 | 2.26E-01 | 1.31E+05 | 0.23 | 7.01E-02 | 4.06E+04 |
| 3 | GT271 | 0.462 | 1.10E-01 | 6.39E+04 | 0.425 | 9.59E-02 | 5.56E+04 | 0.209 | 1.46E-02 | 8.49E+03 |
| 4 | GT195 | 0.427 | 4.70E-02 | 2.73E+04 | 0.353 | 3.39E-02 | 1.96E+04 | 0.197 | 2.88E-03 | 1.67E+03 |
| 5 | EV094 | 0.395 | 1.86E-02 | 1.08E+04 | 0.291 | 9.84E-03 | 5.70E+03 | 0.134 | 3.86E-04 | 2.24E+02 |
| 6 | GATA098 | 0.411 | 7.65E-03 | 4.43E+03 | 0.322 | 3.17E-03 | 1.84E+03 | 0.131 | 5.04E-05 | 2.92E+01 |
| 7 | GT575 | 0.398 | 3.04E-03 | 1.76E+03 | 0.295 | 9.37E-04 | 5.43E+02 | 0.108 | 5.47E-06 | 3.17E+00 |
| 8 | GT101 | 0.372 | 1.13E-03 | 6.55E+02 | 0.243 | 2.28E-04 | 1.32E+02 | 0.094 | 5.16E-07 | 2.99E-01 |
| 9 | GATA97408 | 0.362 | 4.09E-04 | 2.37E+02 | 0.223 | 5.09E-05 | 2.95E+01 | 0.077 | 3.96E-08 | 2.30E-02 |
| 10 | GT015 | 0.35 | 1.43E-04 | 8.29E+01 | 0.2 | 1.02E-05 | 5.91E+00 | 0.064 | 2.53E-09 | 1.46E-03 |
| 11 | GT211 | 0.342 | 4.90E-05 | 2.84E+01 | 0.185 | 1.88E-06 | 1.09E+00 | 0.06 | 1.52E-10 | 8.81E-05 |
| 12 | GT023 | 0.341 | 1.67E-05 | 9.68E+00 | 0.182 | 3.43E-07 | 1.99E-01 | 0.057 | 8.70E-12 | 5.04E-06 |
| 13 | TAA031 | 0.341 | 5.70E-06 | 3.30E+00 | 0.182 | 6.26E-08 | 3.63E-02 | 0.056 | 4.90E-13 | 2.84E-07 |
| 14 | EV096 | 0.341 | 1.94E-06 | 1.13E+00 | 0.181 | 1.13E-08 | 6.57E-03 | 0.056 | 2.74E-14 | 1.59E-08 |
| 15 | GT011 | 0.337 | 6.54E-07 | 3.79E-01 | 0.174 | 1.97E-09 | 1.14E-03 | 0.053 | 1.46E-15 | 8.45E-10 |
| 16 | GATA053 | 0.334 | 2.19E-07 | 1.27E-01 | 0.169 | 3.33E-10 | 1.93E-04 | 0.05 | 7.24E-17 | 4.20E-11 |
| 17 | AC087 | 0.334 | 7.30E-08 | 4.23E-02 | 0.167 | 5.58E-11 | 3.23E-05 | 0.047 | 3.41E-18 | 1.98E-12 |
| 18 | GATA417 | 0.321 | 2.34E-08 | 1.36E-02 | 0.142 | 7.90E-12 | 4.58E-06 | 0.035 | 1.19E-19 | 6.92E-14 |
| 19 | EV037 | 0.31 | 7.27E-09 | 4.21E-03 | 0.12 | 9.52E-13 | 5.52E-07 | 0.026 | 3.14E-21 | 1.82E-15 |

Removing all samples typed at fewer than 11 loci (a total of 51 samples) resulted in a final data set at 9,214 samples (incl. the 63 HDR samples) typed at 11 - 19 loci and an overall genotyping rate at 0.992 (i.e., the vast majority of samples were genotyped at all 19 loci). The samples sizes per general area and range of sampling years are summarized in Table 2. It is important to realize that apart from a few areas then the overall sampling effort has been highly heterogenous across space and time.

Table 2. Sample sizes per general area and sampling years

| General area | sampling years | samples |
|-----------------------------------|----------------|---------|
| eastern US sea border | 1990-2019 | 2,546 |
| eastern Canadian sea border | 1990-2019 | 1,157 |
| Central Atlantic | 1991-2017 | 419 |
| eastern North Atlantic sea border | 1988-2019 | 473 |
| western Caribbean | 1989-2005 | 4,538 |
| eastern Caribbean and Cape Verde | 1995-2015 | 78 |
| Miscellaneous | | 3 |

No duplicate samples were identified among the HDR humpback whale samples. A total of 18 HDR samples matched to samples collected elsewhere along the US eastern sea border (Table 3). All samples matched 100% on all loci genotyped in both samples in each pair (i.e., no mismatching genotypes were detected). In addition, as an additional affirmation, the sex and mitochondrial control region DNA sequences agreed for all matching pairs. The sample identification numbers in Table 3 are reference numbers for the matching samples. Additional information regarding each matching individuals can be obtained by contacting the institution that provided the sample. These institutes have also been informed about matches and provided the HDR reference number. Contact information to the relevant person is provided in Table 3. Samples collected by WCS are mainly from the New York coastal area. Although the majority of samples provided by the Center for Coastal Studies (CCS) originate from the Gulf of Maine, CCS also receives samples from their network of collaborators from other areas along the US eastern sea border.

Table 3. HDR humpback whale samples matching to samples collected by other institutions

| Sample ID | Matching reference | Sex | Matching sample reference | # Matching loci |
|-----------|--------------------|--------|---------------------------|-----------------|
| VA150001 | HDR001 | female | WCS001 | 18 |
| VA150006 | HDR002 | female | WCS002 | 19 |
| VA150007 | HDR003 | male | CCS001 | 18 |
| VA150011 | HDR004 | female | CCS002 | 14 |
| VA160009 | HDR005 | male | CCS003 | 19 |
| VA160011 | HDR006 | female | CCS004 | 19 |
| VA160011 | HDR007 | female | CCS005 | 19 |
| VA160014 | HDR008 | female | CCS006 | 18 |
| VA170005 | HDR009 | male | CCS007 | 19 |
| VA170006 | HDR010 | male | CCS008 | 18 |
| VA170009 | HDR011 | female | CCS009 | 19 |
| VA170009 | HDR012 | female | CCS010 | 15 |
| VA170010 | HDR013 | male | CCS011 | 19 |
| VA180001 | HDR014 | male | CCS012 | 19 |
| VA190003 | HDR015 | female | CCS013 | 18 |
| VA190004 | HDR016 | female | CCS014 | 14 |
| VA190005 | HDR017 | female | CCS015 | 19 |
| VA190006 | HDR018 | male | CCS016 | 19 |

Notes: WCS reference samples contact: Dr. Howard Rosenbaum, Wildlife Conservation Society, email: hrosenbaum@wcs.org, CCS reference samples contact: Dr. Jooke Robbins, Center for Coastal Studies, email: jrobbins@coastalstudies.org

Following the above procedures nine loci were deemed sufficient to discern amongst individuals. A single pair of duplicate samples was detected between two fin whale samples (the two HDR fin whale biopsy samples collected in 2015). None of the HDR fin whale samples matched to the 1,789 (incl. HDR samples) samples contained in the North Atlantic genetic fin whale archive genotyped at 21 microsatellite loci at a genotyping rate of 0.9909. The samples in the data base are summarized in Table 4.

Table 4. Sample areas, years and sample sizes for fin whales

| General area | sampling years | total sample size |
|-----------------------------|----------------|-------------------|
| eastern US sea border | 1991-2019 | 193 |
| eastern Canadian sea border | 1990-2019 | 582 |
| Central North Atlantic | 1985-2018 | 479 |
| eastern North Atlantic | 1982-2020 | 535 |

Data availability

All genetic data generated from the HDR samples were transmitted by in attachments along with this report. The sex and microsatellite genotypes are provided in a so-called one-line STRUCTURE format (Pritchard, Stephens, & Donnelly, 2000), where the first column after sample identification number denotes the sex, and a **-9** denotes missing data. The humpback file is named `hdr_mn_genotypes_w_sex.str`. The fin whale genotype data are in file `hdr_bp_genotypes_w_sex.str`. Mitochondrial control region DNA sequences are also provided as an attachment in fasta format. The file for the humpback DNA sequence data is named `hdr_mn_mt_sequences.fas`, and the fin whale DNA sequence data file `hdr_bp_mt_sequences.fas`.

A brief cautionary note on interpretations of matches

While matches identified in this analysis can be viewed as "true" and provide insights into connections between the whales sampled by HDR and other areas, great caution should be applied with regards to absences of matches as well as relative matching rates. As mentioned above the sampling effort varies substantially across time and space. The lack of any matches to the fin whale HDR biopsies in our database is likely due to the observation that the overall abundance of fin whales in the North Atlantic is ~70,000 individuals, which implies that ~ 1,800 samples represent a very low sampling proportion and hence a low chance of a match overall.

Acknowledgements

The work presented here would have been impossible but for our many collaborators who have tirelessly collected and contributed samples over three decades

Appendix I. HDR and corresponding laboratory sample identification numbers and molecular sex determinations

| Original Animal Number This is HDR's ID number | Current Animal Number This is SAMPLE number | Species | MarECon Ids | Sex |
|---|--|------------------------|-------------|--------|
| HDRVAMn009 | 20150102_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150001 | female |
| HDRVAMn010 | 20150106_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150002 | male |
| HDRVAMn011 | 20150106_DTE_Mn_002 | <i>M. novaeangliae</i> | VA150003 | female |
| HDRVAMn013 | 20150111_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150004 | female |
| HDRVAMn014 | 20150111_DTE_Mn_002 | <i>M. novaeangliae</i> | VA150005 | female |
| HDRVAMn015 | 20150111_DTE_Mn_003 | <i>M. novaeangliae</i> | VA150006 | female |
| HDRVAMn023 | 20150120_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150007 | male |
| HDRVAMn024 | 20150122_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150008 | male |
| HDRVAMn025 | 20150122_DTE_Mn_002 | <i>M. novaeangliae</i> | VA150009 | male |
| HDRVAMn005 | 20150129_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150010 | female |
| HDRVAMn027 | 20150129_DTE_Mn_002 | <i>M. novaeangliae</i> | VA150011 | female |
| HDRVAMn029 | 20150209_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150012 | male |
| HDRVABp005 | 20150429_DTE_Bp_001 | <i>B. physalus</i> | VA150013 | male |
| HDRVABp005 | 20150429_DTE_Bp_002 | <i>B. physalus</i> | VA150014 | male |
| HDRVAMn039 | 20151207_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150015 | male |
| HDRVAMn041 | 2015Dec09_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150016 | male |
| HDRVAMn044 | 2015Dec10_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150017 | female |
| HDRVAMn045 | 2015Dec20_DTE_Mn_001 | <i>M. novaeangliae</i> | VA150018 | male |
| HDRVAMn048 | 2015Dec20_DTE_Mn_002 | <i>M. novaeangliae</i> | VA150019 | male |
| HDRVAMn052 | 2016Jan15_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160001 | female |
| HDRVAMn050 | 2016Jan15_DTE_Mn_002 | <i>M. novaeangliae</i> | VA160002 | male |
| HDRVAMn051 | 2016Jan15_DTE_Mn_003 | <i>M. novaeangliae</i> | VA160003 | male |
| HDRVAMn054 | 2016Jan15_DTE_Mn_004 | <i>M. novaeangliae</i> | VA160004 | male |
| HDRVAMn063 | 2016Feb09_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160005 | male |
| HDRVAMn061 | 2016Feb17_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160006 | male |
| HDRVAMn069 | 2016Nov01_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160007 | female |
| HDRVAMn071 | 2016Nov01_DTE_Mn_002 | <i>M. novaeangliae</i> | VA160008 | male |
| HDRVAMn031 | 2016Nov03_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160009 | male |
| HDRVAMn059 | 2016Nov03_DTE_Mn_002 | <i>M. novaeangliae</i> | VA160010 | male |
| HDRVAMn049 | 2016Nov18_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160011 | female |
| HDRVAMn064 | 2016Dec13_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160012 | female |
| HDRVAMn012 | 2016Dec21_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160013 | female |
| HDRVAMn082 | 2016Dec21_DTE_Mn_002 | <i>M. novaeangliae</i> | VA160014 | female |
| HDRVAMn081 | 2016Dec28_DTE_Mn_001 | <i>M. novaeangliae</i> | VA160015 | female |
| HDRVAMn084 | 2017Jan01_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170001 | female |
| HDRVAMn084? | 2017Jan01_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170002 | female |
| HDRVAMn066 | 2017Jan01_DTE_Mn_002 | <i>M. novaeangliae</i> | VA170003 | male |
| HDRVAMn092 | 2017Jan11_DTE_Mn_001 | | | |
| HDRVAMn095 | 2017Jan16_DTE_Mn_001*Jan17 on tube | <i>M. novaeangliae</i> | VA170004 | male |
| HDRVAMn092 | 2017Jan19_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170005 | male |
| HDRVAMn090 | 2017Jan21_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170006 | male |
| HDRVAMn101 | 2017Jan21_DTE_Mn_002 | <i>M. novaeangliae</i> | VA170007 | male |
| HDRVAMn102 | 2017Jan25_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170008 | male |
| HDRVAMn007 | 2017Feb01_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170009 | female |
| HDRVAMn091 | 2017Feb02_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170010 | male |
| HDRVAMn099 | 2017Feb02_DTE_Mn_002 | <i>M. novaeangliae</i> | VA170011 | female |
| HDRVAMn088 | 2017Feb06_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170012 | male |
| HDRVAMn093 | 2017Feb06_DTE_Mn_002 | <i>M. novaeangliae</i> | VA170013 | female |
| HDRVAMn098 | 2017Feb06_DTE_Mn_003 | <i>M. novaeangliae</i> | VA170014 | female |
| HDRVAMn104 | 2017Feb17_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170015 | female |
| HDRVAMn105 | 2017Feb17_DTE_Mn_002 | <i>M. novaeangliae</i> | VA170016 | female |
| HDRVAMn111 | 2017Feb24_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170017 | female |
| HDRVAMn096 | 2017Feb24_DTE_Mn_002 | <i>M. novaeangliae</i> | VA170018 | male |
| HDRVAMn112 | 2017Mar21_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170019 | male |
| HDRVAMn109 | 2017Mar21_DTE_Mn_002 | <i>M. novaeangliae</i> | VA170020 | male |
| HDRVABp019 | 2017May10_DTE_Bp_001 | <i>B. physalus</i> | VA170021 | female |
| HDRVABp026 | 2017Aug17_DTE_Bp_001 | <i>B. physalus</i> | VA170022 | male |
| HDRVABp027 | 2017Aug17_DTE_Bp_002 | <i>B. physalus</i> | VA170023 | male |
| HDRVAMn120 | 2017Dec22_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170024 | male |
| HDRVAMn122 | 2017Dec29_DTE_Mn_001 | <i>M. novaeangliae</i> | VA170025 | male |
| HDRVAMn126 | 2018Feb09_DTE_Mn_001 | <i>M. novaeangliae</i> | VA180001 | male |
| HDRVAMn132 | 2018Jul31_DTE_Mn_001 | <i>M. novaeangliae</i> | VA180002 | female |
| HDRVAMn146 | 2019Jan04_DTE_Mn_001 | <i>M. novaeangliae</i> | VA190001 | female |
| HDRVAMn153 | 2019Feb03_DTE_Mn_001 | <i>M. novaeangliae</i> | VA190002 | female |
| HDRVAMn154 | 2019Feb03_DTE_Mn_002 | <i>M. novaeangliae</i> | VA190003 | female |
| HDRVAMn151 | 2019Jan31_DTE_Mn_001 | <i>M. novaeangliae</i> | VA190004 | female |
| HDRVAMn152 changed to HDRVAMn156 | 2019Feb14_DTE_Mn_001 | <i>M. novaeangliae</i> | VA190005 | female |
| HDRVAMn154 changed to HDRVAMn152 | 2019Mar02_DTE_Mn_001 | <i>M. novaeangliae</i> | VA190006 | male |
| HDRVAMn163 | 2019May04_DTE_Mn_001 | <i>M. novaeangliae</i> | VA190007 | female |
| HDRVABp049 | 2018Apr22_DTE_Bp_001 | <i>B. physalus</i> | VA180003 | male |
| HDRVABp048 | 2018Apr22_DTE_Bp_002 | <i>B. physalus</i> | VA180004 | male |
| HDRVABp046 | 2018Apr22_DTE_Bp_003 | <i>B. physalus</i> | VA180005 | male |

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