2020-01-01

Specimens Of Opportunity Reveal Novel Information On The Sowerby's Beaked Whale (Mesoplodon bidens)

Kerri Jean Smith
University of Texas at El Paso

SPECIMENS OF OPPORTUNITY REVEAL NOVEL INFORMATION

ON THE SOWERBY'S BEAKED WHALE

(*MESOPLODON BIDENS*)

KERRI JEAN SMITH

Doctoral Program in Environmental Science and Engineering

APPROVED:

Markus J. Peterson, Ph.D., Chair

Philip Lavretsky, Ph.D.

Tarla Rai Peterson, Ph.D.

Craig E. Tweedie, Ph.D

Stephen L. Crites, Jr., Ph.D.
Dean of the Graduate School

Dedication

To Fynn and Zuley, who started this journey with me and were faithful companions.

SPECIMENS OF OPPORTUNITY REVEAL NOVEL INFORMATION ON THE SOWERBY'S BEAKED WHALE (*MESOPLODON BIDENS*)

by

KERRI JEAN SMITH, B.S., M.S.

DISSERTATION

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

Department of Environmental Science and Engineering

THE UNIVERSITY OF TEXAS AT EL PASO

May 2020

Acknowledgements

"Science is a participation sport; choose your team wisely." – Rebecca Barnes

The work and collaborative effort that went into this dissertation exemplifies Barnes' quote. This research would not have been possible without the incredible team who supported me throughout this endeavor. First and foremost, I want to thank my Ph.D. chair, Dr. Markus J. Peterson, for his endless mentoring and support. Thank you, Dr. Peterson, for taking a chance on this project, giving me the space and support to run with it, and for your patience and encouragement throughout this process. None of this would have been possible without you.

My committee members, Drs. Tarla Rai Peterson, Philip Lavretsky, and Craig Tweedie have been invaluable throughout this process. Thank you for the guidance, brainstorming sessions, and support over the last 5 years. I could not have assembled a better science team. Thank you to Dr. James Mead, emeritus marine mammal curator at the National Museum of Natural History and all-around beaked whale expert, for your friendship and enthusiasm. Being quizzed on beaked whale species from photos of beach cast carcasses will remain amongst my fondest memories. To Dr. Clive N. Trueman, thank you for the stable isotope support, 6-hour time zone difference brainstorming sessions, and helping me work through partially constructed ideas. To Dr. Jed Sparks and Kim Sparks, thank you for teaching me how to extract stable isotope data, for hosting me in your lab, and allowing me to stay in your home.

I am eternally grateful to all the museum curators who answered my emails, provided recommendations and referrals, and opened their collections to this project. And to the thousands of individuals, modern and historical, who collected these and millions of other biological specimens, saw their value when others may not, and preserved them and the information on their collection. This is a labor of love all too often unrecognized.

Along the way I have received the support and friendship of so many: Dr. Katie Wedemeyer-Strombel, Allie Alvis, Amorita Armendariz, Perry Houser, Stacy Stoops, Allison Wilkes St. Clair, Aishwarya Pillai, and Mike Aebly, to name only a few. Thank you! And finally,

thank you to my mom and sister, who supported me every step of the way and have taken wonderful care of my pups during my extended absences

Abstract

Conservation science requires quickly acquiring information and taking action in order to protect species at risk of extinction. Elusive wildlife, however, present challenges to effective conservation measures because it is often difficult to collect enough data on these species to recognize their conservation needs and implement management plans. As a result, researchers must identify alternative means of gathering sufficient data on these species. Specimens of opportunity, such as museum specimens, provide a way to improve knowledge on these species, and these specimens have already proven valuable by increasing information on biodiversity, habitat and range, and population structure in many species.

Beaked whales (family Ziphiidae) are a prime example of elusive species, and as a result little is known about their biology and ecology. This speciose group of cetaceans is challenging to locate and distinguish in situ due to the elusive behavior and similar appearance amongst species. Thus, specimens of opportunity may be the most efficacious means of gathering enough information on beaked whales to make informed conservation decisions.

In this study I utilized specimens of opportunity from museum and research institutions to increase knowledge and generate data on the Sowerby's beaked whale (*Mesoplodon bidens*). First described in 1804, little is known about this species' basic biology or ecology. I employed snowball sampling to identify museums with specimens and collected data on 180 specimens from 24 museum and research institutions. I collected data on specimen collection date and location, sex, age class, and 7 skull and mandibular measurements. I quantified skull and mandibular measurements and found that specimens collected in the west Atlantic demonstrated significantly greater median values for total skull length, proximal beak width, total mandibular length, and mandibular symphysis to distal end length. Quadratic discriminant analysis of skull

and mandibular measurements successfully assigned specimens to their collection location in 78.6% of specimens, suggesting this species may be comprised of ≥2 distinct populations.

For 178 specimens I collected a bone or soft tissue sample for stable isotope analysis. It was not possible to sample the same location in each specimen, which necessitated a means to account for variation in isotope values among tissues, and especially across multiple skeletal elements. To quantify carbon and nitrogen intraskeletal variation I sampled the same eight skeletal elements from 72 cetacean skeletons from 14 cetacean species. Isotope variation across skeletons was greater than anticipated based on previous studies. Carbon intraskeletal ranges varied from 0.4 to 7.6‰, with 84.7% (n = 61) of skeletons having a range >1‰, and 55.5% (n = 40) exhibiting a range >2‰. Similarly, nitrogen intraskeletal ranges varied from 0.4 to 5.2‰, with 59.7% (n = 43) of skeletons exhibiting a range >1‰, and 15.3% (n = 11) with a range >2‰. For Sowerby's beaked whale, I identified a median carbon intraskeletal variation of ~4.1‰ and nitrogen variation of ~1.3‰.

For soft tissue samples I needed to verify that the current lipid extraction methods were appropriate for the samples I had collected. Some cetacean tissues, such as skin and muscle, are depleted in ^{13}C compared to synthesized proteins, so that the presence of lipids within protein samples tends to reduce bulk tissue $\delta^{13}C$ values and influence stable isotope analysis. However, extraction methods can also alter stable isotope ratios, especially nitrogen. Thus, I trialed two extraction methods, chloroform-methanol and cyclohexane, and identified the appropriate extraction for each soft tissue type. For low or moderate lipid proportion tissues, such as kidney and muscle, cyclohexane should be used, and chloroform:methanol for higher lipid proportion tissues such as skin.

Finally, I conducted stable isotope analysis for $\delta^{13}C$ and $\delta^{15}N$ on Sowerby's beaked whale bone, muscle, and skin tissues. I found consistent trends in isotope values across all three tissue types. Specimens collected in the east Atlantic had less enriched $\delta^{13}C$ and $\delta^{15}N$ than west Atlantic specimens, and median isotope values were significantly different between regions. Quadratic discriminant analysis considering $\delta^{13}C$ and $\delta^{15}N$ simultaneously correctly assigned 92.0, 90.0, and 80.3% of skin, muscle, and bone samples, respectively, to their collection location. These results indicate the Sowerby's beaked whale specimens in this study exhibited short- and long-term regional site fidelity to the region from which they were collected.

These stable isotope data from bone, muscle, and skin samples, combined with the significant differences in median skull measurements between regions, strongly suggests the Sowerby's beaked whale exhibits a metapopulation structure. This information lays the groundwork for future studies in this species and provides critical knowledge regional and international conservationist scientists need.

Table of Contents

List of Tables

List of Figures

Note to Readers

I have divided this dissertation into 5 chapters: Chapters 1–4 are the body of the dissertation, and Chapter 5 summarizes the main findings and provides recommendations for future research. All chapters are written in the first-person plural because they are a result of collaborative effort; however, I conducted the majority of project design, data collection, analyses, and am lead author on the manuscripts. Chapter 2 has been published in the peer-reviewed journal *Frontiers in Marine Science*, and Chapter 3 has been accepted for publication in the peer-reviewed journal *Rapid Communications in Mass Spectrometry*. Chapter 1 provides justification for this project, describes the sampling method for identifying museum specimens, and contains information about the specimens in this study. Chapter 2 examines intraskeletal isotopic variation in cetacean skeletons. Chapter 3 compares lipid removal methods from cetacean soft tissue samples. And Chapter 4 compares carbon and nitrogen isotope values from specimens collected throughout the species' range. This format led to some overlap in the introductory material of Chapters 1–4, as well as some overlap in the discussion among these chapters. I compiled all references into a comprehensive list at the end of this dissertation.

Chapter 1: Learning from specimens of opportunity: the Sowerby's beaked whale (*Mesoplodon bidens*) as a case study

1.1 Abstract

Elusive species are challenging to study and conserve because basic elements of their biology may be unknown. Specimens of opportunity provide a means of collecting information on these species and may be critical for elusive species conservation. Beaked whales, such as Sowerby's beaked whale (*Mesoplodon bidens*) are prime examples of elusive species because they are challenging to locate and study in situ. We used snowball sampling to identify Sowerby's beaked whale specimens in museums and research institutions. We collected data on specimen collection date and location, sex, age class, and 7 skull and mandibular measurements where possible. Snowball sampling proved highly effective, as we located 180 specimens from 24 institutions in North America and Europe, 62 of which were not listed in online collections databases, resulting in the largest collated dataset for this species. We included original collection location for 174 specimens; sex for 115 specimens; age class for 159 specimens; and skull and mandibular measurements for 112 specimens. We quantified skull and mandibular measurements and found that specimens collected in the west Atlantic demonstrated significantly greater median values for total skull length, proximal beak width, total mandibular length, and mandibular symphysis to distal end length, suggesting there may be population structure in this species. We recommend other researchers consider snowball sampling when designing research projects attempting to identify specimens in collections that may otherwise be overlooked. These data provide critical data on this elusive species, and demonstrate the effectiveness of specimens of opportunity in elusive species research.

1.2 Introduction

1.2.1 Background

Conservation of elusive species is challenging because often there are large gaps in knowledge regarding these species' biology and ecology (Cunningham & Lindenmayer 2005; McKelvey et al. 2008). As a result, the conservation needs of elusive species may be unknown; thus, such species may not receive adequate protection and management. Researchers face many challenges in trying to fill the knowledge gaps associated with elusive species because traditional field research methods, such as mark recapture or satellite tagging, may be ineffective, particularly if the species is rare, difficult to capture, has a large range, or actively avoids researchers (Kalton & Anderson 1986; Green & Young 1993; McDonald 2004; Meek et al. 2014b). For many elusive species, specimens of opportunity may be the most effective way to fill these knowledge gaps, and for some species they may be the only source of reliable knowledge (Robbirt et al. 2011; Roberts et al. 2016). Specimens of opportunity, such as salvaged carcasses and museum specimens, have proved essential in identifying new species, clarifying a species' range and population structure, and in retrospective analyses of biodiversity (Newbold 2010; Holmes et al. 2016; Baus et al. 2019; Coombs et al. 2019; Schwartz et al. 2020). As many museums and research institutions face financial pressure to justify maintenance and upkeep of research collections, it is increasingly important to highlight the value of these collections to wildlife and conservation studies.

Beaked whales (family Ziphiidae) are some of the most elusive and poorly understood mammals. Comprised of at least 23 species, this diverse group of whales accounts for >25% of extant whale and dolphin species, and most information on their biology has come from specimens of opportunity (Dalebout et al. 2004; Mead 2007, 2009). Beaked whales are difficult

to locate at sea due to a variety of factors: they often occur in smaller groups than many other whale and dolphin species, are gray in coloration with a streamlined fusiform body shape lacking a prominent dorsal fin, and may actively avoid research vessels (MacLeod et al. 2005; Ellis et al. 2017). They are thought to prefer deeper, pelagic habitats, which when coupled with their general lack of aerial displays, can make it nearly impossible to locate and study them in situ. When they are located, it is challenging to identify most species, and many at-sea sightings can only be reliably classified to the genus level, with possibly a suggestion of the species. Thus, specimens of opportunity provide the most reliable means of collecting species-specific information for many beaked whales.

Specimens of opportunity have proven critical to increasing our knowledge and understanding of beaked whale diversity, morphology, and biology. Genetic analyses of museum specimens has identified four new species of beaked whales in the last 20 years, and three species of beaked whales—*Mesoplodon bowdoini*, *M. traversii*, and *M. hotaula*—are known only from morphological and genetic analyses of stranded specimens (Dalebout et al. 2002; van Helden et al. 2002; Dalebout et al. 2014; Morin et al. 2017; Yamada et al. 2019). Phylogenies have been generated using specimens of opportunity, resolving questions of relatedness among certain species (Dalebout et al. 2008; Einfeldt et al. 2019b); comparative anatomy of beach-cast carcasses has helped to identify species-specific morphological differences (Mead 2007; Lambert et al. 2011; March et al. 2016); and necropsies have shed light on beaked whale parasites and diseases, sexual maturity, and gestation times (Besharse 1971; Auriolesgamboa 1992; Macleod & Herman 2004; Landrau-Giovannetti et al. 2020).

The Sowerby's beaked whale (*M. bidens*) was the first mesoplodont beaked whale to be described (Sowerby 1806), yet little has been learned about its biology or behavior in >200

years. This species strands relatively frequently compared to other beaked whales, and specimens have been stored in museums in North America and Europe. Most of what is known about Sowerby's beaked whales has come from specimens of opportunity, yet no studies have compiled information about these specimens. In this study we: (1) summarize the existing literature on Sowerby's beaked whales and identify critical gaps in knowledge; (2) detail our sampling method and discuss how it can be applied to other studies; and (3) characterize the specimens we located and provide new data on this species.

1.2.2 Literature summary

In 1804, James Sowerby described the Sowerby's beaked whale from a single male specimen that stranded in the Moray Firth, Scotland, naming it "bidens" because of the presence of only two teeth, both in the lower jaw (Sowerby 1806). This species is thought to exclusively inhabit the North Atlantic, ranging from Norway to the Canary Islands in the east and from Newfoundland to the northeast of the United States, although specimens have been collected as far south in the United States as Florida and Georgia (Dix et al. 1986; Bonde & Oshea 1989; Lien et al. 1990; Carlstrom et al. 1997; Martin et al. 2011). Strandings often are a single animal or a mother-calf pair, and when observed at sea they occur in small groups of ≤10 animals. In 2011, attempts were made to tag Sowerby's beaked whales in the Azores, a location where they are reported to be regularly sighted, especially during the summer. A single animal was successfully tagged, but the animal immediately dislodged the tag (Visser 2012). Recently, a few acoustic recordings have been made of Sowerby's beaked whales, laying the groundwork for passive acoustic monitoring for the presence of this species (Cholewiak et al. 2013; Stanistreet et al. 2016; Stanistreet et al. 2017; Clarke et al. 2019). There are no data regarding this species' abundance or movement behavior anywhere in its range.

Males and females are sexually dimorphic, with males displaying a single pair of tusks in the middle of the lower jaw; smaller versions of these teeth are present in females, but they do not erupt (Macleod & Herman 2004). Tooth placement is a distinguishing feature among beaked whales and is the best diagnostic tool for differentiating Sowerby's beaked whales from other mesoplodont beaked whales in the North Atlantic. The exact purpose of these tusks is unknown but based on the extensive scarring pattern observed on males it is assumed they are important in sexual displays and competitions, where the males scratch, or rake, each other with their tusks. Most other morphological characteristics have not been well defined in this species; however, Mead (2007) identified differences in stomach anatomy among beaked whales, and found that Sowerby's beaked whales have a derived stomach anatomy most similar to 2 other North Atlantic species, *M. europaeus* and *M. mirus*. Body and fluke measurements are available from a single individual (Bose et al. 1990). There are no data on most aspects of this species' reproductive biology, including age of sexual maturity in either males or females, gestation time, or how long calves remain with their mothers.

Little genetic information is available for this species. Mitochondrial and nuclear analyses suggest the Sowerby's beaked whale may be the most basal member of the genus (Einfeldt et al. 2019b; Mcgowen et al. 2019). Regionally distinct and overlapping mitochondrial haplotypes were identified in east and west Atlantic specimens; however, the results of this analysis were never published (COSEWIC 2006). No studies have investigated population structure or genetic diversity in Sowerby's beaked whales.

1.3 Materials and methods

1.3.1 Locating specimens of opportunity

We used a common social science technique called snowball sampling to identify museums or research institutions with Sowerby's beaked whale specimens (Goodman 1961; Wright & Stein 2005). In this method, a set of informants is identified from a larger population; these individuals then are asked to refer others to participate in the study. In 2015, we used GBIF, VertNet, and the Smithsonian National Museum of Natural History (NMNH) mammal collection online databases to identify 42 Sowerby's beaked whale specimens in three museum collections: the NMNH (n = 14), National Museums Scotland (NMS) (n = 15), and the Natural History Museum, London (NHM) (n = 13). We began sampling in 2016 at the NMNH and during this trip met a curator from the University Museum of Bergen, Norway, who confirmed the presence of Sowerby's beaked whales in that museum's collection, despite their not being listed in an online database, and invited us to sample them. This trip then led to recommendations of other collections to contact, and each subsequent visit to a museum or research institution led to additional suggestions of collections to visit.

1.3.2 Data collection and evaluation

We collected data from specimens originating in both the east and west Atlantic, here defined as the specimen's original collection location being on either side of longitude 35 west. For each specimen we recorded sex, age class, collection location, and the condition of the specimen (Appendix Table 1.1). For some specimens, portions of this information were missing, particularly sex and age class. We evaluated tooth size, overall specimen size, and degree of skeletal suture fusion to infer sex and age class in some specimens; however, in many this was not possible, and we recorded this information as "unknown." Where possible, we used the same

soft 72 cm tape measure to measure seven skull and mandibular elements: total skull length (TSL); braincase width (BCW); proximal beak width (PBW); beak length (BL); total mandibular length (TML); mandibular skull to symphysis length (MSSL); and mandibular symphysis to distal length (MSDL) (Figure 1.1). We collected measurements from 62% of specimens (n = 112); however, some of these skulls and mandibles were incomplete and we could only collect certain of these measurements (Appendix Table 1.1). For specimens we did not measure, the skulls and mandibles were not collected or preserved, were on display and thus inaccessible, or were broken or disassembled.

We used descriptive statistics to quantify the seven skull and mandibular measurements. Next, we used unpaired Wilcoxon tests to evaluate differences in median measurement values by specimen sex and region of specimen collection; we considered p-values ≤ 0.05 significant. We then evaluated the ratio of total mandibular length that is comprised of the MSDL. The mandibular symphysis is the site of tusk eruption in males, which is the primary means of distinguishing morphologically similar beaked whales, and this ratio may provide a method for estimating age class and serve as an efficient species identification tool. Finally, we conducted linear and quadratic discriminant analyses to determine whether we could use skull and mandibular measurements to accurately assign specimens to their original collection region or sex. Analyses were performed using R (R Core Team 2018) with RStudio (RStudio Team 2016) and JMP (SAS 2019).

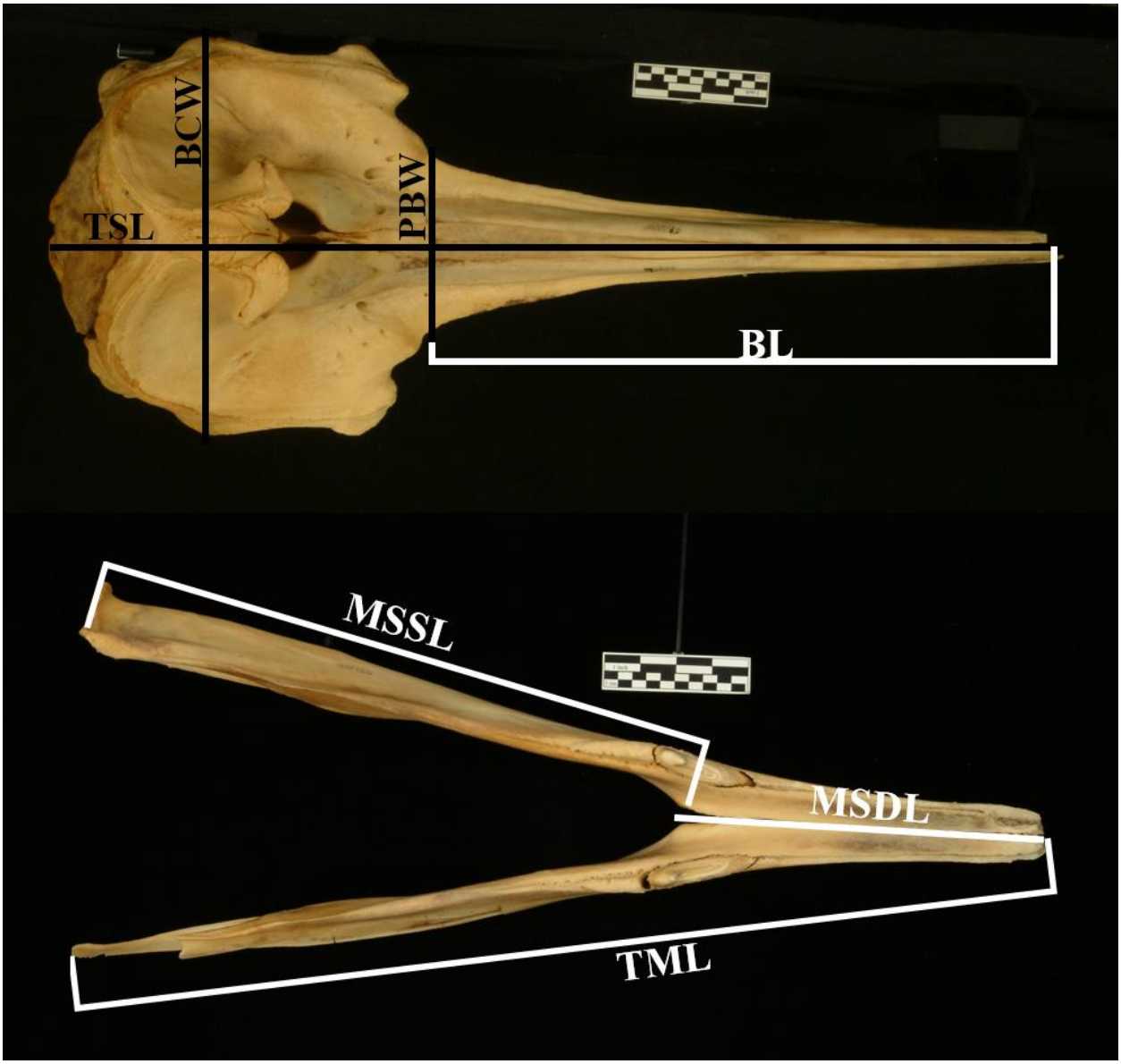

Figure 1.1 Skull and mandibular measurements of Sowerby's beaked whales (Mesoplodon bidens). TSL = Total Skull Length; BCW = Braincase Width; PBW = Proximal Beak Width; BL = Beak Length; TML = Total Mandibular Length; MSSL = Mandibular Skull to Symphysis Length; MSDL = Mandibular Symphysis to Distal Length. Photographed specimen is USNM 572008.

1.4 Results

1.4.1 Specimen sampling

We ultimately located and collected data on 180 specimens from 24 museums and stranding programs in North America and Europe (Appendix Table 1.1). We identified an additional 11 potential Sowerby's beaked whale specimens from three museums but were unable to obtain access to the specimens and could not confirm their identification. Thus, we did not include them in this study. We expect there are additional collections with samples that we did not identify. We concluded our sampling in 2019 and know that a few additional specimens identified as Sowerby's beaked whales have been recovered and added to museum and research collections since, but we have not seen these specimens.

1.4.2 Age and sex of specimens

Specimen sex was either recorded, or we were able to determine sex through a combination of specimen size and tooth morphology, in 64% of specimens (n = 115) (Figure 1.2). Of these, 60 were female and 55 male. Female specimens were predominantly adults (n = 44), but a sizable minority (n = 12) were subadults. Male specimens were also predominantly adult (n= 44) but included calves and juveniles (n = 2) in addition to subadults (n = 7). For both sexes there was a small subset of specimens for which we could not determine age class (females = 4; males = 2). Sex could not be determined for 65 specimens; this group was primarily subadults (n = 26) and juveniles and calves (n = 14) but also included 10 adult specimens and 15 specimens of unknown age. Both of these groups were comprised of specimens with missing or damaged skulls and mandibles, so we could not collect measurements or evaluate suture fusion (Figure 1.2).

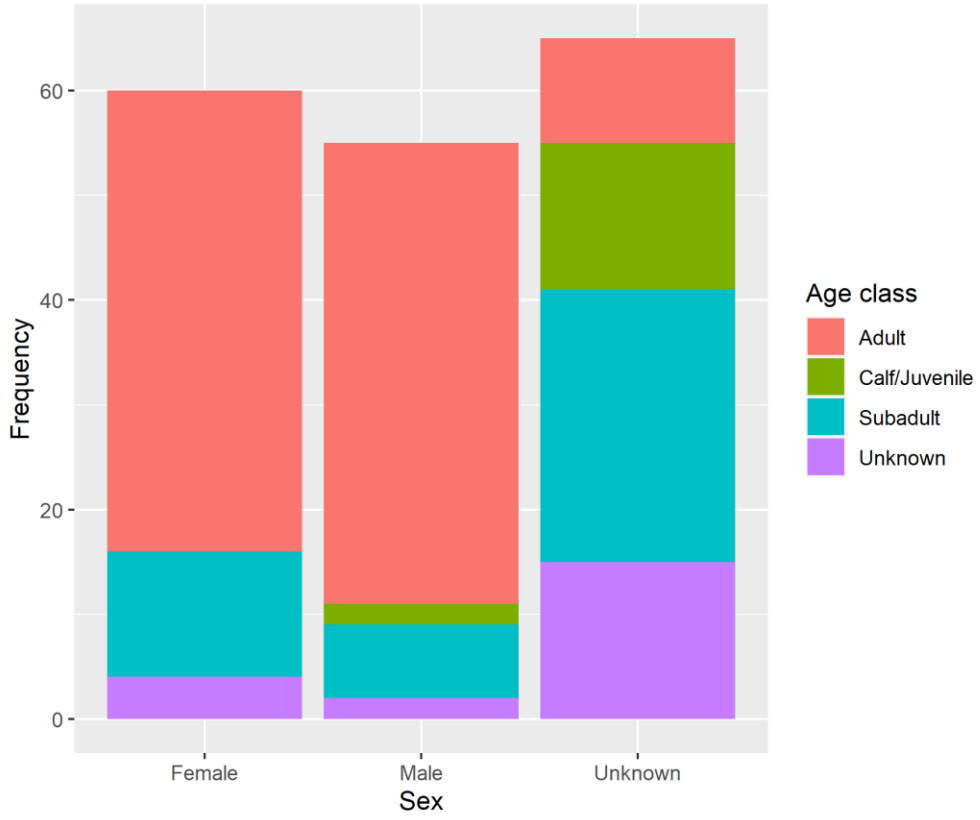

Figure 1.2 Sex and age class frequencies for 180 Sowerby's beaked whale (*Mesoplodon bidens*) specimens housed in museum or research institutions.

1.4.3. Specimen collection location

Original specimen collection location information was available for 97% of specimens (n = 174) (Figure 1.3). Of these, 45 were collected in the west Atlantic and 129 in the east Atlantic. In the west Atlantic, 21 specimens were bycaught in the swordfish (*Xiphias gladius*) pelagic drift gillnet fishery of the western North Atlantic (Wenzel et al. 2013) and 24 were beach cast carcasses. In the east Atlantic, 7 specimens were recovered during dredging operations in the North Sea, and 122 specimens were beach cast carcasses. This group also contained the type specimen, originally collected in 1800 (Sowerby 1806). For some of the historical specimens collected in the early 1800s, the original records list collection method as "caught;" however, there are no records suggesting that Sowerby's beaked whales were intentionally hunted. We

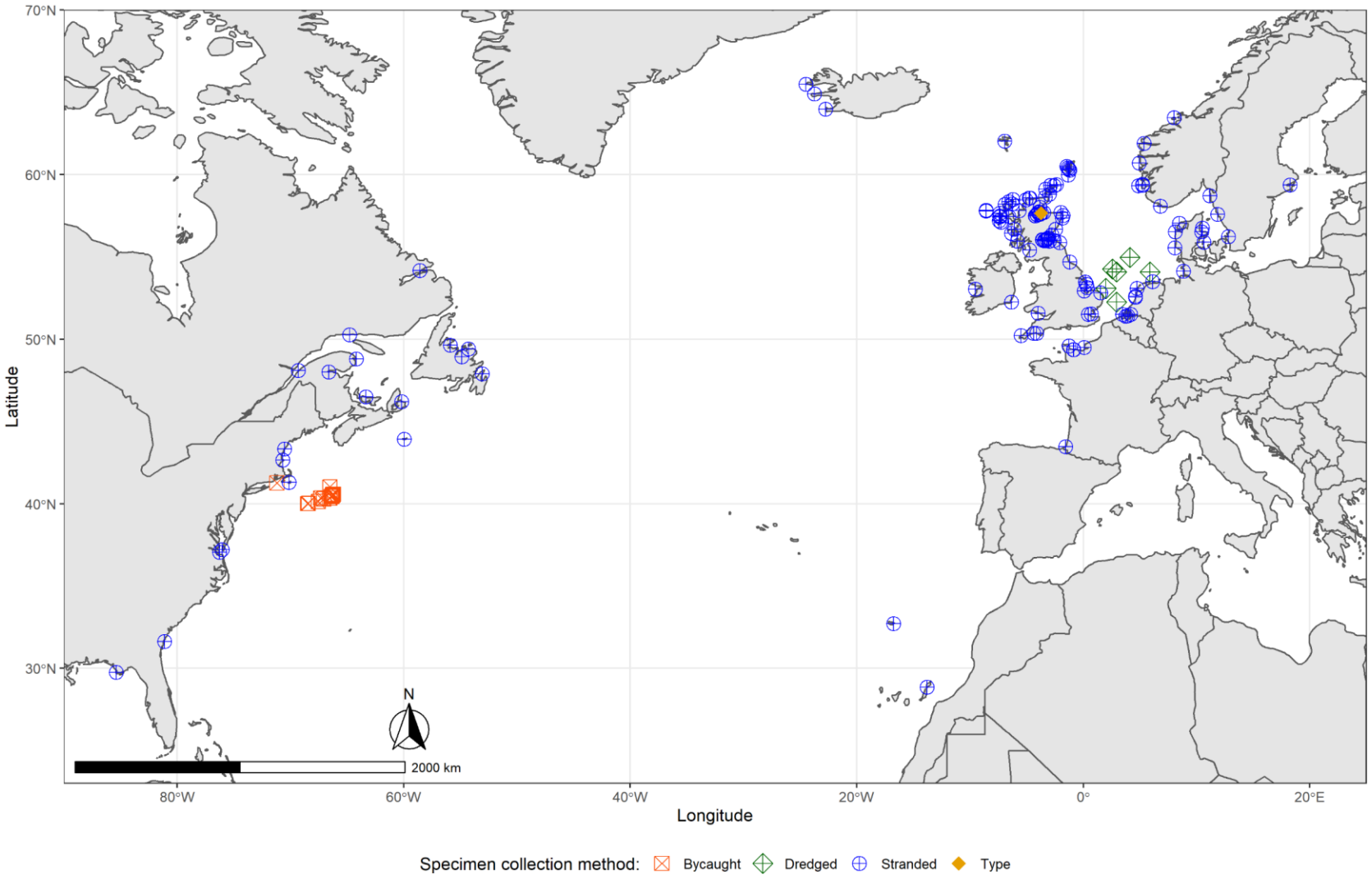

Figure 1.3 Collection location and method for 174 Sowerby's beaked whale (*Mesoplodon bidens*) specimens housed in museums or research institutions; 45 specimens were collected in the west Atlantic and 129 specimens were collected in the east Atlantic.

think this could mean bycaught in fishing nets, be an alternative term for stranded, or in some cases, the animal may have already been sick and close to shore and was opportunistically hunted.

1.4.4 Skull and mandible measurements

We quantified median skull and mandibular measurement values for all specimens from which we could collect measurements (Table 1.1, Appendix Table 1.1). We also quantified these measurements according to sex and region of original specimen collection (east and west Atlantic Ocean). Median measurements were similar between males and females. However, we found considerable variation between east and west Atlantic median values. West Atlantic median values were consistently greater than east Atlantic values, and 4 measurements (TSL, PBW, TML, and MSDL) were significantly different between regions (Table 1.1).

We found a positive correlation ($R^2 = 0.64$; $p = <0.001$) between the percent ratio of TML that is comprised of MSDL, and TML (Figure 1.4). As TML increases, the percent ratio of that length that is MSDL also increases. For juvenile and calf specimens TML was <45cm, and the mean percent ratio of MDSL to TML was 22.9% (SD = 5.0%). In subadult and adult animals TML was >45cm and the mean percent ratio of MDSL to TML increased to 31.2% (SD = 4.3%).

Not surprisingly, no skull and mandibular measurements had high linear discriminant percent probabilities on their own. Assignment percent probabilities by sex ranged 46.8 – 55.1, and by region ranged 47.8 - 67.0. Quadratic discriminant analysis of a partial combination of measurements, and for all measurement variables combined, also had low correct percent assignment probability for sex (<70.0%). Quadratic discriminant analysis for region, however, had a relatively high (78.6%) correct percent assignment probability when all measurement variables were combined. Quadratic discriminant analysis of the combination of 4 measurements

Table 1.1 Median measurement values for 7 skull and mandibular elements of Sowerby's beaked whale (*Mesoplodon bidens*) specimens housed in museum or research institutions. We were able to take measurements from 112 specimens; however, not all specimens had all elements present. *P*-values are for unpaired Wilcoxon tests comparing median measurement values between female and male, and east and west specimens.

Measurements (cm)	Female		Male			East Atlantic		West Atlantic			All specimens	
	median	***n***	**median**	***n***	*P*	**median**	***n***	**median**	***n***	*P*	**median**	***n***
Total Skull length	73.2	31	71.5	30	0.564	70.4	56	75.9	19	0.037	71.2	78
Braincase width	29.1	39	29.8	35	0.24	28.4	75	29.9	19	0.055	28.7	98
Proximal beak width	13.4	40	12.9	36	0.42	12.5	76	13.8	20	0.006	12.7	100
Beak length	47.2	31	43.9	32	0.26	43.8	58	47.8	20	0.164	43.8	82
Total mandibular length	67.8	34	63.9	31	0.24	61.3	67	70.3	19	0.004	62.6	91
Mandibular skull to symphysis length	44.2	36	44.4	32	0.94	43.2	71	44.4	19	0.163	43.6	95
Mandibular symphysis to distal end length	22.8	36	20.4	33	0.17	17.9	71	23.9	20	<0.001	19.5	96

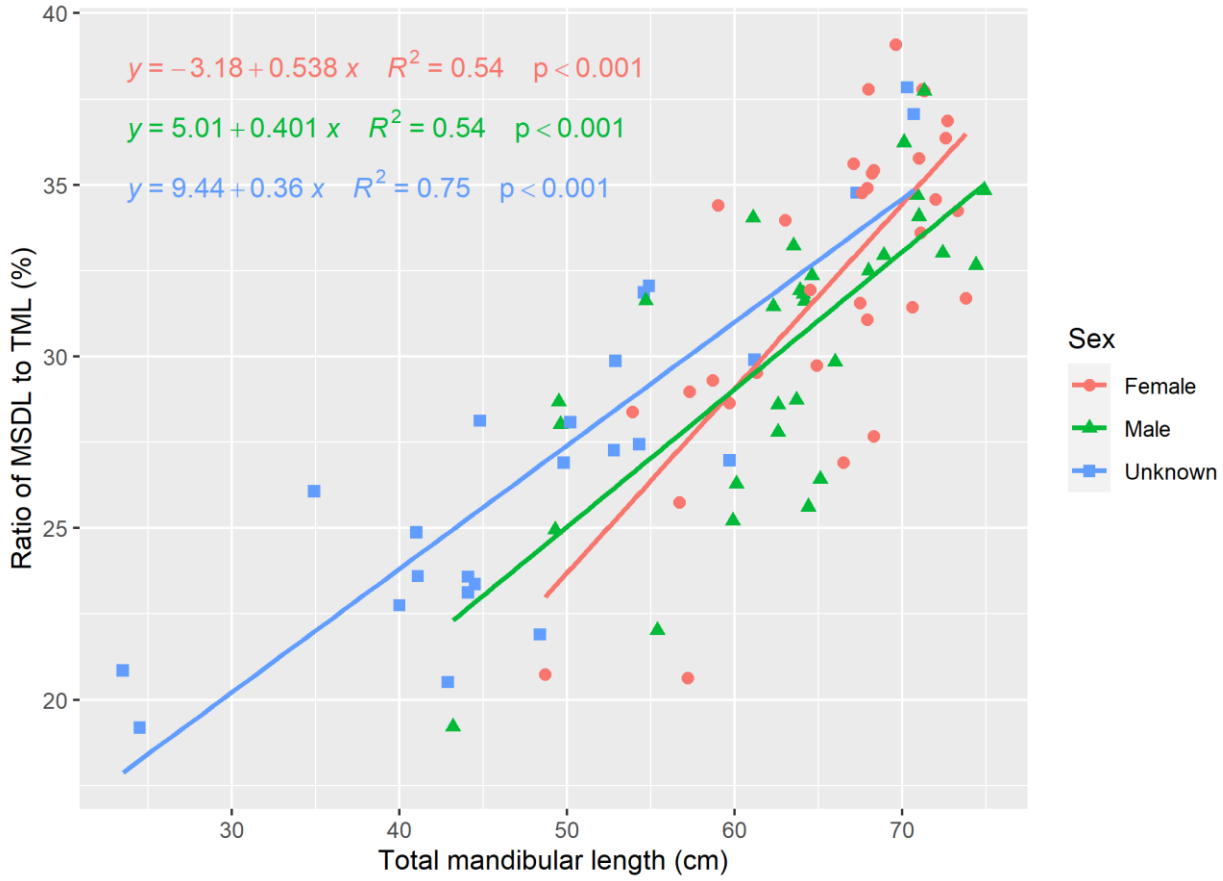

Figure 1.4 Percent ratio of mandibular symphysis to distal end length (MSDL) to total mandibular length (TML) plotted by total mandibular length for 33 female, 30 male, and 24 unknown sex (total = 87) Sowerby's beaked whale (*Mesoplodon bidens*) specimens housed in museum or research institutions.

with significantly different median values between regions (TSL, PBW, TML, and MSDL) correctly assigned 76.8% of specimens to their collection location; however, no other combination of variables had assignment percent probabilities >70%.

1.5 Discussion

1.5.1 Sampling efficacy

Snowball sampling (Goodman 1961; Wright & Stein 2005) proved highly successful at locating museums with specimens that we otherwise would not have identified. Although many museums maintain their own online collections database, the number of museums we would have had to individually search to identify specimens would have been time prohibitive. Additionally, many museums do not have online searchable databases, and we would have had to personally contact each of these museums to inquire about specimens. To complicate matters further, some museums do not provide contact details for curators, and only provide a general inquiry form that can be submitted to customer relations. By employing snowball sampling, we were able to tap into the curator network and learn about samples that we otherwise would have missed. Had we not used this method we probably would have missed 62 samples from 11 museums and research institutions. These were primarily smaller, regional museums that may not be able to invest time and funds into collection digitization. However, samples from these institutions often are accompanied by detail-rich records that provide critically important context, and active efforts to identify these institutions and include them in studies should be made.

At many institutions, we identified and/or located additional specimens in the collection, including at the three original museums we identified through online databases. This included 1 additional specimen at the NMNH, 39 at the NMS, and 9 at the NHM. In one example of identifying additional specimens when visiting the collection, we were invited to give a lecture to

museum staff and visitors; after the lecture we were approached by a curator who had been given a Sowerby's beaked whale mandible by a beachcomber several years earlier. Collection data and coordinates for the specimen had been recorded by the beachcomber on scratch paper, and both had been stored away by the receiving curator. Our visit reminded the curator of the specimen, they recovered it from storage, and the specimen was accessioned during our visit and made available for sampling.

1.5.2 Specimen demographics

Male and female specimens were represented roughly equally in our study; however, there was a sizeable number of specimens for which sex could not be determined. Most of these were juveniles or calves in which teeth would not have erupted, making it nearly impossible to determine sex if the sex organs were absent when the specimen was collected. Genetic analysis should be used to determine sex of these specimens, which may provide more information on sex-specific stranding patterns. Additionally, Einfeldt et al. (2019a) identified XXY aneuploidy in 3 North Atlantic beaked whale specimens (2 *Hyperoodon ampullatus* and 1 *M. mirus*); these specimens displayed a mixture of male and female sexual characteristics, suggesting that in a minority of specimens external sexual morphology may not be a reliable indicator of sex, and therefore sex-specific behavior, necessitating sex identification by genetic analysis.

Specimens were collected more often in the east Atlantic than west; this could be due to oceanic currents carrying specimens away from versus towards shore. In the west Atlantic, the Gulf Stream may be carrying distressed and dead animals away from shore, resulting in a smaller number of strandings. Conversely, in the east Atlantic the North Atlantic Drift Current may carry distressed or dead animals towards shore and explain the high proportion of stranding in the United Kingdom, particularly Scotland. Additionally, MacLeod et al. (2005) argued that the

North Sea may be acting as a shallow trap for pelagic and deep diving beaked whales, confusing them and resulting in a large number of deaths and strandings on the surrounding shoreline.

The high proportion of strandings in the east Atlantic versus the west has resulted in a few previous studies and reports suggesting that Sowerby's beaked whales may be less common in the west Atlantic than east (e.g, MacLeod et al. 2005; COSEWIC 2019). However, nearly half of the west Atlantic specimens in our study were bycaught in the swordfish drift gillnet fishery. That such a high number of specimens were bycaught in a relatively small area over a short period suggests that this may be an important habitat for west Atlantic members of this species, and supports the idea that Sowerby's beaked whales may be similarly abundant in the west Atlantic as east, but become beach cast less often.

Our dataset includes 4 extralimital strandings of Sowerby's beaked whales in the east Atlantic (Figure 1.3; Appendix Table 1.1). These specimens stranded in the United States, in the states of Florida, Georgia, and Virginia. Previous extralimital strandings in the east Atlantic, such as in the Canary Islands, prompted reconsideration of the species accepted range in that region (Martin et al. 2011). These four strandings in the west Atlantic, in addition to photographs of animals strongly resembling Sowerby's beaked whales stranding in Brazil and the Caribbean, may warrant further investigation and reconsideration of the accepted range for Sowerby's beaked whales in the west Atlantic.

1.5.3 Specimen morphology

Prior to our study, few data were available on Sowerby's beaked whale skull and mandibular measurements and morphology beyond simple characteristics, such as tooth eruption location. By collecting and collating skull and mandibular measurements, in addition to total body length and body weight data for a small subset of specimens (Appendix Table 1.1), we generated a

robust data set that can be used to better investigate morphological variation amongst Sowerby's beaked whales, and amongst north Atlantic beaked whale species. In particular, the ratio of MSDL to TML may prove highly useful in assigning specimens to an age class, improve our understanding of Sowerby's beaked whale growth and development, and serve as an efficient means of distinguishing amongst beaked whales specimens if they are damaged or incomplete.

We recorded significant differences in median skull and mandibular measurements for 4 variables (TSL, PBW, TML, and MSDL) between east and west Atlantic specimens, and quadratic discriminant analysis had a high rate of success at assigning specimens to their collection location when considering all 7 skull and mandibular elements (Table 1.1). This suggests that east and west Atlantic Sowerby's beaked whales are comprised of $\geq$2 distinct populations with differing skull and mandibular morphology. However, because east Atlantic specimens were more highly represented in our dataset, these differences may be artifacts of unequal sampling. Previous mitochondrial analysis of 14 specimens produced both regionally distinct and regionally overlapping mitochondrial haplotypes (COSEWIC 2006). To better explore population structure in Sowerby's beaked whales, both stable isotope analysis and whole genome analysis should be conducted. Stable isotope analysis should provide insight into foraging behavior and habitat use, while whole genome analysis will provide more data regarding population structure and genetic relatedness in this species.

Despite tooth morphology acting as a sexually dimorphic characteristic, we found no other mandibular or skull measurements that were sex specific or indicated sexual dimorphism. This was somewhat surprising because male tusks are larger than female teeth and erupt from the mandible, and we expected some sex-specific variation in mandibular measurements to accommodate male tusks. The trend we observed in increasing percent ratio of MSDL to TML in

both males and females may help to explain this from an evolutionary perspective. In 1953, Fraser (1974) used radiographs to identify vestigial teeth in the mandible of a male subadult Sowerby's beaked whale; we also observed vestigial teeth in one specimen in the course of this study. This information, in addition to the presence of a full set of functional teeth in the modern Shepherd's beaked whale (*Tasmacetus shepherdi*) suggests that a basal form of Sowerby's beaked whale had functional teeth. Perhaps MSDL continued to grow in the basal form to accommodate those teeth, and modern MSDL growth in both males and females is an evolutionary holdover. Advancing genetic analysis techniques may provide a way to identify functional genes related to mandibular growth, providing more information on this interesting pattern.

1.6 Conclusions

Specimens of opportunity are vital resources for biological studies, and snowball sampling proved an efficacious means of identifying these specimens. By compiling data on 180 disperse Sowerby's beaked whale specimens of opportunity, we: (1) identified significant differences in 4 median skull and mandible measurements between specimens collected in the east and west Atlantic, which suggests this species may be comprised of ≥2 distinct populations; (2) provided the first quantified skull and mandibular measurements for a sizable group of this species; (3) identified an interesting trend in mandibular growth patterns; and (4) provided a spatial distribution of Sowerby's beaked whale specimen collection locations, which may support expanding the species' accepted range in the west Atlantic. These data will aid future research into this species' distribution, morphology, and behavior, and directly contribute to conservation plans for this species. As museums face shrinking budgets, the digitization of collection records

may have to be put on hold, necessitating alternative means of locating specimens of opportunity. We encourage snowball sampling be employed by other researchers attempting to locate museum specimens.

Chapter 2: Cetacean skeletons demonstrate ecologically relevant variation in intraskeletal stable isotopic values[1]

2.1 Abstract

Conservation science requires quickly acquiring information and taking action in order to protect species at risk of extinction. Stable isotope measurements are one way to rapidly gather data regarding species' foraging ecology and habitat use, and passively collected samples limit additional stress to at-risk species. For these samples to be useful, however, we must know how representative they are of the stable isotope ratios of the entire organism. Bone tissue, often stored in museum collections or research centers, may be the most readily available tissue from rare, endangered, or extinct vertebrates, but using bone requires practitioners to understand intraskeletal stable isotope variation. We sampled the same eight skeletal elements from 72 cetacean skeletons from 14 species to evaluate intraskeletal variation in carbon and nitrogen isotope values. We found considerably more variation than anticipated. Carbon intraskeletal ranges varied from 0.4 to 7.6‰, with 84.7% (n = 61) of skeletons having a range >1‰, and 55.5% (n = 40) exhibiting a range >2‰. Similarly, nitrogen intraskeletal ranges varied from 0.4 to 5.2‰, with 59.7% (n = 43) of skeletons exhibiting a range >1‰, and 15.3% (n = 11) with a range >2‰. There were differences in which bones contributed most to intraskeletal variation; however, we advise against using humeri and mandibles as these bones presented the most consistent trends in deviation from the intraskeletal means for both isotopes. The large intraskeletal variation we observed is likely due to changes in foraging behavior or habitat use being reflected differently in bone isotope ratios due to differences in bone turnover rates. We suggest that for cetaceans, intraskeletal carbon isotope ranges >1‰ and nitrogen ranges >2‰ are

[1] Published as: Smith KJ, Sparks JP, Timmons ZL and Peterson MJ (2020) Cetacean Skeletons Demonstrate Ecologically Relevant Variation in Intraskeletal Stable Isotopic Values. Front. Mar. Sci. 7:388. doi: 10.3389/fmars.2020.00388

ecologically relevant, and that using different bones from animals of the same population may produce false positive differences in foraging behavior or habitat within the population if intraskeletal variation is not considered. Future studies should use the same bones from each animal and conduct species-specific analyses of intraskeletal variation, if possible, when using specimens of opportunity. Failure to consider this variation could lead to erroneous conclusions regarding a species range or key habitats, jeopardizing conservation efforts.

2.2 Introduction

Conservation science is a crisis discipline because conservation action typically must be taken for species at risk of extinction before practitioners are confident in the sufficiency of their data (Soulé 1985). One fundamental difficulty in wildlife conservation is rapidly understanding how species interact with, and utilize, their habitat (Aberg et al. 2000; Cristescu & Boyce 2013). A variety of tools and research methods have been developed and employed to gain insight into habitat use. Typically, these methods require directly interacting with the animal in some manner, such as radio telemetry, capture or sedation to collect biological samples, or long-term observation; all of which can alter animal behavior (Brigham 1989; Pietz et al. 1993; Guthery & Lusk 2004; Brooks et al. 2008; Rachlow et al. 2014). Less invasive methods, such as camera traps and drones, still may alter animal behavior, as many animals identify the device in their habitat and interact with it (Meek et al. 2014a; Meek et al. 2016; Mulero-Pazmany et al. 2017). Methods with no animal interactions, such as shore-based marine mammal observational studies, require thousands of observer hours, can be implemented over only limited spatial areas, are restricted in insight regarding surface-based activities, and are susceptible to observer error (Rugh et al. 1990; Aragones et al. 1997).

Stable isotope analysis (SIA) is an innovative technique for investigating wildlife habitat use, such as providing insight into foraging behavior, niche segregation, individual-level resource utilization, and diet shifts (Hobson 1999; West et al. 2006; Newsome et al. 2007). Studies incorporating SIA can employ a variety of tissue types, each providing unique temporal snapshots of ecological or dietary conditions reflecting the timeframe when the tissue was generated. Although many SIA studies use actively collected samples that require direct human–animal interaction, such as biopsy plugs or blood samples, one of the most powerful aspects of this technique is the ability to gain insight from passively collected samples, such as molted feathers or fur (McKechnie 2004; Thompson et al. 2005). Feathers, for example, often incorporate the isotopic value of the water and food resources in the region where they are grown, and are excellent samples for identifying migratory or breeding grounds without requiring human–bird interaction (Chamberlain et al. 1997; Hobson et al. 2001; Guillemain et al. 2019). Thus, SIA of specimens of opportunity provides a powerful monitoring system that minimizes invasive activities, limits impacts on animal behavior, and can be rapidly completed. Opportunistically collected samples provide a valuable alternative to capturing or harassing wildlife, but present new challenges. For rare or difficult to locate animals, small sample size or less than ideal samples can complicate analyses (Ben-David & Flaherty 2012; Hopkins & Ferguson 2012). If researchers are attempting to gain insight into longer-term behavior from passively collected samples, such as feathers, bone remains from archaeological sites, or more recent skeletal remains, they must determine if the sample evaluated accurately reflects the tissue's value for the animal as a whole.

In rare, endangered, or extinct vertebrate species, bone is often the only tissue available for SIA, and is routinely stored in museum and research collections. Despite the ubiquitous

availability of bone tissue from vertebrates, comparatively few studies have focused on bone SIA (Vander Zanden et al. 2015), and only a small subset of these studies has examined isotopic variation among different bones from the same organism (Table 2.1). Bone tissue is slow to grow and regenerate, thus incorporating and reflecting diet isotopic signatures at a slower rate than other tissues (Newsome et al. 2007; Vander Zanden et al. 2015). Controlled feeding studies have been completed that examine bone isotopic values, many with the purpose of coupling isotopic values in bone with soft tissues (BenDavid et al. 1997; Hong et al. 2000; Phillips & Eldridge 2006). However, these studies only examined matching bones, or the same bones in each study organism. Bone tissue is replaced and repaired at different rates depending on the bone's function, density, and size (Kohn & Cerling 2002; Lafage-Proust et al. 2015). Due to these differing turnover rates in bone, different bones sampled from the same animal may have different isotopic values. In the case of rare or extinct species, it may be impossible to acquire complete skeletons, compelling researchers to compare isotopic values from different bones among conspecifics. These non-matching bones may suggest different diets, water sources, or other environmental parameters, not because individuals in a population were utilizing different resources, but because the bones reflect dietary or habitat shifts at different rates. Failure to consider isotopic variation among different bones of conspecific individuals may result in erroneous conclusions regarding environmental conditions and dietary habits.

In this study, we investigated intraskeletal stable isotope variation of $\delta^{13}C$ and $\delta^{15}N$ in 14 cetacean species using skeletons from the National Museums Scotland osteological collection. Cetaceans are a quintessential example of the challenges conservation scientists face: studying cetaceans in situ is often invasive, requiring locating the animal, following it, and interacting with it in some way (Taylor et al. 2008; Ballance 2009). These processes are time consuming

Table 2.1 Summary of data from previous studies that investigated intraskeletal $\delta^{13}C$ and $\delta^{15}N$ isotopic variation relevant to our research.

Reference	Species	Sample locations	Number of individuals	Avg Intraskeletal Range (‰)	
				$\delta^{13}C$	$\delta^{15}N$
Deniro and Schoeniger (1983)	Mink (unknown species)	Femur & humerus	15	0.6	0.7
Deniro and Schoeniger (1983)	Rabbit (unknown species)	Femur, humerus, mandible, radius, scapula, distal tibia, ulna	3	0.3	0.3
Jorkov et al. (2009)	*Homo sapiens*	Rib, femur, temporal	57	0.4	0.8
Jorkov et al. (2009)	*Homo sapiens*	Rib, femur, temporal, molar	16	0.8	1.0
Riofrío-Lazo and Aurioles-Gamboa (2013)	*Mirounga angustirostris*	Mandible, tooth, maxilla	14	1.8	1.1
Riofrío-Lazo and Aurioles-Gamboa (2013)	*Mirounga angustirostris*	Mandible & maxilla	17	0.8	0.5
Olsen et al. (2014)	*Homo sapiens*	Rib, fibula, metacarpal	6	0.6	1.6
Webb et al. (2016)	*Sus domesticus*	Rib & femur	48	NA	0.3
Cheung et al. (2017)	*Homo sapiens*	Femur & fibula	11	1.0	0.6
Cheung et al. (2017)	*Homo sapiens*	Femur & radius	6	1.2	0.5
Cheung et al. (2017)	*Homo sapiens*	Femur & tibia	1	1.1	1.2
Cheung et al. (2017)	*Homo sapiens*	Femur & ulna	1	1.8	0.3
Clark et al. (2017)	*Odobenus rosmarus divergens*	Cranium & mandible	11	0.1	0.3
Clark et al. (2017)	*Pusa hispida*	Calcaneus, mandible, femur, humerus, innominate, phalanx, rib, scapula, metatarsal, vertebra	1	0.9	1.2
Clark et al. (2017)	*Phoca sp.*	Cranium, femur, humerus, innominate, phalanx, rib, scapula, metatarsal, vertebra	1	0.5	1.1
Clark et al. (2017)	*Enhydra lutris*	Mandible, femur, humerus, innominate, rib, scapula, metatarsal, vertebra	1	1.2	0.7
Fahy et al. (2017)	*Homo sapiens*	Femur, tibia, rib, radius, occipital, metacarpal, humerus, thoracic vertebrae, pelvis, clavicle	10	0.9	1.6
Bas et al. (2019)	*Otaria byronia*	Atlas, humerus, basioccipital	14	0.8	1.3
Bas et al. (2019)	*Lagenorhynchus obscurus*	Atlas, humerus, basioccipital	15	1.4	0.3

and have numerous logistical challenges beyond required permitting. Cetacean skeletons, however, have been collected and housed in museum collections for hundreds of years, providing a large specimen-of-opportunity cache for researchers. By sampling multiple bones from the same skeleton, we can establish an understanding regarding how representative a given bone is of the entire skeleton, thus increasing the power of skeleton-based SIA studies, and providing a valuable contribution to passive habitat use studies.

2.3 Materials and methods

To test for intraskeletal isotopic variation, we sampled the same 8 bone locations from 72 cetacean specimens (14 species) housed in the National Museums Scotland osteological collection (Table 2.2, Appendix Table 2.1). In order to consistently sample the same location for each bone among individuals and species, we compared bone size and selected the same proportional sampling site. We selected these specimens because they were complete or near-complete skeletons, were well represented in the collection so small-scale destructive sampling would not hinder future studies and encompassed the breadth of physiological and ecological variation in cetaceans. We aimed to limit our sampling to adult (n = 49) or subadult (n = 11) specimens, but due to the limited number of specimens that contained all sampling locations we included 12 juvenile specimens in order to increase sample size.

We used a battery powered handheld drill to remove 1g of bone tissue and subsampled 200mg for collagen extraction. Our extraction protocol was adapted from Ambrose (1990) and Jorkov et al. (2007). We ground subsamples with mortar and pestle and performed lipid extractions in a 2:1 chloroform:methanol solution 3 times for 30 minutes each; if the supernatant was not clear after 3 washes, additional washes were carried out as needed. The mineral

Table 2.2 Number of individual animals sampled per species for each skeletal sampling location. We sampled the same locations by bone among individuals to reduce the effects of natural bone variability, and samples contained a mixture of cortical and trabecular bone.

Species	**Skeletal sample location**							
	Occipital condyle	Mandibular ramus	Thoracic vertebral body	Thoracic vertebral spinous process	Proximal rib	Distal rib	Scapula	Humeral head
Balaenoptera acutorostrata	3	3	3	3	3	3	2*	3
Delphinus delphis	5	5	5	5	5	5	5	5
Globicephala melas	4	4	4	4	4	4	4	4
Grampus griseus	5	5	5	5	5	5	5	5
Hyperoodon ampullatus	5	5	5	5	5	5	5	5
Kogia breviceps	3	3	3	3	3	3	3	3
Lagenorhynchus acutus	5	5	5	5	5	5	5	5
Lagenorhynchus albirostris	5	5	5	5	5	5	5	5
Mesoplodon bidens	10	10	10	10	10	10	10	10
Orcinus orca	4	4	4	4	4	4	4	4
Phocoena phocoena	5	5	5	5	5	5	5	5
Stenella coeruleoalba	5	5	5	5	5	5	5	5
Tursiops truncatus	10	9*	10	10	10	10	10	10
Ziphius cavirostris	3	3	3	3	3	3	3	3

* One *Balaenoptera acutorostrata* skeleton was without scapulae and one *Tursiops truncatus* skeleton was without mandibles.

component was removed using a 30 minute 0.5M HCl bath followed by 3 deionized water rinses, and a 30 minute 0.1M NaOH bath followed by 3 deionized water rinses. Previous studies demonstrated that bone tissue lipid extraction and acidification demineralization does not significantly alter δ^{15}N values (Tomaszewicz et al. 2015; Tatsch et al. 2016). We added 7ml of pH 3 water to each sample and incubated at 80°C for 24hrs. The supernatant was collected and freeze-dried, resulting in purified collagen. Between 0.85 and 1.15mg of collagen was loaded in 3x5mm tin capsules and submitted for C and N stable isotope analysis.

Stable isotope analysis was completed at the Cornell Isotope Laboratory at Cornell University using a Thermo Delta V isotope mass spectrometer interfaced with a NC2500 elemental analyzer (ThermoFisher Scientific, 168 Third Avenue Waltham, MA USA 02451). We calibrated our results using 2 primary reference scales: Vienna Pee Dee Belemnite for δ^{13}C, and Atmospheric Air for δ^{15}N. To ensure accuracy and precision, we analyzed an in-house standard (δ^{13}C: -20.16 ±0.03‰ and δ^{15}N: 6.35 ±0.05‰) between every 10 samples. As an additional measure of extraction method and analysis accuracy and repeatability, we randomly selected 2 bones, subsampled 4 additional 200 mg samples each, and followed the methods described above to extract collagen and analyze for stable isotope ratios (δ^{13}C: -14.93 ±0.02‰ and δ^{15}N: 10.98 ±0.08‰; δ^{13}C: -13.79 ±0.05‰ and δ^{15}N: 11.55 ±0.06‰). We also evaluated collagen sample composition (percent carbon, percent nitrogen, and C/N ratio) and collagen percent yield to monitor sample quality.

We employed descriptive statistics to explore intraskeletal variation among bone sampling locations for both δ^{13}C and δ^{15}N. Because we are not making comparisons among animals, we did not have to consider the Suess effect, which is long-term incorporation of

isotopically light carbon into the marine ecosystem due to fossil fuel use (Keeling 1979). Analyses were performed using R (R Core Team 2018) with RStudio (RStudio Team 2016).

2.4 Results

We found a high degree of variation in the isotopic values among different bones taken from the same animal. For example, internal skeletal ranges for $\delta^{13}C$ varied from 0.4 to 7.6‰, with 84.7% (n = 61) of skeletons having a range >1‰, and 55.5% (n = 40) exhibiting a range >2‰ (Figure 2.1). Similarly, skeletal ranges for $\delta^{15}N$ varied from 0.4 to 5.2‰, with 59.7% (n = 43) of skeletons exhibiting a range >1‰, and 15.3% (n = 11) with a range >2‰. For all skeletons, and for both isotopes, at least one bone was ≥1 SD from the skeletal mean, and in most skeletons multiple bones were ≥1 SD from the mean. For $\delta^{13}C$, the number of skeletons with 1, 2, 3, and 4 bones ≥1 SD from the mean was 23, 31, 14, and 4, respectively. For $\delta^{15}N$, the number of skeletons with 1, 2, 3, and 4 bones ≥1 SD from the mean was 10, 41, 17, and 4, respectively. In a subset of skeletons (n = 31 for $\delta^{13}C$; n = 17 for $\delta^{15}N$), 1 bone was ≥2 SD from the skeletal mean and one bottlenose dolphin (*Tursiops truncatus)* skeleton had 4 bones ≥2 SD from the mean for $\delta^{15}N$.

There were no consistent trends regarding which specific bones within an individual animal differed in isotopic values from the skeletal mean across specimens. However, the proximal rib sampling location demonstrated the lowest levels of deviation, with 6 specimens (8.3%) ≥1 SD from *intraskeletal mean* $\delta^{13}C$ values, and 7 specimens (9.7%) ≥1 SD from $\delta^{15}N$ intraskeletal mean values. For $\delta^{13}C$, 50.0% (n = 36) of humeral heads were ≥1 SD lower than the skeletal mean (no humeral heads were +1 SD). Mandibular rami (40.3%; n = 29) and scapulae (16.7%; n = 12) had the second and third highest rates of deviation from mean

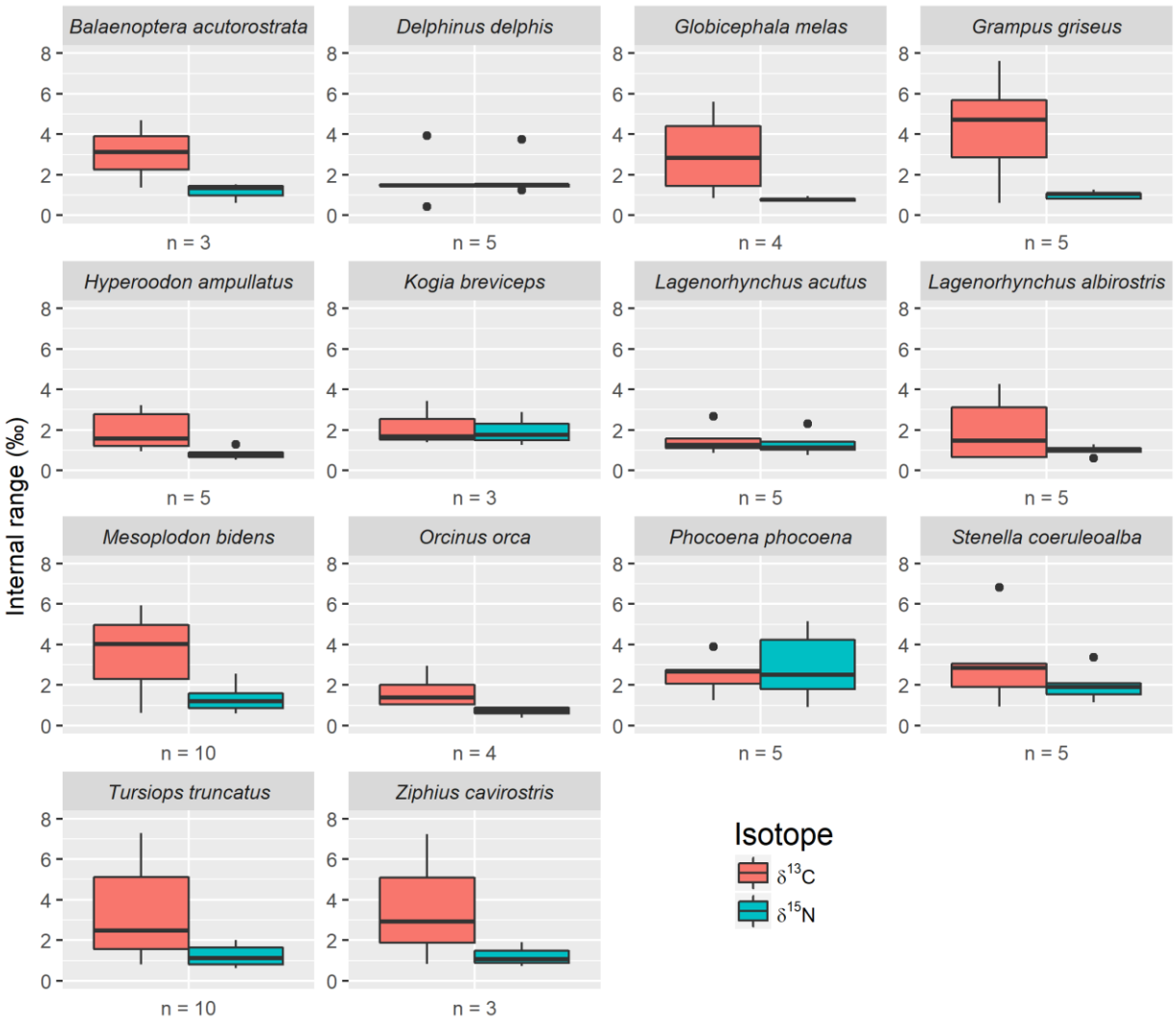

Figure 2.1 Individual intraskeletal range for $\delta^{13}C$ and $\delta^{15}N$, grouped by species (*n* = number of individual animals evaluated). Boxes present median and interquartile range and whiskers represent 95% confidence intervals.

skeletal δ^{13}C, and all bone sample locations had at least one representative with ≥1 SD. We found 56.9% (n = 41) of mandibular rami were ≥1 SD from the skeletal δ^{15}N mean; specifically, of these 22.0% (n = 9) were greater than the mean, whereas 78.0% (n = 32) were less than the mean. Humeral heads (30.0%; n = 28) and occipitals (25.0%; n = 18) had the second and third highest rates of deviation from the skeletal δ^{15}N average, and all bones had at least one representative ≥1 SD from the mean.

Mean collagen yield was 10.6%, with a range of 1.8–37.5%. This includes 37 samples with artificially low percent yield due to a freeze dryer malfunction resulting in partial loss of the sample or producing collagen that was challenging to recover from the vial, which prevented obtaining an accurate weight. Mean %C and %N were 29.56 and 10.19, respectively, with a mean C/N ratio of 3.49.

2.5 Discussion

Intraskeletal isotopic variation has not been well investigated across a variety of taxa, and only one other study has considered this topic for cetacean skeletons (Vander Zanden et al. 2015; Bas et al. 2019). Studies of this type are challenging due to difficulty in locating large numbers of intact skeletons of the same species. As a result, previous studies typically had small sample sizes, low numbers of sampling locations, or a combination of both (Table 2.1). We addressed this deficiency by combining a large sample size from five cetacean families with many bone sampling locations per skeleton (Table 2.2). We documented much greater intraskeletal isotopic variation than has previously been reported (Figure 2.1; Table 2.1), suggesting that if analyses were to be expanded to other taxa, similar results may be observed.

We identified some noteworthy patterns in stable isotope values that can be used to better inform the design of intraskeletal isotope studies in cetaceans. The proximal rib demonstrated the lowest rate of deviation from both $\delta^{13}C$ and $\delta^{15}N$ intraskeletal means and may be the best bone from our sampling locations to use for comparative studies. The $\delta^{13}C$ isotopic value of half of all humeral heads was ≥1 SD from the skeletal mean, so we advise against using this bone as representative of the skeleton as a whole. The mandibular ramus probably also should be avoided as we documented large, but inconsistent deviation from the skeletal mean for both isotopes. These two sampling locations represent two different bone types with different turnover rates. The humeral head is part of the humeral long bone and forms the shoulder joint with the scapula. However, cetacean skeletal and muscle anatomy studies have found that the humeral head is largely vestigial, and flipper movement is limited and related to maintaining balance and aiding in swimming speed (Cooper et al. 2007; Sanchez & Berta 2010). As a result, this bone is under less ecophysiological pressure than other more mobile bones and joints. In contrast, the mandibular ramus is part of the dense mandibular irregular bone with a high degree of turnover and remodeling (Matsuura et al. 2014; Shadwick et al. 2017). In cetaceans, the mandible serves as the primary method of interacting with each other and the environment and is more susceptible to damage than other bones. These two bones represent distinct functions and turnover rates, and this may explain why they exhibit the greatest difference from the skeletal mean.

For 13 of 14 species in our study, $\delta^{13}C$ was more variable than $\delta^{15}N$ (Figure 2.1). This trend is similar to cetacean intraskeletal isotopic variation reported by Bas et al. (2019), who compared $\delta^{13}C$ and $\delta^{15}N$ isotope values among three sampling locations from 15 specimens (Table 2.1). They reported $\delta^{13}C$ intraskeletal isotopic variation that fell within the lower range of

variation in our study, and we suspect that had additional skeletal elements been compared, then variation found by our two studies may have been similar. Greater $\delta^{13}C$ than $\delta^{15}N$ intraskeletal variation is also consistent with Riofrío-Lazo and Aurioles-Gamboa (2013), who found variation in northern elephant seal skeletons (*Mirounga angustirostris*) and for sea otter (*Enhydra lutris*) skeletons in Clark et al. (2017). Many human (*Homo sapiens)* archeological studies also reported this trend (Table 2.1), but these authors typically compared only two or three sampling locations. The study most similar in design to ours is Fahy et al. (2017); they compared $\delta^{13}C$ and $\delta^{15}N$ isotope values between 10 sample locations in 10 humans. They found $\delta^{15}N$ intraskeletal variability was greater than $\delta^{13}C$ variation; however, $\delta^{15}N$ variation was similar to values in our study. Only a few other studies examined intraskeletal variation in terrestrial vertebrates (Table 2.1). The differences observed between terrestrial and marine mammal studies may be due, in part, to different physiological pressures placed on bones in terrestrial versus aquatic and semiaquatic environments.

Newsome et al. (2010) documented that younger marine mammals exhibit higher bone turnover rates of carbon and nitrogen stable isotopes, possibly contributing to intraskeletal variation. We did not observe this pattern. In fact, adult animals demonstrated some of the highest levels of intraskeletal variation. For example, harbor porpoises (*Phocoena phocoena*) had relatively low levels of $\delta^{13}C$ intraskeletal variation despite including one subadult individual, and the outlier animal was an adult (Figure 2.1). Atlantic white sided dolphins (*Lagenorhynchus acutus*) displayed generally lower levels of $\delta^{13}C$ intraskeletal variation compared to white beaked dolphins (*L. albirostris*), even though we sampled five adult specimens for each species. Amongst beaked whales, Sowerby's beaked whales (*Mesoplodon bidens*) displayed the greatest median $\delta^{13}C$ intraskeletal variation despite including only adult animals, while both northern

bottlenose whale (*Hyperoodon ampullatus*) and Cuvier's beaked whale (*Ziphius cavirostris*) samples included juvenile animals. No data on cetacean bone tissue turnover rates is available, but Newsome et al. (2006) estimated complete bone collagen turnover in yearling seals and sea lions at 8-10 months. If young cetaceans exhibit a similar pattern, then the moderate variation we observed in younger animals is logical because their bones are reflecting a shorter time span, and therefore less environmental variability than seen in older age classes. Thus, age class of the specimen does not seem to drive the variation we observed. Likewise, collection date and storage time of the specimens did not contribute to intraskeletal variation. All our specimens, with the exception of two, were collected since 1989, and the two older specimens demonstrated similar intraskeletal variation as modern specimens.

We were consistent in our sampling locations in each skeleton to reduce the introduction of additional variation due to natural differences throughout the bone. Each sample contained a mixture of mineralized cortical bone and spongy trabecular bone, and the inherent unequal ratios of these bone types at different sampling sites, and the differences in their turnover rates, may contribute to some of our observed intraskeletal variation (Manolagas 2000; Clarke 2008). However, if this was a major contributing factor, we would expect to see animals of the same species demonstrating similar trends in variation; instead, we saw considerable variation at the individual animal level. This suggests a combination of physiological and ecological factors driving isotopic variation. Carbon and nitrogen isotope ratios in a skeleton reflect habitat and diet, respectively (Ben-David & Flaherty 2012). Organisms in controlled settings, such as in feeding studies or laboratories, show little isotopic variation when fed a consistent diet, even when considering bone turnover rates (Deniro & Schoeniger 1983). Therefore, differences we

observed in intraskeletal isotope ratios suggest differences in foraging behavior and individual-level resource utilization over time.

As animals switch habitats or consume different food sources, the rate isotopes from these sources are incorporated will vary among bones due to bone-specific turnover rates (Newsome et al. 2010). This combination of changing environmental isotope ratios and physiological mechanisms leads to ecologically relevant intraskeletal isotopic variation—that is, the isotopic values from different bones from the same individual could lead to different conclusions regarding an animal's life history if considered independently. This is especially important for studies forced to use non-matching bones for analyses. The amount of intraskeletal range that is ecologically relevant depends on the specific questions being asked, but we suggest that $\delta^{13}C$ ranges >1‰ and $\delta^{15}N$ ranges >2‰ are ecologically significant for cetacean studies. Isoscape models built using specific prey resources of the species in this study do not yet exist, but we still can characterize how variation might affect researcher's conclusions by considering isoscapes already available. For example, an isoscape model built from jellyfish collected in waters around the British Isles demonstrates a 1–2‰ difference in $\delta^{13}C$ values across the study area (Glew et al. 2019). Based on this, the $\delta^{13}C$ variation we observed within the skeletons in our study would indicate different foraging locations along the U.K shelf sea if the bone sample locations were considered independently. If a study is trying to identify important foraging or breeding habitats to make conservation recommendations and must make use of non-matching bones, a 1‰ difference may appear to suggest different regions of importance yet may simply represent differences among bones sampled from the same skeleton. Similarly, nitrogen isotope values in animals are enriched at rate of 3–4‰ for each increase in trophic level (Post 2002), yet we observed $\delta^{15}N$ intraskeletal range values up to 5.16‰.

Bone turnover rates and changes in habitat use or foraging behavior could explain much of the intraskeletal variation in carbon and nitrogen isotope ratios we observed, but there is still considerable unexplained variation. This could be due to physiological factors that are beyond the scope of our study, such as metabolic rates or bone disease/injury, both of which can alter bone growth patterns (Manolagas 2000; Clarke 2008; Olsen et al. 2014). We did not sample animals that had obvious signs of bone disease or injury remodeling, but there is little information regarding the individual life history of most specimens in our study. Thus, we do not know their movement patterns and habitat use, beyond general species information, or the specifics of their age or health. However, even amongst closely related species, such as Atlantic white sided dolphins and white beaked dolphins, which have overlapping habitats and grow to a similar size, we saw considerable differences in intraskeletal variation (Weinrich et al. 2001; Galatius & Kinze 2016). For some species, such as bottlenose dolphins, specimens in our study may have come from different populations, with different foraging behavior and habitat use, further contributing to intraskeletal isotopic variation. Although we sampled a large breadth of cetacean species, we were limited to relatively small species sample sizes due to difficulty in acquiring complete skeletons. In two cases, we chose to include animals that were missing one of eight sampling locations to increase sample size for that species; if this study was repeated with much larger species sample sizes, further trends in variation may become apparent. Regardless, to truly understand factors driving individual and species variation, we would need data from hundreds of tagged animals of the same species—where all their life movement data is available—to more systematically evaluate stable isotope variation for the species. Because this is not feasible for most cetacean studies, we instead must acknowledge that considerable variation exists within individual animals.

Specimens of opportunity are a critical resource for ecological studies, but they do present unique challenges that must be considered. Because opportunistically collected skeletons are often incomplete, necessitating comparisons between unmatched bones among animals, there is a need to understand intraskeletal isotopic variation. Our study demonstrates that substantial intraskeletal variation is present for the cetacean species we evaluated. Thence, we recommend that future studies using opportunistic bone tissue for stable isotope analysis conduct species-specific evaluations for intraskeletal variation. Failure to identify or consider this variation could have serious implications for studies that use bone isotope values to explore animal ecology. When the results of such studies are used to inform conservation action, it is imperative to consider that different bones from the same animals may suggest different habitats or resource use when none existed. Accounting for this intraskeletal variation in stable isotopes values produces more robust analyses and thus better-informed conservation management plans.

Chapter 3: Evaluation of two lipid removal methods for stable carbon and nitrogen isotope analysis in whale tissue[2]

3.1 Abstract

The presence of lipids in animal tissues can influence the interpretation of stable isotope data, particularly in lipid-rich tissues such as the skin and muscle of marine mammals. The traditionally employed chloroform:methanol delipidation protocol has the potential to alter $\delta^{15}N$ values in proteinaceous tissues. Our objective was to determine whether cyclohexane is an alternative extraction method, effectively removing lipids without altering $\delta^{15}N$ values. Kidney, liver, muscle, and skin samples were collected from beach-cast Sowerby's beaked whales (*Mesoplodon bidens*). Control subsamples were processed without delipidation extraction, and duplicate subsamples were extracted with either chloroform:methanol or cyclohexane. $\delta^{13}C$, $\delta^{15}N$, and C:N values were determined by continuous-flow elemental analysis isotope ratio mass spectrometry. Paired Wilcoxon tests were used to evaluate the change in isotope values after extraction, and unpaired Wilcoxon tests were used to evaluate difference in isotope values between extractions. Cyclohexane is an effective delipidation technique for tissues with low and moderate lipid content. Chemical delipidation influenced $\delta^{15}N$ values; extracted samples generally showed an increase in $\delta^{15}N$ values which varied 0.0‰ to 1.7‰. Chloroform:methanol extraction resulted in alterations to $\delta^{15}N$ values greater than analytical precision for all analyzed tissues. Changes to $\delta^{15}N$ values after cyclohexane extraction were at or near analytical precision in liver and muscle but greater than analytical precision for kidney and skin. We recommend

[2] Accepted for publication in *Rapid Communications in Mass Spectrometry* as: **Smith, KJ**, Trueman, CN, France, CAM, and Peterson, MJ. Evaluation of two lipid removal methods for stable carbon and nitrogen isotope analysis in whale tissue.

processing duplicate subsamples for stable isotope analysis, one with and one without extraction in order to obtain accurate values for each isotope. Prolonged chemical extractions are not necessary to effectively remove lipids. When samples are limited, we suggest using cyclohexane for tissues with low or moderate lipid content, and chloroform:methanol for lipid-rich tissues.

3.2 Introduction

Stable isotope analysis (SIA) of animal tissues is a rapidly expanding tool applied to a variety of environmental, ecological, anthropological, and forensic problems; however, interpretation of stable isotope data can be confounded by a suite of variables related to sample design, collection, preparation, and analysis (Keeling 1979; Peterson & Fry 1987). Animal tissues are comprised of multiple compound classes (e.g., proteins) and compounds (e.g., amino acids), each with potentially different isotopic compositions (Ben-David & Flaherty 2012). The isotopic composition of bulk (whole) tissue is an average of the isotopic composition of the constituent molecules weighted by their relative proportion (Phillips & Eldridge 2006; Vander Zanden et al. 2015). If the relative proportion of isotopically distinct tissue components varies among bulk samples, then tissue composition will contribute to measured population stable isotope means and distributions. Wildlife and anthropological studies addressing questions of spatial origin, movement behavior, or diet commonly focus on largely proteinaceous tissues such as muscle, feather, hair keratin, or bone collagen for isotopic analyses (Hobson 1999; Cristescu & Boyce 2013). Such tissues commonly also contain lipids, potentially influencing $\delta^{13}C$ values and C:N ratios (West et al. 2006; Post et al. 2007; Elliott et al. 2017). On average, synthesized body lipids tend to be depleted in ^{13}C compared to synthesized proteins, so that the presence of lipids within protein samples tends to reduce bulk tissue $\delta^{13}C$ values. The degree of isotopic differentiation

can vary depending on lipid and protein composition, nutritional status, and other physiological effects (DeNiro & Epstein 1977; Post et al. 2007; Elliott & Elliott 2016). Soft tissues such as muscle, liver, and subcutaneous connective tissues frequently act as physiological lipid stores. Lipid contents in these tissues may be high and markedly variable among individuals (Ryan et al. 2012). Failure to consider lipid content when conducting tissue-based studies can therefore bias data interpretation and lead to erroneous conclusions about diet or movement patterns (Logan & Lutcavage 2008; Bergamo et al. 2016; Elliott et al. 2017). Two approaches have been proposed to address the problem of lipid content in mixed tissue isotope analyses: statistical isotopic correction models and chemical removal of lipids.

Statistical isotopic correction models aim to account for the influence of ^{13}C depleted lipids retrospectively using C:N ratios as predictors of lipid content and mass balance approaches to correct measured values (Wahl et al. 2004). These models typically are established by statistical regression between measured $\delta^{13}C$ values and C:N ratios and may also utilize measured or estimated end member values for pure lipid, pure protein, or expected protein:lipid offsets. The coefficients associated with statistical lipid correction models are likely to vary according to tissue type, physiology, and metabolic status. Therefore, while a variety of models are available, they do not generate consistent results between and within species and tissue types (Ryan et al. 2012; Elliott et al. 2014; Skinner et al. 2016; Giménez et al. 2017; Taylor et al. 2017). Thus, lipid correction models must be parameterized for each study and may still yield inconsistent results (Cloyed et al. ; Sweeting et al. 2006; Logan et al. 2008; Lesage et al. 2010; Yurkowski et al. 2015).

Chemical lipid extraction provides a rapid and consistent means of ensuring lipid removal. The most common method for lipid extraction is a polar solvent solution of

chloroform:methanol. This technique, in use for more than 60 years, is effective at removing lipids. However, the process is relatively aggressive, potentially also influencing the relative proportions of amino acids present because of the higher solubility of the polar amino acids in polar solutes (Bligh & Dyer 1959; Elliott et al. 2017). As $\delta^{13}C$ and $\delta^{15}N$ values vary among individual amino acids, altering the relative proportions of amino acids present in a protein following chloroform:methanol extractions can alter the isotopic compositions of both carbon and nitrogen in bulk protein analyses. Non-polar solvents, such as hexane and diethyl ether, provide an alternative means of lipid removal. All amino acids are relatively insoluble in non-polar solvents, so the use of non-polar solvents for lipid extraction carries less risk of unintentional alteration of amino acid and bulk protein isotopic compositions (Logan & Lutcavage 2008; Elliott & Elliott 2016). Despite years of study and the rise in the use of stable isotope analyses of animal tissues, the relative performance of different chemical extraction approaches as applied to specific tissues of different species is still not well characterized. As a result, there is a conflicting body of evidence about the effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values and a lack of consistency in extraction methods employed across studies. In addition to avoiding the potential effects of chemical extraction on target protein isotopic compositions, it may be beneficial to avoid chemical extraction for simple time and cost considerations.

For any given species, tissue, and study there is often uncertainty regarding: (1) whether tissue lipid extraction is a necessary step prior to stable isotope analyses; and if so, (2) the magnitude of undesirable isotopic alteration that should be expected associated with different chemical extraction methods. This is especially problematic in the case of poorly studied species, tissues with few case studies in the literature, and tissues with high and variable lipid contents.

In this study we evaluated two methods of lipid removal, chloroform:methanol and cyclohexane, and their effects on $\delta^{13}C$, $\delta^{15}N$, and C:N values in four tissue types collected from Sowerby's beaked whales (*Mesoplodon bidens*). Cyclohexane is a nonpolar solvent frequently used to extract lipids for lipid research studies but has only occasionally been used in stable isotope analyses (Monteiro-Riviere et al. 2001; Kojadinovic et al. 2008; Chouvelon et al. 2014; Li et al. 2014; Anthony & Stuart 2015; Howa et al. 2016). Whale tissue, especially skin, is lipid-rich and has proven particularly challenging to evaluate with statistical isotopic correction models (Lesage et al. 2010; Ryan et al. 2012; Giménez et al. 2017). Thus, it is often assumed to be necessary to use a chemical extraction method when processing whale tissue. Here, we assessed the necessity of using a chemical lipid extraction method in tissue for this whale species, the degree to which each method altered isotope ratios, and how any changes to isotope values may influence interpretation of these values.

3.3 Methods

3.3.1 Sampling, sample preparation, and stable isotope analysis

We obtained samples of kidney (n = 18), liver (n = 17), muscle (n = 18), and skin (n = 24) from 26 stranded Sowerby's beaked whales (n = 77 total tissue samples). Samples were opportunistically collected from beach-cast carcasses from various locations along the Scottish coastline by the Scottish Marine Animal Stranding Scheme and stored at -20 ℃. We collected ~0.5 g subsamples of frozen tissues and preserved them in 95% ethanol for <1 week for transport. Ethanol is a commonly used preservative for soft tissues that can contribute to lipid removal and increase $\delta^{13}C$ values in the tissues of some species, but typically has small and insignificant effects on $\delta^{15}N$ values (Kaehler & Pakhomov 2001; Sarakinos et al. 2002; Hogsden

& McHugh 2017; Javornik et al. 2019). Prior to analyses we removed excess ethanol, subsampled each tissue sample, freeze dried the samples individually for 16 hours, and ground dried tissues with mortar and pestle. We subsampled 10 samples from each tissue type to serve as an unextracted control; these samples were submitted for stable isotope analysis without lipid extraction. We selected these tissues for the control because there was enough of each sample for pre- and post-extraction analysis and duplicate analysis, if needed. For each of the 77 tissue samples, we extracted one subsample with 2:1 chloroform:methanol for 30 minutes, manually agitating samples every 5 minutes. We repeated this process with a duplicate sample for cyclohexane extraction. Lipid extraction timelines vary among studies from minutes to days; we employed a single 30-minute extraction to keep extraction methods consistent between our two protocols. Longer extraction times, particularly for chloroform:methanol, are often employed on tissues (Post et al. 2007; Logan & Lutcavage 2008; Elliott & Elliott 2016). However, it is unclear if prolonged extraction is necessary to effectively remove lipids, especially on finely ground materials. Lipid extracted samples were dried at 60 ℃ for 16 hours post extraction. Between 0.5 and 0.8 mg of each sample was loaded in 3x5mm tin capsules and submitted for C and N stable isotope analysis.

Stable isotope analysis was completed at the Smithsonian Institution Museum Conservation Institute Stable Isotope Mass Spectrometry Laboratory using a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to an Elementar vario ISOTOPE Cube Elemental Analyzer via a Thermo Conflo IV (ThermoFisher Scientific, 168 Third Avenue Waltham, MA USA 02451). Raw sample values were calibrated to V-PDB and Air (δ^{13}C and δ^{15}N, respectively) via an in-house Costech Acetanilide (Costech Analytical, 26074 Avenue Hall, Suite 14 Valencia, CA USA 91355) and Urea-UIN3, both calibrated to USGS40 and

USGS41 L-glutamic acids (Schimmelmann et al. 2009). The in-house standards were included between every 10 samples to ensure accuracy and precision, with an analytical precision of +/- 0.2‰ (1σ). Weight percent carbon and nitrogen values were calibrated to the in-house acetanilide standard with an analytical precision of +/- 0.5%.

3.3.2 Data analysis

Our data analyses addressed four questions: (1) are both lipid removal techniques effective; (2) how much variance is there between chloroform:methanol and cyclohexane extracted samples; (3) does delipidation extraction significantly change $\delta^{13}C$, $\delta^{15}N$, and C:N values; and (4) do extraction methods change isotope values in similar ways? To answer question (1), we evaluated the C:N ratios post extraction for all samples (n = 77) because the C:N ratio often is used to evaluate the presence of lipids in tissue samples, and previous studies have identified a significant relationship between larger C:N ratios, higher lipid proportions, and lower $\delta^{13}C$ values in some animal tissues (Post et al. 2007). We used these same 77 samples to address question (2), employing paired Wilcoxon tests to compare $\delta^{13}C$, $\delta^{15}N$, and C:N values between each subsample of chloroform:methanol and cyclohexane extracted tissue. We then used a subset of these samples (n = 40; 10 of each tissue type) to address questions 3 and 4, comparing $\delta^{13}C$, $\delta^{15}N$, and C:N values of the unextracted control samples to those same tissues post extraction. To address question (3), we used paired Wilcoxon tests to evaluate differences in pre- and post-extraction values for each extraction method to explore how extraction method changed isotope values ($\delta^{13}C$ and $\delta^{15}N$) and their relationship to each other (C:N ratios). For question (4), we used unpaired Wilcoxon tests to compare the degree and direction of change in values between the same tissue subsamples extracted with chloroform:methanol and cyclohexane. We considered p-

values ≤ 0.05 significant, and statistical analyses were performed using R (R Core Team 2018) with RStudio (RStudio Team 2016).

We use two delta notations to express our results. The first is the standard delta notation δ, which is the parts per thousand difference between the sample and international standards, expressed as $\delta^{y}X = [(R_{sample} - R_{standard})/(R_{standard})]$, where X is the element, y is the atomic mass of the heavy stable isotope, and R is the ratio of heavy to light isotopes. The second is Δ notation, used to represent the difference between two δ values. In this paper we use it to represent the difference between extracted and unextracted values (e.g. $\Delta^{13}C = \delta^{13}C_{extracted} - \delta^{13}C_{unextracted}$).

3.4 Results and discussion

For question (1), we found both extraction methods effectively removed lipids from tissues with relatively lower initial lipid content. A 30-minute chloroform:methanol extraction effectively delipidated lipid-rich tissues, and a 30-minute cyclohexane extraction was moderately effective at delipidating lipid-rich tissues . C:N ratios were reduced to < 5 in all 77 chloroform:methanol extracted samples, and in all but 1 cyclohexane extracted skin sample (Figure 3.1). There is currently no consensus regarding "correct" marine mammal C:N ratios following delipidation; some sources suggest tissues with C:N (by mass) values > 3.5 contain sufficient lipid to significantly complicate tissue $\delta^{13}C$ interpretations, while others consider values between 4 and 5 acceptable (McConnaughey & McRoy 1979; Post et al. 2007; Ryan et al. 2012). Our chloroform:methanol extracted samples had a mean C:N ratio of 3.4 (range: 3.0 – 4.7), and the cyclohexane extracted mean was 3.6 (range: 3.0 – 6.4). Thus, chloroform:methanol C:N ratios in this study fell within multiple definitions of acceptable C:N ratios, demonstrating that prolonged extraction times, especially on ground tissue, are not necessary. Likewise, cyclohexane C:N

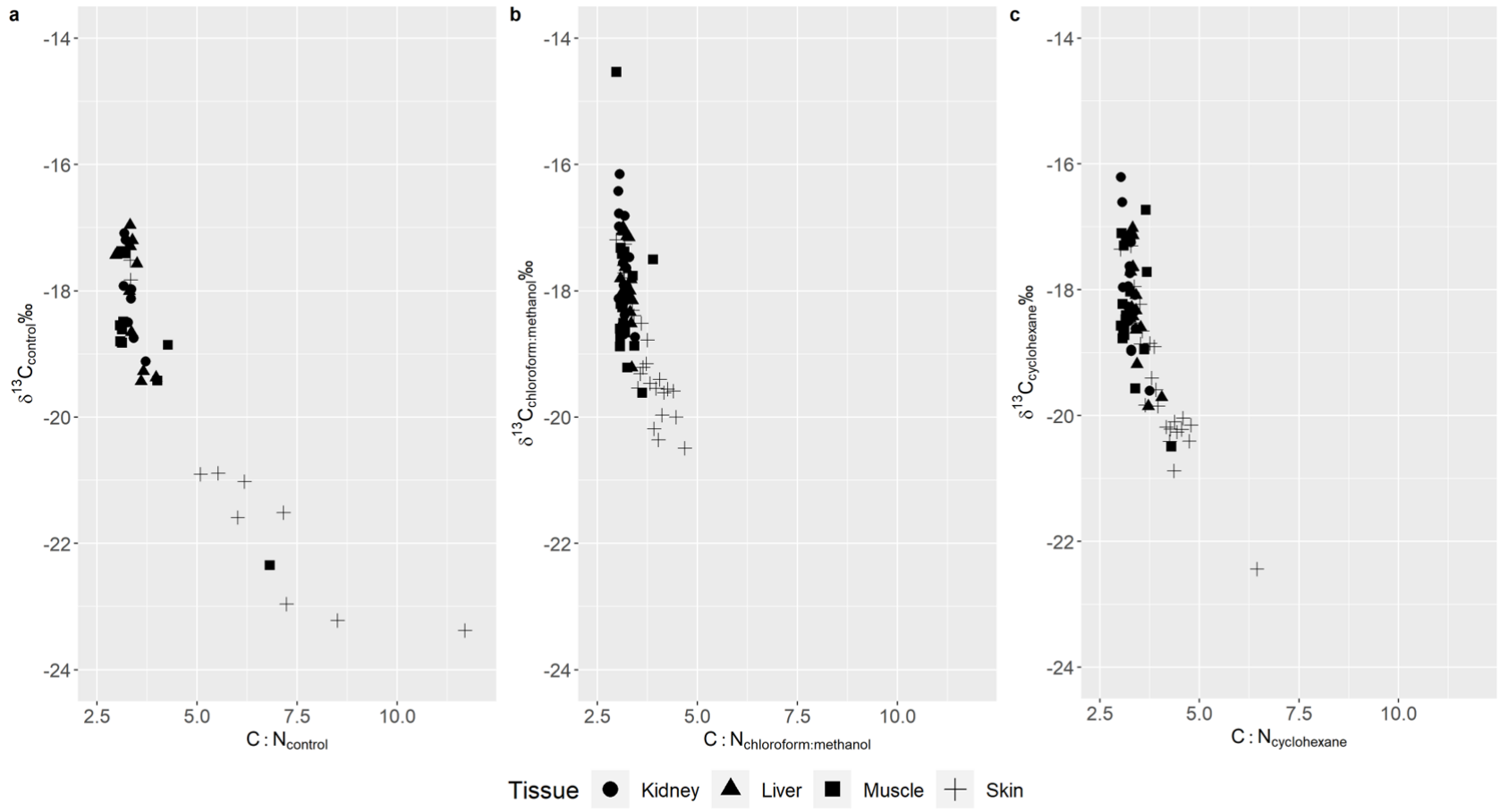

Figure 3.1 Evaluation of extraction method effectiveness in Sowerby's beaked whale tissue samples. 40 subsamples (10 each for kidney, liver, muscle, and skin) were analyzed without chemical delipidation (a); 77 subsamples (18 kidney, 17 liver, 18 muscle, 24 skin) were extracted with 2:1 chloroform:methanol (b) and cyclohexane (c).

ratios for most tissues also fell within acceptable C:N ratios, and longer extractions with this method may only be required on lipid-rich tissues, such as skin.

For both extraction methods, mean skin C:N values were greater than total sample mean (chloroform:methanol = 3.8; cyclohexane = 4.1), and muscle, liver, and kidney C:N mean were less than total sample mean (chloroform:methanol = 3.2, 3.2, and 3.2 respectively; cyclohexane = 3.3, 3.4, and 3.3 respectively) (Figure 3.1). The observed relationship between $\delta^{13}C$ values and C:N ratios post extraction begins to level out when C:N ratios exceed 4, and extrapolation of the relationship to infinite C:N ratios suggests that the $\delta^{13}C$ value of pure lipid in Sowerby's beaked whale tissues is between -20‰ and -25‰. Based on the observed relationship between $\delta^{13}C$ values and C:N ratios (Figure 3.1), together with the assumed C:N ratio of pure protein (Post et al. 2007), we suggest that beaked whale tissue samples with C:N ratios around 3.5 do not require chemical extraction or statistical correction.

Paired Wilcoxon tests for question (2), variance between chloroform:methanol and cyclohexane extracted samples (n = 77), demonstrated that $\delta^{13}C$ values of kidney, liver, and skin subsamples extracted with chloroform:methanol were significantly different than subsamples of those same tissues extracted with cyclohexane, and the difference in muscle tissue values approached significance (Table 3.1). The difference in $\delta^{13}C$ values between subsamples ranged from 0.0‰ to 2.3‰. For $\delta^{15}N$ values, only kidney subsamples were significantly different between the two extraction methods, and differences in values between subsamples ranged from 0.0‰ to 1.7‰. C:N values were significantly different in kidney, liver, and skin subsamples, and the difference in values between subsamples ranged from 0.0‰ to 2.5‰.

Finally, we addressed questions (3) and (4), evaluating the effect of lipid extraction on isotope values and variation in values between differently extracted subsamples of the same

Table 3.1 Mean (± SD) $\delta^{13}C$, $\delta^{15}N$, and C:N values of chloroform:methanol and cyclohexane delipidated Sowerby's beaked whale tissues (n = 77). P values are for paired Wilcoxon tests to evaluate difference in values post extraction method in subsamples of the same tissue sample.

			Chloroform:methanol		Cyclohexane		
	Tissue	*n*	Mean	SD	Mean	SD	*P*
$\delta^{13}C$	Kidney	18	-17.7	0.76	-18.0	0.82	0.014
	Liver	17	-17.8	0.62	-18.2	0.84	0.001
	Muscle	18	-18.1	1.08	-18.3	0.90	0.081
	Skin	24	-19.1	0.93	-19.5	1.15	0.007
$\delta^{15}N$	Kidney	18	13.3	0.80	13.1	0.72	0.012
	Liver	17	13.2	0.88	13.2	0.85	0.712
	Muscle	18	12.6	0.82	12.7	0.96	0.865
	Skin	24	12.7	0.94	12.6	0.91	0.331
C:N	Kidney	18	3.2	0.10	3.3	0.15	0.002
	Liver	17	3.2	0.10	3.4	0.20	<0.001
	Muscle	18	3.2	0.22	3.3	0.32	0.899
	Skin	24	3.8	0.45	4.1	0.69	0.014

tissue sample. Below we summarize the treatment effects and recommendations for each tissue type:

3.4.1 Kidney

Unextracted kidney C:N ratios ranged between 3.2 and 3.7 with a mean of 3.3 and low variation among individuals (Table 3.2). Chloroform:methanol extraction reduced C:N ratios and decreased mean $\delta^{13}C$ values. Both extraction methods increased variation among individuals in $\delta^{13}C$ and $\delta^{15}N$ values. Chloroform:methanol extraction resulted in greater variation among individuals for $\Delta^{13}C$ values, and both extraction methods had similar variation among individuals in $\Delta^{15}N$ and ΔC:N values (Table 3.3, Figure 3.2). Due to the low C:N ratios in unextracted samples and inconsistent changes to variation among individuals in $\delta^{13}C$ and $\delta^{15}N$ values, we recommend avoiding lipid extraction in whale kidney samples.

Table 3.2 Mean (± SD) $\delta^{13}C$, $\delta^{15}N$, and C:N values for unextracted, chloroform:methanol lipid extracted, and cyclohexane lipid extracted Sowerby's beaked whale tissues. *P* values pertain to paired Wilcoxon tests comparing mean values pre and post extraction to evaluate the magnitude of change each extraction method has on values.

			Unextracted		Chloroform:methanol			Cyclohexane		
	Tissue	*n*	Mean	SD	Mean	SD	*P*	Mean	SD	*P*
$\delta^{13}C$	Kidney	10	-18.0	0.70	-17.7	0.92	0.084	-18.0	0.96	0.492
	Liver	10	-18.1	0.98	-17.7	0.65	0.037	-18.0	0.96	0.375
	Muscle	10	-18.9	1.38	-18.4	0.75	0.048	-18.5	0.92	0.193
	Skin	10	-21.1	2.03	-18.9	0.89	0.002	-19.5	1.09	0.004
$\delta^{15}N$	Kidney	10	13.1	0.83	13.2	0.90	0.375	12.9	0.87	0.275
	Liver	10	13.3	0.86	13.2	0.82	0.557	13.3	0.83	0.492
	Muscle	10	12.4	0.75	12.4	0.77	0.769	12.5	0.61	0.375
	Skin	10	12.2	0.73	12.4	0.86	0.106	12.3	0.80	0.232
C:N	Kidney	10	3.3	0.16	3.2	0.11	0.004	3.3	0.17	0.625
	Liver	10	3.4	0.25	3.2	0.09	0.006	3.4	0.23	0.232
	Muscle	10	3.7	1.12	3.3	0.28	0.009	3.3	0.39	0.027
	Skin	10	6.4	2.35	3.7	0.41	0.002	4.1	0.53	0.004

Table 3.3 Mean (± SD) $\Delta^{13}C$, $\Delta^{15}N$, and ΔC:N values between delipidated and unextracted Sowerby's beaked whale tissues (extracted value – unextracted value). *P* values pertain to unpaired Wilcoxon tests to evaluate difference in the change to isotope values by delipidation method.

			Chloroform:methanol		Cyclohexane		
	Tissue	*n*	Mean	SD	Mean	SD	*P*
$\Delta^{13}C$	Kidney	10	0.7	0.53	0.0	0.39	0.123
	Liver	10	0.4	0.48	0.1	0.45	0.143
	Muscle	10	0.5	0.85	0.3	0.63	0.529
	Skin	10	2.2	1.39	1.6	1.16	0.248
$\Delta\delta^{15}N$	Kidney	10	0.2	0.48	-0.2	0.49	0.315
	Liver	10	-0.1	0.36	-0.1	0.31	1.000
	Muscle	10	0.1	0.41	0.1	0.20	0.853
	Skin	10	0.2	0.26	0.1	0.32	1.000
ΔC:N	Kidney	10	-0.1	0.11	0.0	0.10	0.075
	Liver	10	-0.3	0.19	-0.1	0.14	0.015
	Muscle	10	-0.4	0.94	-0.4	0.74	0.739
	Skin	10	-2.7	2.26	-2.3	2.17	0.529

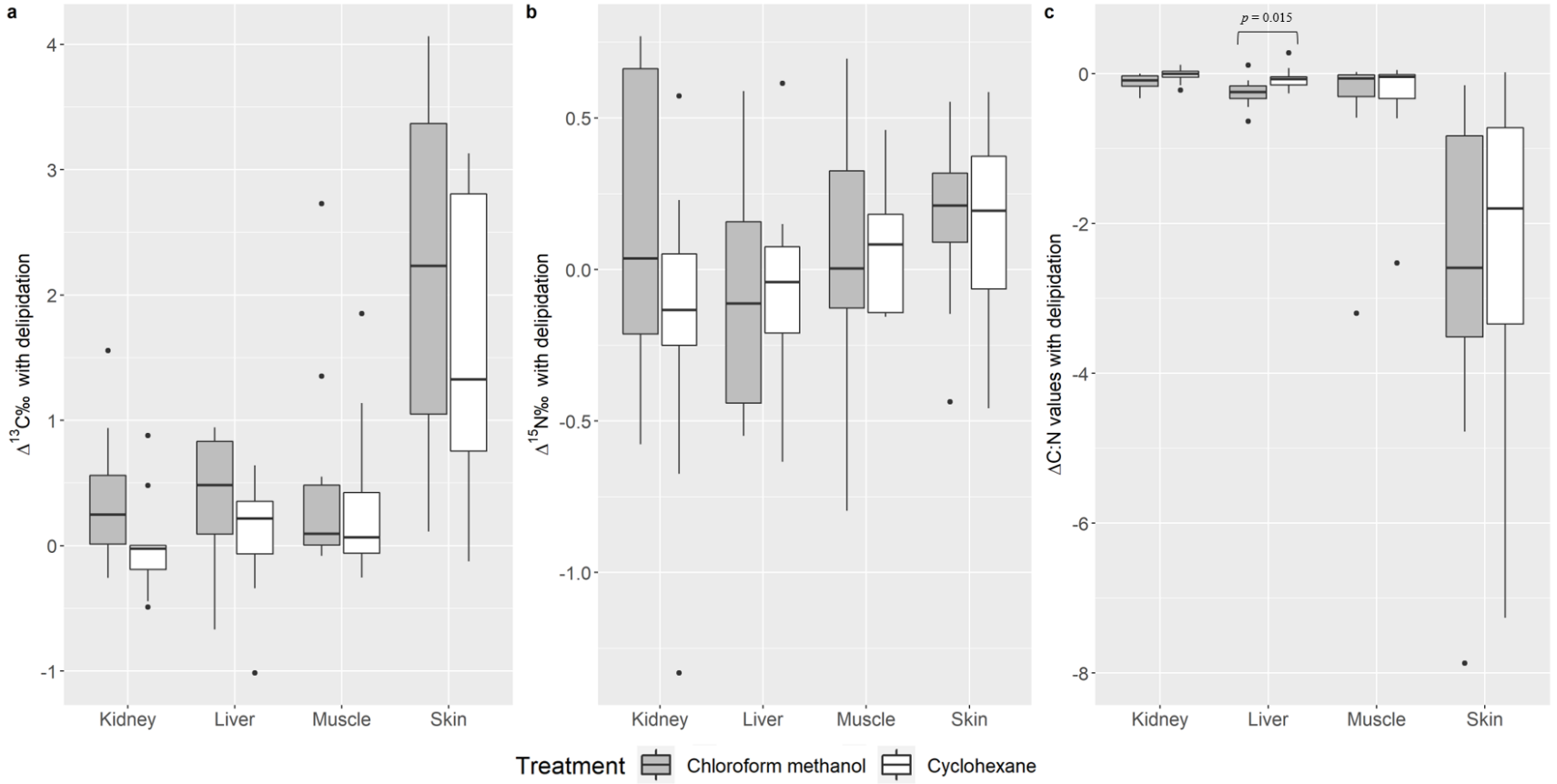

Figure 3.2 Boxplot comparison of the difference in changes to $\delta^{13}C$ (a), $\delta^{15}N$ (b), and C:N (c) values in Sowerby's beaked whale tissues according to lipid extraction method for 10 samples of each tissue type per extraction. Boxes represent median and interquartile range and whiskers represent 95% confidence intervals. Unpaired Wilcoxon tests were used to test for significant differences between extraction methods. The only significant difference between extraction methods was in liver C:N ratios ($p = 0.015$).

3.4.2 Liver

Unextracted C:N ratios ranged between 3.2 and 4.0 with a mean of 3.4 and a small variation among individuals (Table 3.2). Chloroform:methanol extraction reduced C:N ratios and decreased mean $\delta^{13}C$ values and variation among individuals in $\delta^{13}C$ values. $\delta^{15}N$ values and variation among individuals remained largely unchanged after both extraction methods. Both extraction methods had similar variation among individuals in $\Delta^{13}C$, $\Delta^{15}N$, and $\Delta C:N$ values; however, mean $\Delta C:N$ between extraction methods was significantly different (Table 3.3, Figure 3.2). Due to low unextracted C:N ratios we recommend avoiding lipid extraction in whale liver samples. However, due to the reduction in variation among individuals in $\delta^{13}C$ values and relatively low effect on $\delta^{15}N$ and $\Delta^{15}N$ values post extraction, a short extraction with chloroform:methanol may be useful in some studies.

3.4.3 Muscle

Unextracted C:N ratios ranged between 3.1 and 6.8 with a mean of 3.7 and a large variation among individuals (Table 3.2). Both extraction methods effectively reduced mean C:N ratios below 3.5 and reduced among individual variability in $\delta^{13}C$ values. Both extraction methods increased mean $\delta^{15}N$ values to a similar extent, but chloroform:methanol resulted in greater variation among individuals. Chloroform:methanol extraction resulted in greater variation among individuals in $\Delta^{13}C$, $\Delta^{15}N$, and $\Delta C:N$ values (Table 3.3, Figure 3.2). We therefore recommend cyclohexane extraction for whale muscle samples.

3.4.4 Skin

Unextracted C:N ratios ranged between 3.3 and 11.7 with a mean of 6.4 and a large variation among individuals (Table 3.2). Both extraction methods significantly reduced mean C:N ratios and reduced variation among individuals in $\delta^{13}C$ values, though variation among individuals post

cyclohexane extraction was greater than post chloroform:methanol extraction. Both extraction methods increased mean $\delta^{15}N$ values to a similar extent, but chloroform:methanol extraction resulted in increased variation among individuals. Chloroform:methanol extraction resulted in greater variation among individuals for both in $\Delta^{13}C$ and $\Delta C:N$ values, whereas cyclohexane extraction resulted in greater variation among individuals in $\Delta^{15}N$ values (Table 3.3, Figure 3.2). We therefore recommend subsampling whale skin samples and submitting one sample for stable isotope analysis without lipid extraction to obtain an accurate $\delta^{15}N$ value, and one after extraction with chloroform:methanol for an accurate $\delta^{13}C$ value.

3.5 Conclusions and recommendations

Our results indicate that cyclohexane is an effective delipidation technique for tissues with low and moderate lipid content, but not as effective as chloroform:methanol with lipid-rich tissues, such as whale skin. In the sampled Sowerby's beaked whale tissues, the $\delta^{13}C$ value of lipids is between -20‰ and -25‰, and tissues with lower C:N ratios, such as kidney and liver, do not require delipidation (Table 3.2). Samples extracted with cyclohexane resulted in generally lesser changes to $\delta^{15}N$ compared to chloroform-methanol extraction, with some differences being at or near analytical precision, suggesting that this extraction method is less likely to alter the abundance of amino acids in the sample.

It is possible to aggressively delipidate tissues multiple times to obtain a desired C:N ratio, but increasingly aggressive extractions dramatically increase the risk of altering amino acid compositions and associated bulk protein $\delta^{13}C$ and $\delta^{15}N$ values. We found that a single 30-minute extraction effectively removed lipids in most tissue samples, suggesting that prolonged lipid extraction of hours or days may be unnecessary, especially for ground tissues. Thus, we recommend avoiding aggressive delipidation when possible except in lipid-rich tissues such as

whale skin. For these Sowerby's beaked whale tissues, C:N values < 5 indicate lipids have been removed while preserving the relative abundance of amino acids; we anticipate repeating this analysis on the same tissue types from other whale species would yield comparable results.

Lipid content in tissue samples and how the presence of lipids effects $\delta^{13}C$ is an important consideration when designing animal studies. Our work provides insight into selecting the appropriate delipidation technique, if applicable, for a variety of tissues with varying levels of lipid content. When ample tissue is available and funding permits, we recommend reporting isotope values from both unextracted and chloroform:methanol extracted samples. Researchers would then consider $\delta^{15}N$ values from the unextracted sample and $\delta^{13}C$ from the extracted sample in studies. However, for rare or scarce tissues, or when funding limits processing to one sample, we recommend using cyclohexane for tissues with low or moderate lipid content, and chloroform:methanol for lipid-rich tissues.

Chapter 4: Stable isotope analysis of specimens of opportunity reveals site fidelity in an elusive North Atlantic species, the Sowerby's beaked whale (*Mesoplodon bidens*)

4.1 Abstract

Elusive wildlife present challenges to effective conservation measures because it is often challenging to collect enough data on these species to implement management and conservation plans. Specimens of opportunity, such as museum specimens, provide a way to improve knowledge on these species, and these specimens have already proven valuable by increasing information on biodiversity, habitat and range, and population structure in many species. Stable isotope analysis (SIA) if a powerful tool to investigate foraging behavior and habitat use and can be used in elusive wildlife studies. In this study we conducted SIA on Sowerby's beaked whale (*Mesoplodon bidens*) specimens of opportunity. Beaked whales are a specious group of cetaceans that are challenging to study in situ, and although Sowerby's beaked whale was discovered >200 years ago, little is known about its biology. We collected bone, muscle, and skin tissue from 103 Sowerby's beaked whales collected in the east and west Atlantic Ocean and conducted $\delta^{13}C$ and $\delta^{15}N$ analyses. We found consistent trends in isotope values across all three tissue types. East Atlantic specimens had less enriched $\delta^{13}C$ and $\delta^{15}N$ than west Atlantic specimens, and median isotope values were significantly different between regions. Quadratic discriminant analysis considering $\delta^{13}C$ and $\delta^{15}N$ simultaneously correctly assigned 92.0%, 90.0%, and 80.3% of skin, muscle, and bone samples, respectively, to their collection location. Our results indicate these Sowerby's beaked whale specimens exhibited short- and long-term regional site fidelity to the region from which they were collected, and suggests the species exhibits a metapopulation structure. This information lays the groundwork for future studies in this species and provides critical knowledge regional and international conservationist scientists need. Our results

demonstrate the effectiveness of SIA in specimens of opportunity, which can be applied to other beaked whale species, and other elusive species.

4.2 Introduction

Effective wildlife conservation requires understanding the ecology of species to be conserved, yet often species of concern are rare or elusive, resulting in large gaps in knowledge regarding their biology and ecology (Cunningham & Lindenmayer 2005; McKelvey et al. 2008). Elusive wildlife species present a unique set of challenges to researchers and managers because they typically are rarely encountered, live in remote or inaccessible habitats, or actively avoid human researchers and equipment (Kalton & Anderson 1986; Green & Young 1993; McDonald 2004; Meek et al. 2014a). This can make it challenging or impossible to gain reliable information on even basic elements of their life history through traditional field techniques (Piggott & Taylor 2003; Joseph et al. 2006). Thus, it is necessary to identify alternative ways to gather data on these species.

Specimens of opportunity offer a potentially efficacious pathway for collecting data on rare and elusive species (Robbirt et al. 2011; Roberts et al. 2016). These sources include museum specimens, salvaged carcasses, or specimens collected in the wildlife trade or for human consumption and sold in the marketplace. Museums are critically important repositories of biological data, and museum specimens have been used in comparative studies, to identify new species, and to understand historical biodiversity (Newbold 2010; Holmes et al. 2016; McDonough et al. 2018; MacLean et al. 2019). Salvaged carcasses, such as roadkill or stranded marine mammals, have provided new information on range and population structure (Baus et al. 2019; Coombs et al. 2019; Schwartz et al. 2020). Similarly, animals collected for the pet trade or

human consumption have yielded new species and information on hybridization events (Erdmann 1999; Baker et al. 2007; Endo et al. 2012; Ebert et al. 2019). As research tools develop, the quantity and quality of information that can be gained from these specimens of opportunity grows, providing an invaluable resource to investigate the biology and ecology of elusive species.

Stable isotope analysis (SIA) is a powerful and efficient tool that can be used on specimens of opportunity for addressing biological and ecological questions that may otherwise be challenging or impossible to answer (McKechnie 2004; MacKenzie et al. 2011). Using a variety of tissues representing different timespans of the animal's life, researchers can investigate an animal's natal grounds, movement behavior, and foraging ecology (Phillips & Eldridge 2006; Vander Zanden et al. 2015). SIA conducted on specimens of opportunity has been used to better understand animal migrations, the spatial origin of wildlife products, and even historical human diets (Chamberlain et al. 1997; Hobson 1999; Hopkins & Ferguson 2012; Cheung et al. 2017; Guillemain et al. 2019). Two of the most commonly used isotopes for wildlife studies are carbon ($\delta^{13}C$), used to evaluate habitat range and latitudinal shifts, and nitrogen ($\delta^{15}N$), which is used for obtaining foraging and trophic information (Ben-David & Flaherty 2012). As the body of literature on the application of SIA to wildlife studies grows, in conjunction with more high-resolution maps documenting the spatial relationship of stable isotope abundance (i.e., isoscapes), SIA creates more opportunities to utilize specimens of opportunity to increase knowledge regarding elusive species (Vander Zanden et al. 2018).

Although they comprise >25% of extant whale and dolphin species, beaked whales are a group of poorly understood and elusive species, with most questions regarding their basic biology unanswered (Dalebout et al. 2004; Mead 2007, 2009). This paucity of data is largely

attributable to the challenge of locating beaked whales and distinguishing species due to their elusive behavior and similar appearance (MacLeod et al. 2005). Consequently, visually identifying and studying beaked whales in situ is challenging, and many field sightings of beaked whales can only be reliably identified to genus, with possibly a suggestion of species. Specimens of opportunity already have proven critical to increasing our understanding of beaked whale diversity and ecology. For example, three species (*Mesoplodon bowdoini*, *M. traversii*, and *M. hotaula*) are known only from beach cast carcasses, and several other species are known primarily from strandings and a few unconfirmed sightings. Similarly, during the last 20 years four new species were discovered by reexamining museum specimens (Dalebout et al. 2002; van Helden et al. 2002; Dalebout et al. 2014; Morin et al. 2017; Yamada et al. 2019).

The Sowerby's beaked whale (*M. bidens*) was first described in 1804, yet in >200 years since its original description little has been learned about its life history (Waller 2013; Ellis et al. 2017). Due to difficulty in locating and identifying Sowerby's beaked whales, most information on the species' basic biology, such as its spatial and foraging ecology, is still largely unknown, explaining why it is considered "data deficient" by the IUCN and a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (Taylor et al. 2008; COSEWIC 2019). Although individuals of this species have been observed and collected from both North American and European waters, it is unknown if this is one continuous and highly mobile population, or if the species is structured into subpopulations (Macleod 2000; MacLeod et al. 2003; MacLeod et al. 2005).

Based on this lack of data on the population structure and spatial ecology of Sowerby's beaked whales, the management needs of this species are unclear. Site fidelity has been recorded in other beaked whales, such as Cuvier's (*Ziphius cavirostris*) and Blainville's (*M. densirostris*),

but this has not been investigated or documented in Sowerby's beaked whales (McSweeney et al. 2007). Although mitochondrial DNA analysis of 14 individuals identified shared haplotypes between animals collected from both sides of the Atlantic Ocean, the population connectivity of this species is largely unknown (COSEWIC 2006). Additional data regarding the spatial ecology of Sowerby's beaked whale is needed to identify conservation threats and aid in the development of management plans.

In this study we evaluated carbon and nitrogen stable isotope values in tissues from Sowerby's beaked whale specimens of opportunity from the east and west Atlantic. Although previous studies have used specimens of opportunity to increase knowledge on rare and elusive species, these studies have primarily relied on museum or herbarium collections. Our research brings together three tissues types from museum specimens, beach cast carcasses, and bycaught animals to create a robust and diverse collection of specimens of opportunity. Our objectives were to: (1) evaluate the efficacy of specimens of opportunity in spatial ecology studies; (2) identify and characterize regional patterns in isotopic values among Sowerby's beaked whale individuals; and (3) determine if isotope values from specimens of opportunity can be used to illuminate the spatial ecology of Sowerby's beaked whale across multiple timescales.

4.3 Methods

4.3.1 Sampling

We sampled 103 opportunistically collected Sowerby's beaked whale specimens from museums, stranding programs, and research centers for bone, muscle, and skin tissue. In this study we define east and west Atlantic Ocean as being on either side of longitude 35 west. However, all east Atlantic specimens (n = 65) were beach cast, whereas west Atlantic specimens (n = 38) were

either beach cast or bycaught in the swordfish (*Xiphias gladius*) pelagic drift gillnet fishery of the western North Atlantic (Wenzel et al. 2013). In order to reduce the influence of the Seuss effect (Keeling 1979), especially regarding bone tissue, we only sampled specimens collected since 1980. We collected bone tissue from 71, muscle tissue from 41, and skin tissue from 50 specimens. For bone samples, we used a battery powered handheld drill to remove 1g of bone tissue from the occipital bone, when available. In 17 specimens this bone was not available, and we sampled an alternate location approved by the museum. Soft tissue samples were stored at -20 ℃ prior to sampling; we removed 0.5g samples and stored them in 95% ethanol for transportation. Soft tissues are commonly preserved in ethanol, and studies have demonstrated that this preservation technique can contribute to lipid removal but has small and insignificant effects on $\delta^{13}C$ and $\delta^{15}N$ values (Kaehler & Pakhomov 2001; Sarakinos et al. 2002; Javornik et al. 2019).

4.3.2 Stable isotope analysis

We ground bone samples with mortar and pestle and subsampled 200 mg for collagen extraction. Our extraction protocol followed that outlined in Chapter 2, including lipid extraction and HCl and NaOH baths to remove the mineral component. Carbon and nitrogen analyses were completed at the Cornell Isotope Laboratory at Cornell University using a Thermo Delta V isotope mass spectrometer interfaced with a NC2500 elemental analyzer (ThermoFisher Scientific, 168 Third Avenue Waltham, MA USA 02451). We calibrated our results using 2 primary reference scales: Vienna Pee Dee Belemnite for $\delta^{13}C$, and Atmospheric Air for $\delta^{15}N$. To ensure accuracy and precision, we analyzed an in-house standard ($\delta^{13}C$: -20.16 ±0.03‰ and $\delta^{15}N$: 6.35 ±0.05‰) between every 10 samples.

Soft tissue samples were subsampled and freeze dried individually for 16 hours. We ground dried tissues with mortar and pestle and extracted with 2:1 chloroform:methanol for 30 minutes, manually agitating every 5 minutes; additional extractions were performed as necessary if the supernatant was not clear. We dried samples at 60 ℃ for 16 hours post extraction and loaded between 0.5 and 0.8 mg in 3x5mm tin capsules and submitted for C and N SIA. Analysis was completed at the Smithsonian Institution Museum Conservation Institute Stable Isotope Mass Spectrometry Laboratory using a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to an Elementar vario ISOTOPE Cube Elemental Analyzer via a Thermo Conflo IV (ThermoFisher Scientific, 168 Third Avenue Waltham, MA USA 02451). Two standards, an in-house Costech Acetanilide (Costech Analytical, 26074 Avenue Hall, Suite 14 Valencia, CA USA 91355) and Urea-UIN3, calibrated to USGS40 and USGS41 (L-glutamic acid), were included between every 10 samples, with an analytical precision of +/-0.2‰ (1σ). Weight percent carbon and nitrogen values were calibrated to the in-house acetanilide standard with an analytical precision of +/- 0.5%.

We use delta notation (δ) to express our stable isotope results. This is the parts per thousand difference between the sample and international standards, expressed as $\delta^{y}X = [(R_{sample}-R_{standard})/(R_{standard})]$, where X is the element, y is the atomic mass of the stable isotope, and R is the ratio of heavy to light isotopes.

4.3.3 Data analysis

We first created carbon-nitrogen biplots for each tissue type and visually identified apparent patterns in isotope values for samples collected from the east and west Atlantic. We next performed quadratic discriminant analysis to determine whether samples could be correctly assigned to their collection region based on $\delta^{13}C$ values alone, $\delta^{15}N$ values alone, or $\delta^{13}C$ and

$\delta^{15}N$ values simultaneously. We then created box and whisker plots with 95% confidence intervals to visualize difference in tissue $\delta^{13}C$ or $\delta^{15}N$ values between regions and performed Mann Whitney U test to evaluate differences in median tissue isotope values between regions and considered p-values ≤ 0.05 significant. Finally, we used descriptive statistics to compare differences in mean isotope values between regions. We performed our analyses using R (R Core Team 2018) with RStudio (RStudio Team 2016) and JMP (SAS 2019).

4.4 Results

Carbon and nitrogen biplots suggested that Sowerby's beaked whales collected from the east and west Atlantic had differing isotope values (Figure 4.1). Bone tissue had the most overlap in isotope values between regions, whereas both skin and muscle samples appeared as two more distinct regional groups.

Quadratic discriminant analysis assigned specimens to their collection location with a high degree of success (Table 4.1). Analysis that simultaneously considered both $\delta^{13}C$ and $\delta^{15}N$ values was more successful at correctly assigning specimens to their collection location than analysis of either single isotope separately. Skin, muscle, and bone samples analyzed simultaneously for $\delta^{13}C$ and $\delta^{15}N$ were correctly assigned in 92.0, 90.2, and 80.3% of the time, respectively. Single isotope assignment percent probabilities for all tissue types were >70.0% (70.4–82.9%; Table 4.1). We found no consistent trends in sex, age, or collection location among mis-assigned samples.

Box and whisker plots demonstrated differences in median isotope values in all three tissues between collection regions, and that specimens collected in the west Atlantic consistently

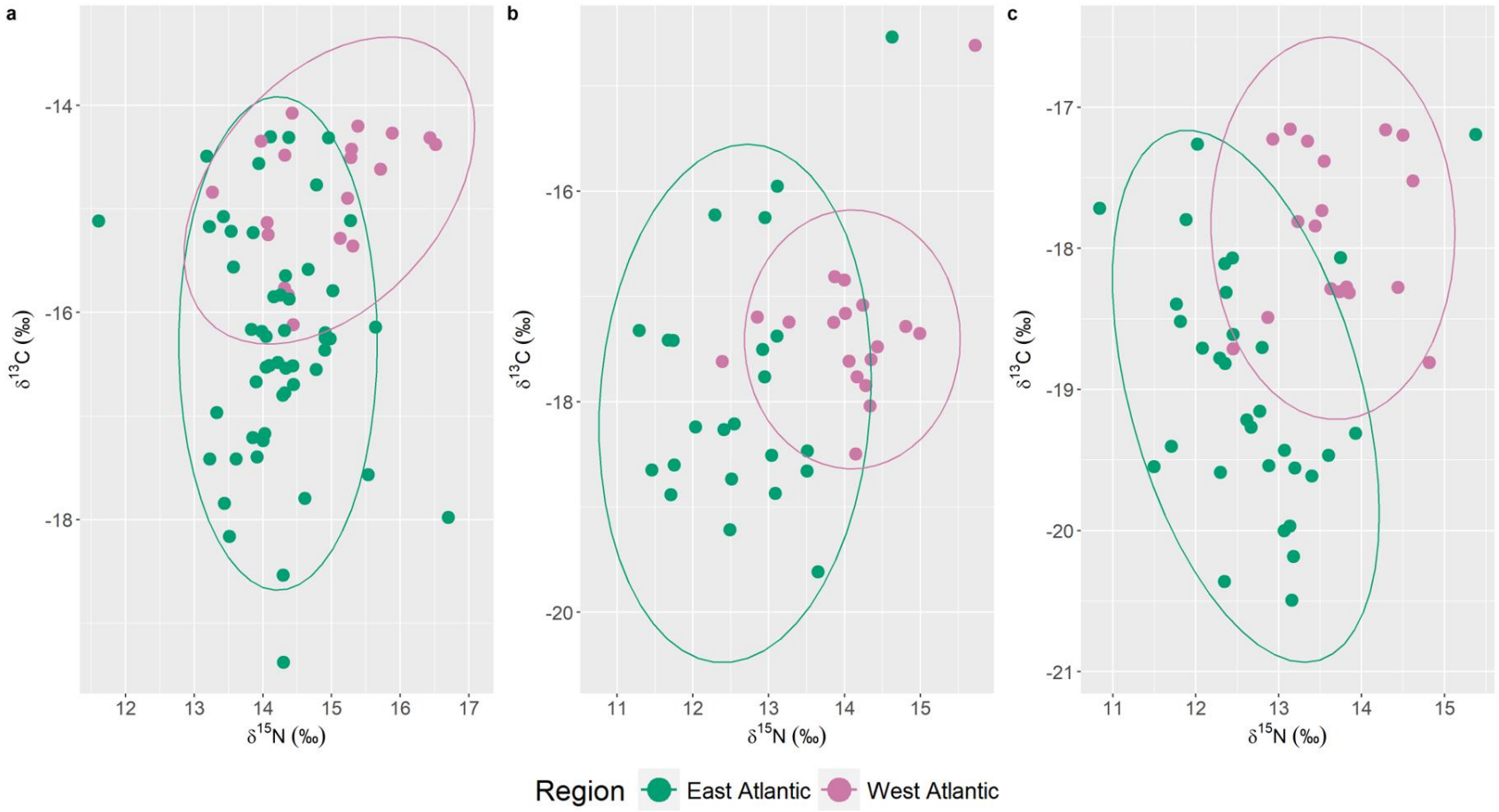

Figure 4.1 $\delta^{13}C$ and $\delta^{15}N$ biplots of (a) bone (n = 71), (b) muscle (n = 41), and (c) skin (n = 50) samples from Sowerby's beaked whale (*Mesoplodon bidens*) specimens of opportunity collected in the east and west Atlantic Ocean basin, 1980–2019. Ellipses are 95% normal confidence ellipses.

Table 4.1 Quadratic discriminant analysis assignment percent probabilities for $\delta^{13}C$, $\delta^{15}N$, and simultaneous $\delta^{13}C$ and $\delta^{15}N$ values in three tissue types of Sowerby's beaked whale (*Mesoplodon bidens*) specimens of opportunity collected in the east and west Atlantic Ocean basins, 1980–2019.

Tissue	*n*	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$ & $\delta^{15}N$
Bone	71	76.0	70.4	80.3
Muscle	41	70.7	82.9	90.2
Skin	50	76.0	76.0	92.0

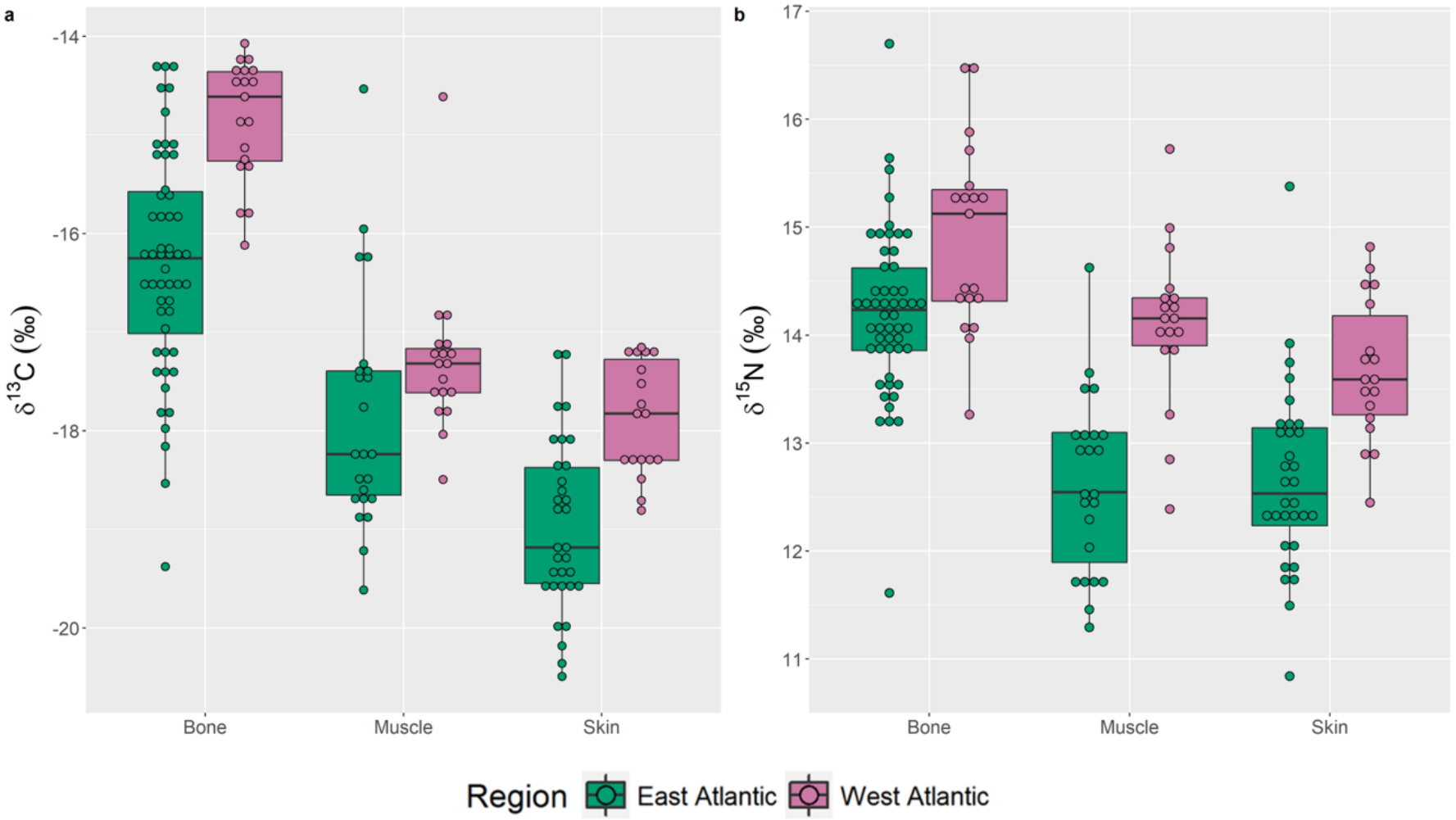

Figure 4.2 Distribution of $\delta^{13}C$ and $\delta^{15}N$ values in three tissues of Sowerby's beaked whale (*Mesoplodon bidens*) specimens of opportunity collected in the east (n = 65) and west (n = 38) Atlantic Ocean basin, 1980–2019. Boxes present median and interquartile range and whiskers represent 95% confidence intervals.

displayed more enriched median isotope values (Figure 4.2). Mann Whitney U tests demonstrated significant differences in median $\delta^{13}C$ and $\delta^{15}N$ values between east and west Atlantic samples in all three tissue types (Table 4.2). Mean $\delta^{13}C$ and $\delta^{15}N$ values in west Atlantic specimens were more enriched than east Atlantic specimens in all tissue types (Table 4.2). East Atlantic specimens exhibited a larger range in $\delta^{13}C$ and $\delta^{15}N$ values than west Atlantic specimens except in $\delta^{15}N$ muscle values, which was the same between regions. In both regions and for both isotopes, bone tissue was more enriched than muscle and skin tissue. For $\delta^{13}C$ values, muscle was more enriched than skin in both regions, but for $\delta^{15}N$ values skin was slightly more enriched than muscle in east Atlantic specimens and less enriched than muscle in west Atlantic specimens (Table 4.2).

Table 4.2 Mean, standard deviation, and range of $\delta^{13}C$ and $\delta^{15}N$ values in three tissues of Sowerby's beaked whale (*Mesoplodon bidens*) specimens of opportunity collected in the east and west Atlantic Ocean basin, 1980–2019. *P* values pertain to Mann Whitney U test used to evaluate differences in median tissue isotope values by region of collection.

		East Atlantic				West Atlantic				
Isotope	Tissue	*n*	Mean ‰	SD	Range ‰	*n*	Mean ‰	SD	Range ‰	*P*
$\delta^{13}C$	Bone	52	-16.3	1.13	5.1	19	-14.8	0.61	2.0	<0.001
	Muscle	23	-17.7	1.20	5.1	18	-17.3	0.79	3.9	0.018
	Skin	32	-19.0	0.87	3.3	18	-17.9	0.57	1.6	<0.001
$\delta^{15}N$	Bone	52	14.2	0.77	5.1	19	14.9	0.87	3.2	0.002
	Muscle	23	12.6	0.81	3.3	18	14.1	0.75	3.3	<0.001
	Skin	32	12.7	0.85	4.6	18	13.7	0.66	2.3	<0.001

4.5 Discussion

Our results suggest that Sowerby's beaked whales exhibit short- and long-term regional site fidelity. The distinct differences in $\delta^{13}C$ and $\delta^{15}N$ values across three tissue types with different growth and turnover rates indicates these Sowerby's beaked whales were not only present in the region from which they were collected during the final months of their lives, but on a continuously long term, possibly decadal, scale. Exact tissue growth and turnover times are species-dependent and influenced by animal health and body condition, where the sample was taken from the carcass, and environmental factors such as temperature. These values are not known for Sowerby's beaked whales or most cetaceans due to the inability to conduct feeding studies for these species; however, we can make broad approximations based on other marine mammals, which experience similar ecophysiological pressures, and large terrestrial mammals (Newsome et al. 2010; Vander Zanden et al. 2015).

4.5.1 Stable isotope values by tissue type

Skin is the fastest growing tissue we evaluated, representing short-term movement and foraging behavior. Skin can be relatively easily sampled in wild populations using biopsy darts, and its growth and isotope incorporation rate has been studied in captive bottlenose dolphins (*Tursiops truncatus*) and killer whales (*Orcinus orca*). Hicks et al. (1985) measured skin turnover in bottlenose dolphins at 73 days, and Williams et al. (2008) found that captive bottlenose dolphins and killer whales fed controlled diets for 5–7 months had reached isotope equilibrium in their skin and had isotope values that reflected their diets. Thus, we estimate that the skin isotope signatures in the Sowerby's beaked whales in our study reflect habitat and foraging behavior ~3 months prior to sampling.

East Atlantic skin samples demonstrated less enriched mean $\delta^{13}C$ than west Atlantic specimens, corresponding to Atlantic Ocean isoscape models (Magozzi et al. 2017). The consistency of $\delta^{13}C$ and $\delta^{15}N$ isotope values among animals collected from the same region, the clear distinction in median values between east and west Atlantic specimens, and the high assignment percent probability from discriminant analysis all suggest that the animals in our study were living and foraging in the region from which they were collected several months prior to their deaths (Tables 4.1–4.2, Figure 4.2). This indicates that animals were not moving between the east and west Atlantic during the months prior to their collections, suggesting regional site fidelity on the order of months at a time.

Muscle is a more challenging tissue to study than skin due the invasive nature required to collect samples, which is often limited to animals that have been sacrificed in feeding studies or have died and been opportunistically sampled. As a result, there is a lack of information on cetacean muscle growth and isotope turnover time. Vander Zanden et al. (2015) found a positive correlation between body mass and isotope half-life in mammal muscle tissue, and that muscle had a higher half-life than other internal organs. Muscle isotope turnover rate has been studied in cattle, which provide the best current approximation to Sowerby's beaked whales due to similar body mass (i.e. ~700 kg). Bahar et al. (2009) switched diets of beef cattle 5 months before slaughter and found that carbon and nitrogen isotopic equilibrium was not reached in that time, thus demonstrating that muscle turnover time and isotopic integration in mammals of this size takes >5 months. They suggested it may take a ≥1 year for sampled muscle tissue to reflect diet. For these reasons, we estimate that muscle isotope signatures in Sowerby's beaked whales reflect foraging and habitat use from ~1 year prior to sampling.

We found that muscle tissue followed the same isotopic patterns as skin tissue, with east Atlantic mean values less enriched than west Atlantic mean values for both isotopes, clear distinction in median values by region, and high assignment percent probabilities (Tables 4.1–4.2, Figure 4.2). These results suggest that animals were in the region of collection at least one year prior to sampling. Combined with the shorter temporal snapshot of skin, muscle tissue strongly suggests that Sowerby's beaked whales are not frequently moving between the east and west Atlantic and instead demonstrate regional site fidelity for at least a year.

No data are available on cetacean bone growth and turnover rates. However, in other large mammals bone can represent a decade or more of growth, and has a turnover rate of 5–10% per year in adults (Bronner 2008, Chapter 2). Thus, bone tissue can provide a "smear" of isotopic signatures from several years, making this the most complex tissue to analyze in our study. Despite this complexity, bone tissue followed the same patterns as skin and muscle, with east Atlantic specimens less enriched in both isotope values, and with distinct isotopic median values between regions (Table 4.1, Figure 4.2). Bone tissue had the lowest assignment percent probability; however, with an assignment percent probability >80%, variation in this slow turnover tissue may reflect changing ecosystem isotope values rather than trans-Atlantic movement patterns (Table 4.2). Ecosystem variables, such as the Atlantic meridional mode, which contributes to interannual and decadal variation in Atlantic Ocean sea surface temperature, may drive bone isotope variation (Doi et al. 2010). Additionally, in Chapter 2 we found that Sowerby's beaked whale skeletons exhibit median intraskeletal $\delta^{13}C$ variation of ~4‰, which may explain some of the isotope variation and mis-assigned specimens in our study because we could not sample the occipital bone in 17 specimens. Thus, the bone isotopic values in our study demonstrate that bone tissue is largely being grown in a single geographic region, and even in

this complex and slow growing tissue we see long-term east and west Atlantic site fidelity, with the possibility of infrequent broader movements.

4.5.2 Spatial population structuring

When considered alone, $\delta^{15}N$ values were better at correctly assigning specimens to their collection region than $\delta^{13}C$ values. This is likely due to $\delta^{13}C$ values being more geographically variable due to a confluence of shallow and deep-water currents, particularly in the western Atlantic, and to globally changing $\delta^{13}C$ values due to the Seuss effect (Reverdin et al. 2003; Hakkinen & Rhines 2009; Lorrain et al. 2020).

Distinct $\delta^{15}N$ values were observed between east and west Atlantic specimens in all tissue types, suggesting long-term differences in foraging behavior between these groups (Table 4.1, Figure 4.2). Few data are available regarding Sowerby's beaked whale foraging as most specimens strand without stomach contents. In the east Atlantic, stomach contents have been analyzed from specimens that stranded in the Azores and the Bay of Biscay, where both studies found that small to medium mid-water fish species, such as hake and cod (e.g., *Micromesistius poutassou*, *Trisopterus spp.*, and *Merluccius merluccius*) comprised the majority of stomach contents (Pereira et al. 2011; Spitz et al. 2011). In the west Atlantic, stomach contents from healthy Sowerby's beaked whales bycaught in the pelagic driftnet fishery revealed similar prey items: fish comprised the majority of stomach contents, with short beard codling (*Laemonema barbatulum*), Cocco's lanternfish (*Lobianchia gemellarii*), marlin-spike grenadier (*Nezumia bairdii*), lanternfishes (*Lampanyctus spp*.), and longfin hake (*Phycis chesteri*) being most abundant (Wenzel et al. 2013). Despite the similarities in types of prey items between east and west Atlantic specimens, the differences we observed in $\delta^{15}N$ values strongly suggest that Sowerby's beaked whales demonstrate long-term fidelity in their foraging locations.

Distinct east and west Atlantic $\delta^{13}C$ ratios in our specimens indicate long-term regional fidelity for Sowerby's beaked whales rather than continuous or seasonal movement throughout the Atlantic Ocean basin (Table 4.1, Figure 4.2). We observed a pattern of less enriched east Atlantic $\delta^{13}C$ values than west Atlantic values across all three tissue types; this trend is consistent with $\delta^{13}C$ isoscape models of the Atlantic Ocean basin (Magozzi et al. 2017; Trueman & St John Glew 2019). It is important to consider that $\delta^{13}C$ values are subject to the Seuss effect, the long-term increase in isotopically light carbon being incorporated into marine ecosystems due to fossil fuel use (Keeling 1979). Though we limited our samples to a 40-year window in order to reduce the influence of the Seuss effect in our study, recent studies have shown declines in marine ecosystem $\delta^{13}C$ values of up to 2.5‰ over only 15 years (Lorrain et al. 2020). These changing global $\delta^{13}C$ values could account for some of the $\delta^{13}C$ variation we observed in specimens collected from the same region, and partially explain why $\delta^{13}C$ was not as good a predictor of collection location as $\delta^{15}N$. However, $\delta^{13}C$ values alone still successfully assigned >70% of specimens to their collection region (Table 4.2) and aligned with trends in measured regional $\delta^{13}C$ isoscape values, suggesting that if it we had sufficient ecosystem data to account for environmental fluctuations in $\delta^{13}C$ values for these samples, assignment percent probability would increase.

4.5.3 Conservation implications

These results provide the first evidence for spatial structuring in Sowerby's beaked whale populations and suggests there may be limited movement between these two regions. Coupled with previously identified shared mitochondrial haplotypes between east and west Atlantic animals (COSEWIC 2006), our results suggest that Sowerby's beaked whales exhibits a metapopulation structure. However, to further explore whether there are two or more Sowerby's

beaked whale distinct population segments, or a homogenous species with habitat preference among individuals and regional mixing for mating, genetic analysis also is needed. The Atlantic Ocean basin is a complex ecosystem and environmental factors such as seasonal productivity, temperature, and ocean currents likely all influence Sowerby's beaked whale spatial distribution. Future studies focused on exploring the nuances of these factors, and on evaluating how Sowerby's beaked whale isotope values align with seasonally changing Atlantic isoscapes, are needed. East Atlantic specimens are better represented than west Atlantic specimens in our dataset; this may be due to multiple oceanic currents in the west Atlantic acting to carry distressed animals and carcasses away from shore. For example, the Gulf Stream may be carrying specimens east and out to sea, resulting in less beach-cast carcasses in the west Atlantic. We do not think that west Atlantic carcasses are being carried to strand in the east Atlantic, as the level of decomposition in most strandings had not progressed sufficiently to suggest long-term drift. In the east Atlantic, the North Atlantic Drift Current may explain why Sowerby's beaked whales strand in the United Kingdom, particularly Scotland, with such a high frequency as compared to other locations.

Our results provide critical data regarding spatial structuring in Sowerby's beaked whale populations, demonstrate the value of specimens of opportunity for conservation science, and illustrate the usefulness of SIA for elusive species research. The methods we used can be applied to other beaked whales, providing much needed information about this enigmatic group of animals. Due to the paucity of data on beaked whales in general, analysis of specimens of opportunity for some species may be the only way to garner sufficient baseline data to reliably inform future research and conservation plans for beaked whales. For beaked whales assumed to have large distributions, SIA of specimens of opportunity can provide an efficient and

inexpensive means to test this assumption and thus provide insight into population units or regional fidelity among groups or individuals.

Specimens of opportunity are vital sources of biological information regarding elusive species, and SIA is an efficacious means of quickly generating data to address wildlife and ecological questions. The methods used in this study can be applied to an array of other marine or terrestrial animals, narrowing the knowledge gap for elusive species, and aiding in the development of wildlife conservation plans. Museum and research institutions often store multiple tissues from specimens of opportunity, and with the increase in frozen tissue repositories researchers have access to multiple temporal snapshots and can reconstruct short- and long-term foraging and movement behavior. Our results demonstrate the usefulness of these samples to elusive species research and provide a framework to apply these methods to other species.

Chapter 5: Conclusions and future directions

5.1 Sowerby's beaked whales

Sowerby's beaked whales (*Mesoplodon bidens*) remain an elusive species, but our research helped shed light on important aspects of their biology and ecology. Foremost among these are the stable isotope and morphological data that suggest population structuring in this species. Previously, this species has been treated as a continuous population due to a lack of data, though some management plans have mentioned that population structuring may exist (e.g., COSEWIC 2019). Our study provides strong stable isotope evidence for short- and long-term regional site fidelity (Table 4.1, Figure 4.2), further substantiated by significant skull and mandibular morphological variation between specimens from the east and west Atlantic (Table 1.1). Due to difficulty locating animals in situ, and their strong aversion to the attachment of tracking devices (Visser 2012), extensive field studies with current research methods may never be possible.

Molecular analysis of beach cast and museum specimens can be conducted to better understand population structure and connectivity throughout the species' range. Additionally, there exists ample opportunities to conduct additional stable isotope analyses, such as compound specific analysis, which provides more fine scale resolution data on foraging behavior. Sowerby's beaked whales strand relatively frequently in Europe, and a network of stranding volunteers routinely collects material from these carcasses. Researchers should strive to collaborate with these programs to gain access to samples that are all too often overlooked in favor of boat-based field work.

Our collated database of specimens (Appendix Table 1.1) provides an opportunity to explore skull and mandibular morphology, especially age- and sex-specific characteristics, such as TML and the proportion of the mandibular length that is mandibular symphysis. We found

that calf and juvenile Sowerby's beaked whales were characterized by TML <45cm with a mean percent ratio of MSDL to TML of 22.9%, compared to TML >45cm with a mean percent ratio of 31.2% in adult specimens. As Sowerby's beaked whales mature, their TML increases, as expected, but the proportion of that length that is mandibular symphysis increases as well. Further investigation of how this varies between males and females will provide much needed information on growth and sexual maturity in this species. We expect that there was some systematic error in our measurements due to the tape measure we used, and random error as a result of each museum having slightly different conditions under which we were taking measurements. Therefore, selecting a subset of specimens to measure with calibrated calipers and under more controlled conditions should be considered for a future study.

Finally, the extralimital strandings in the west Atlantic should be more closely evaluated to see if a range expansion in this region is warranted. Through snowball sampling we were connected to researchers who have photographs of beaked whales that strongly resemble Sowerby's beaked whales in Brazil and the Caribbean. Unfortunately, no bone or soft tissues were retained from these animals and their identification cannot be confirmed. The animal that stranded in Florida has previously been considered an extreme extralimital example, but this may not be the case. The southwest portion of the north Atlantic may be less populated by Sowerby's beaked whales, may only be used by the species on a seasonal basis, and/or currents in the area may severely reduce the probability of Sowerby's beaked whale carcasses stranding. Any of these scenarios could account for the few sightings and strandings in this region; however, ample strandings have occurred outside of the accepted range along the southern US coastline for this to be reconsidered as part of the species' range.

5.2 Cetacean stable isotopes

We found considerable intraskeletal isotopic variation among cetacean species; this was an unanticipated result based on previous studies (Table 2.1). We have proposed bones to use in cetacean skeletal isotope studies (i.e. proximal rib) and bones to avoid (i.e. humeral head and mandibular ramus). However, this should be further explored. Specifically, species-specific studies with many sampling locations (e.g., 20) across numerous specimens (e.g., 100) encompassing both sexes across all age classes. Though it is easier to use soft tissue than bone for stable isotope analysis, quite often bone is the only tissue available. Researchers then are faced with the dilemma of assuming any single bone of an animal represent the bone tissue of that animal as a whole or excluding these valuable samples because they cannot be standardized. Species-specific analysis will help researchers to better integrate these tissues into their studies.

5.3 Specimens of opportunity

Specimens of opportunity were vital to the success of our research, and they should be utilized in other elusive species research. We found that museum curators are eager to work with researchers, and employing a snowball sampling approach similar to ours will facilitate making these connections and locating specimens.

References

Aberg J, Jansson G, Swenson JE, Mikusinski G. 2000. Difficulties in detecting habitat selection by animals in generally suitable areas. Wildlife Biology **6**:89-99.

Ambrose SH. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. Journal of Archaeological Science **17**:431-451.

Anthony R, Stuart B. 2015. Solvent Extraction and Characterization of Neutral Lipids in Oocystis sp. Frontiers in Energy Research **2**.

Aragones LV, Jefferson TA, Marsh H. 1997. Marine mammal survey techniques applicable in developing countries. Pages 15-39 in Smith BD, and Perrin WF, editors. Asian Marine Biology. Hong Kong University Press, Hong Kong.

Auriolesgamboa D. 1992. Notes on a mass stranding of Baird's beaked whales in the Gulf of California, Mexico. California Fish and Game **78**:116-123.

Bahar B, Moloney AP, Monahan FJ, Harrison SM, Zazzo A, Scrimgeour CM, Begley IS, Schmidt O. 2009. Turnover of carbon, nitrogen, and sulfur in bovine longissimus dorsi and psoas major muscles: Implications for isotopic authentication of meat. Journal of Animal Science **87**:905-913.

Baker CS, Cooke JG, Lavery S, Dalebout ML, Ma Y-U, Funahashi N, Carraher C, Brownell Jr RL. 2007. Estimating the number of whales entering trade using DNA profiling and capture-recapture analysis of market products. Molecular Ecology **16**:2617-2626.

Ballance LT. 2009. Cetacean Ecology. Pages 196-201 in Perrin WF, Wursig B, and Thewissen JGM, editors. Encyclopedia of Marine Mammals, 2nd Edition.

Bas M, Garcia NA, Crespo EA, Cardona L. 2019. Intraskeletal variability in stable isotope ratios of C and N among pinnipeds and cetaceans. Marine Mammal Science:11.

Baus I, Mino CI, Monge O. 2019. Current trends in the application of non-invasive genetic sampling to study Neotropical birds: Uses, goals, and conservation potential. Avian Biology Research **12**:125-132.

Ben-David M, Flaherty EA. 2012. Stable isotopes in mammalian research: a beginner's guide. Journal of Mammalogy **93**:312-328.

BenDavid M, Flynn RW, Schell DM. 1997. Annual and seasonal changes in diets of martens: Evidence from stable isotope analysis. Oecologia **111**:280-291.

Bergamo TF, Botta S, Copertino M. 2016. Lipid extraction in stable isotope analyses of juvenile sea turtle skin and muscle. Aquatic Biology **25**:1-6.

Besharse JC. 1971. Maturity and sexual dimorphism in skull, mandible, and teeth of beaked whale, *Mesoplodon densirostris*. Journal of Mammalogy **52**:297-315.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology **37**:911-917.

Bonde RK, Oshea TJ. 1989. Sowerbys beaked whale (*Mesoplodon bidens*) in the Gulf of Mexico. Journal of Mammalogy **70**:447-449.

Bose N, Lien J, Ahia J. 1990. Measurements of the bodies and flukes of several cetacean species. Proceedings of the Royal Society B-Biological Sciences **242**:163-173.

Brigham RM. 1989. Effects of radio transmitters on the foraging behavior of barn swallows. Wilson Bulletin **101**:505-506.

Bronner F. 2008. Chapter 25 - Metals in Bone: Aluminum, Boron, Cadmium, Chromium, Lanthanum, Lead, Silicon, and Strontium. Pages 515-531 in Bilezikian JP, Raisz LG, and Martin TJ, editors. Principles of Bone Biology (Third Edition). Academic Press, San Diego.

Brooks C, Bonyongo C, Harris S. 2008. Effects of global positioning system collar weight on zebra behavior and location error. Journal of Wildlife Management **72**:527-534.

Carlstrom J, Denkinger J, Feddersen P, Oien N. 1997. Record of a new northern range of Sowerby's beaked whale (*Mesoplodon bidens*). Polar Biology **17**:459-461.

Chamberlain CP, Blum JD, Holmes RT, Feng XH, Sherry TW, Graves GR. 1997. The use of isotope tracers for identifying populations of migratory birds. Oecologia **109**:132-141.

Cheung C, Jing ZC, Tang JG, Weston DA, Richards MP. 2017. Diets, social roles, and geographical origins of sacrificial victims at the royal cemetery at Yinxu, Shang China: New evidence from stable carbon, nitrogen, and sulfur isotope analysis. Journal of Anthropological Archaeology **48**:28-45.

Cholewiak D, Baumann-Pickering S, Van Parijs S. 2013. Description of sounds associated with Sowerby's beaked whales (*Mesoplodon bidens*) in the western North Atlantic Ocean. Journal of the Acoustical Society of America **134**:3905-3912.

Chouvelon T, Chappuis A, Bustamante P, Lefebvre S, Mornet F, Guillou G, Violamer L, Dupuy C. 2014. Trophic ecology of European sardine Sardina pilchardus and European anchovy Engraulis encrasicolus in the Bay of Biscay (north-east Atlantic) inferred from delta C-13 and delta N-15 values of fish and identified mesozooplanktonic organisms. Journal of Sea Research **85**:277-291.

Clark CT, Horstmann L, Misarti N. 2017. Quantifying variability in stable carbon and nitrogen isotope ratios within the skeletons of marine mammals of the suborder Caniformia. Journal of Archaeological Science-Reports **15**:393-400.

Clarke B. 2008. Normal Bone Anatomy and Physiology. Clinical Journal of the American Society of Nephrology **3**:S131-S139.

Clarke E, Feyrer LJ, Moors-Murphy H, Stanistreet J. 2019. Click characteristics of northern bottlenose whales (*Hyperoodon ampullatus*) and Sowerby's beaked whales (*Mesoplodon bidens*) off eastern Canada. Journal of the Acoustical Society of America **146**:307-315.

Cloyed CS, DaCosta KP, Hodanbosi MR, Carmichael RH. The effects of lipid extraction on δ13C and δ15N values and use of lipid-correction models across tissues, taxa and trophic groups. Methods in Ecology and Evolution **n/a**.

Coombs EJ, et al. 2019. What can cetacean stranding records tell us? A study of UK and Irish cetacean diversity over the past 100 years. Marine Mammal Science **35**:1527-1555.

Cooper LN, Dawson SD, Reidenberg JS, Berta A. 2007. Neuromuscular Anatomy and Evolution of the Cetacean Forelimb. The Anatomical Record **290**:1121-1137.

COSEWIC. 2006. COSEWIC assessment and update status report on the Sowerby's beaked whale *Mesoplodon bidens* in Canada. Canada. CotSoEWi, Ottawa.

COSEWIC. 2019. COSEWIC assessment and status report on the Sowerby's Beaked Whale *Mesoplodon bidens* in Canada. Canada CotSoEWi, Ottawa.

Cristescu B, Boyce MS. 2013. Focusing Ecological Research for Conservation. AMBIO **42**:805-815.

Cunningham RB, Lindenmayer DB. 2005. Modeling count data of rare species: Some statistical issues. Ecology **86**:1135-1142.

Dalebout ML, Baker CS, Mead JG, Cockcroft VG, Yamada TK. 2004. A comprehensive and validated molecular taxonomy of beaked whales, family Ziphiidae. Journal of Heredity **95**:459-473.

Dalebout ML, Mead JG, Baker CS, Baker AN, van Helden AL. 2002. A new species of beaked whale *Mesoplodon Perrini* sp. n. (cetacea: Ziphiidae) discovered through phylogenetic analyses of mitochondrial DNA sequences. Marine Mammal Science **18**:577-608.

Dalebout ML, et al. 2014. Resurrection of *Mesoplodon hotaula* Deraniyagala 1963: A new species of beaked whale in the tropical Indo-Pacific. Marine Mammal Science **30**:1081-1108.

Dalebout ML, Steel D, Baker CS. 2008. Phylogeny of the Beaked Whale Genus Mesoplodon (Ziphiidae: Cetacea) Revealed by Nuclear Introns: Implications for the Evolution of Male Tusks. Systematic Biology **57**:857-875.

DeNiro M, Epstein S. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science **197**:261-263.

Deniro MJ, Schoeniger MJ. 1983. Stable carbon and nitrogen isotope ratios of bone-collagen - variations within individuals, between sexes, and within populations raised on monotonous diets. Journal of Archaeological Science **10**:199-203.

Dix L, Lien J, Sergeant DE. 1986. A North-Sea beaked whale, *Mesoplodon bidens*, in Conception Bay, Newfoundland. Canadian Field-Naturalist **100**:389-391.

Doi T, Tozuka T, Yamagata T. 2010. The Atlantic Meridional Mode and Its Coupled Variability with the Guinea Dome. Journal of Climate **23**:455-475.

Ebert DA, Akhilesh KV, Weigmann S. 2019. *Planonasus indicus* sp. n., a new species of pygmy false catshark (Chondrichthyes: Carcharhiniformes: Pseudotriakidae), with a revised diagnosis of the genus and key to the family. Marine Biodiversity **49**:1321-1341.

Einfeldt AL, Orbach DN, Feyrer LJ. 2019a. A method for determining sex and chromosome copy number: sex-by-sequencing reveals the first two species of marine mammals with XXY chromosome condition. Journal of Mammalogy **100**:1671-1677.

Einfeldt AL, Paterson IG, Feyrer LJ. 2019b. Complete mitochondrial genomes and phylogeny of two rare whale species: True's (*Mesoplodon mirus*: True 1913) and Sowerby's (*Mesoplodon bidens*: Sowerby 1804) beaked whales. Mitochondrial DNA Part B-Resources **4**:275-276.

Elliott KH, Davis M, Elliott JE. 2014. Equations for Lipid Normalization of Carbon Stable Isotope Ratios in Aquatic Bird Eggs. PLOS ONE **9**:e83597.

Elliott KH, Elliott JE. 2016. Lipid extraction techniques for stable isotope analysis of bird eggs: Chloroform–methanol leads to more enriched 13C values than extraction via petroleum ether. Journal of Experimental Marine Biology and Ecology **474**:54-57.

Elliott KH, Roth JD, Crook K. 2017. Lipid Extraction Techniques for Stable Isotope Analysis and Ecological Assays. Pages 9-24 in Bhattacharya SK, editor. Lipidomics: Methods and Protocols. Humana Press Inc, Totowa.

Ellis R, Mead JG, Ellis R, Mead JG 2017. Sowerby's beaked whale *Mesoplodon bidens* (Sowerby, 1804). Johns Hopkins Univ Press, Baltimore.

Endo T, Hotta Y, Hisamichi Y, Kimura O, Sato R, Haraguchi K, Funahashi N, Baker CS. 2012. Stable isotope ratios and mercury levels in red meat products from baleen whales sold in Japanese markets. Ecotoxicology and Environmental Safety **79**:35-41.

Erdmann MV. 1999. An account of the first living coelacanth known to scientists from Indonesian waters. Environmental Biology of Fishes **54**:439-443.

Fahy GE, Deter C, Pitfield R, Miszkiewicz JJ, Mahoney P. 2017. Bone deep: Variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans. Journal of Archaeological Science **87**:10-16.

Fraser FC 1974. Report on Cetacea stranded on the British coasts from 1948-1966. British Museum (N.H.), London.

Galatius A, Kinze CC. 2016. *Lagenorhynchus albirostris* (Cetacea: Delphinidae). Mammalian Species **48**:35-47.

Giménez J, Ramírez F, Forero MG, Almunia J, de Stephanis R, Navarro J. 2017. Lipid effects on isotopic values in bottlenose dolphins (Tursiops truncatus) and their prey with implications for diet assessment. Marine Biology **164**:122.

Glew KSJ, Graham LJ, McGill RAR, Trueman CN. 2019. Spatial models of carbon, nitrogen and sulphur stable isotope distributions (isoscapes) across a shelf sea: An INLA approach. Methods in Ecology and Evolution **10**:518-531.

Goodman LA. 1961. Snowball Sampling. Ann. Math. Statist. **32**:148-170.

Green RH, Young RC. 1993. Sampling to detect rare species. Ecological Applications **3**:351-356.

Guillemain M, Bacon L, Kardynal KJ, Olivier A, Podhrazsky M, Musil P, Hobson KA. 2019. Geographic origin of migratory birds based on stable isotope analysis: the case of the greylag goose (*Anser anser*) wintering in Camargue, southern France. European Journal of Wildlife Research **65**.

Guthery FS, Lusk JJ. 2004. Radiotelemetry studies: are we radio-handicapping northern bobwhites? Wildlife Society Bulletin **32**:194-201, 198.

Hakkinen S, Rhines PB. 2009. Shifting surface currents in the northern North Atlantic Ocean. Journal of Geophysical Research: Oceans **114**.

Hicks BD, Staubin DJ, Geraci JR, Brown WR. 1985. Epidermal growth in the bottlenose dolphin, *Tursiops truncatus*. Journal of Investigative Dermatology **85**:60-63.

Hobson KA. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia **120**:314-326.

Hobson KA, McFarland KP, Wassenaar LI, Rimmer CC, Goetz JE. 2001. Linking breeding and wintering grounds of Bicknell's thrushes using stable isotope analyses of feathers. Auk **118**:16-23.

Hogsden KL, McHugh PA. 2017. Preservatives and sample preparation in stable isotope analysis of New Zealand freshwater invertebrates. New Zealand Journal of Marine and Freshwater Research **51**:455-464.

Holmes MW, et al. 2016. Natural history collections as windows on evolutionary processes. Molecular Ecology **25**:864-881.

Hong J, Hipp JA, Mulkern RV, Jaramillo D, Snyder BD. 2000. Magnetic resonance imaging measurements of bone density and cross-sectional geometry. Calcified Tissue International **66**:74-78.

Hopkins JB, Ferguson JM. 2012. Estimating the diets of animals using stable isotopes and a comprehensive bayesian mixing model. Plos One **7**.

Howa JD, Lott MJ, Chesson LA, Ehleringer JR. 2016. Isolation of components of plastic explosives for isotope ratio mass spectrometry. Forensic Chemistry **1**:6-12.

Javornik J, Hopkins JB, III, Zavadlav S, Levanič T, Lojen S, Polak T, Jerina K. 2019. Effects of ethanol storage and lipids on stable isotope values in a large mammalian omnivore. Journal of Mammalogy **100**:150-157.

Jorkov MLS, Heinemeier J, Lynnerup N. 2009. The Petrous Bone-A New Sampling Site for Identifying Early Dietary Patterns in Stable Isotopic Studies. American Journal of Physical Anthropology **138**:199-209.

Jorkov MLS, Heinerneier J, Lynnerup N. 2007. Evaluating bone collagen extraction methods for stable isotope analysis in dietary studies. Journal of Archaeological Science **34**:1824-1829.

Joseph LN, Field SA, Wilcox C, Possingham HP. 2006. Presence–absence versus abundance data for monitoring threatened species. Conservation Biology **20**:1679-1687.

Kaehler S, Pakhomov EA. 2001. Effects of storage and preservation on the d13C and d15N signatures of selected marine organisms. Marine Ecology Progress Series **219**:299-304.

Kalton G, Anderson DW. 1986. Sampling rare populations. Journal of the Royal Statistical Society: Series A (General) **149**:65-82.

Keeling CD. 1979. The Suess effect: 13Carbon-14Carbon interrelations. Environment International **2**:229-300.

Kohn MJ, Cerling TE. 2002. Stable isotope compositions of biological apatite. Pages 455-488 in Kohn MJ, Rakovan J, and Hughes JM, editors. Phosphates: Geochemical, Geobiological, and Materials Importance.

Kojadinovic J, Richard P, Le Corre M, Cosson RP, Bustamante P. 2008. Effects of Lipid Extraction on $\delta^{13}C$ and $\delta^{15}N$ Values in Seabird Muscle, Liver and Feathers. Waterbirds: The International Journal of Waterbird Biology **31**:169-178.

Lafage-Proust MH, Roche B, Langer M, Cleret D, Vanden Bossche A, Olivier T, Vico L. 2015. Assessment of bone vascularization and its role in bone remodeling. Bonekey Reports **4**.

Lambert O, de Buffrenil V, de Muizon C. 2011. Rostral densification in beaked whales: Diverse processes for a similar pattern. Comptes Rendus Palevol **10**:453-468.

Landrau-Giovannetti N, Subramaniam K, Brown MA, Ng TFF, Rotstein DS, West K, Frasca S, Waltzek TB. 2020. Genomic characterization of a novel circovirus from a stranded Longman's beaked whale (*Indopacetus pacificus*). Virus Research **277:19782**.

Lesage V, Morin Y, Rioux È, Pomerleau C, Ferguson SH, Pelletier É. 2010. Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. Marine Ecology Progress Series **419**:249-265.

Li Y, Ghasemi Naghdi F, Garg S, Adarme-Vega TC, Thurecht KJ, Ghafor WA, Tannock S, Schenk PM. 2014. A comparative study: the impact of different lipid extraction methods on current microalgal lipid research. Microbial cell factories **13**:14-14.

Lien J, Barry F, Breeck K, Zuschlag U. 1990. Multiple strandings of Sowerby beaked whales, *Mesoplodon bidens*, in Newfoundland. Canadian Field-Naturalist **104**:414-420.

Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. Journal of Animal Ecology **77**:838-846.

Logan JM, Lutcavage ME. 2008. A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. Rapid Communications in Mass Spectrometry **22**:1081-1086.

Lorrain A, et al. 2020. Trends in tuna carbon isotopes suggest global changes in pelagic phytoplankton communities. Global Change Biology **26**:458-470.

MacKenzie KM, Palmer MR, Moore A, Ibbotson AT, Beaumont WRC, Poulter DJS, Trueman CN. 2011. Locations of marine animals revealed by carbon isotopes. Scientific Reports **1**:21.

MacLean HJ, Nielsen ME, Kingsolver JG, Buckley LB. 2019. Using museum specimens to track morphological shifts through climate change. Philosophical Transactions of the Royal Society B: Biological Sciences **374**:20170404.

MacLeod CD, Errin WFP, Pitman RL, Barlow J, Ballance LT, Gerrodette T, Joyce GP, Mullin KD, Palka DL, Waring GT. 2005. Known and inferred distributions of beaked whale species (Cetacea: Ziphiidae). Journal of Cetacean Research and Management **7**:271-286.

Macleod CD, Herman JS. 2004. Development of tusks and associated structures in *Mesoplodon bidens* (Cetaceae, Mammalia). Mammalia **68**:175-184.

MacLeod CD, Santos MB, Pierce GJ. 2003. Review of data on diets of beaked whales: evidence of niche separation and geographic segregation. Journal of the Marine Biological Association of the United Kingdom **83**:651-665.

Macleod D. 2000. Review of the distribution of *Mesoplodon species* (order Cetacea, family Ziphiidae) in the North Atlantic. Mammal Review **30**:1-8.

Magozzi S, Yool A, Vander Zanden HB, Wunder MB, Trueman CN. 2017. Using ocean models to predict spatial and temporal variation in marine carbon isotopes. Ecosphere **8**:e01763.

Manolagas SC. 2000. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine Reviews **21**:115-137.

March D, Brown D, Gray R, Curthoys I, Wong C, Higgins DP. 2016. Auditory anatomy of beaked whales and other odontocetes: Potential for cochlear stimulation via a "vibroacoustic duct mechanism". Marine Mammal Science **32**:552-567.

Martin V, Tejedor M, Perez-Gil M, Dalebout ML, Arbelo M, Fernandez A. 2011. A Sowerby's beaked whale (*Mesoplodon bidens*) stranded in the Canary Islands: the most southern record in the eastern North Atlantic. Aquatic Mammals **37**:512-519.

Matsuura T, Tokutomi K, Sasaki M, Katafuchi M, Mizumachi E, Sato H. 2014. Distinct characteristics of mandibular bone collagen relative to long bone collagen: relevance to clinical dentistry. Biomed Res Int **2014**:769414.

McConnaughey T, McRoy CP. 1979. Food-Web structure and the fractionation of Carbon isotopes in the bering sea. Marine Biology **53**:257-262.

McDonald LL. 2004. Sampling rare populations. Pages 11-42 in Thompson WL, editor. Sampling rare or elusive species. Island Press, New York.

McDonough MM, Parker LD, Rotzel McInerney N, Campana MG, Maldonado JE. 2018. Performance of commonly requested destructive museum samples for mammalian genomic studies. Journal of Mammalogy **99**:789-802.

Mcgowen MR, et al. 2019. Phylogenomic resolution of the cetacean tree of life using target sequence capture. Systematic Biology **69**:479–501.

McKechnie AE. 2004. Stable isotopes: powerful new tools for animal ecologists. South African Journal of Science **100**:131-134.

McKelvey KS, Aubry KB, Schwartz MK. 2008. Using anecdotal occurrence data for rare or elusive species: The illusion of reality and a call for evidentiary standards. BioScience **58**:549-555.

McSweeney DJ, Baird RW, Mahaffy SD. 2007. Site fidelity, associations, and movements of Cuvier's (*Ziphius cavirostris*) and Blainville's (*Mesoplodon densirostris*) beaked whales off the island of Hawai'i. Marine Mammal Science **23**:666-687.

Mead JG. 2007. Stomach anatomy and use in defining systemic relationships of the cetacean family Ziphiidae (beaked whales). The Anatomical Record **290**:581-595.

Mead JG. 2009. Beaked whales, overview: Ziphiidae. Pages 94-97 in Perrin WF, Würsig B, and Thewissen JGM, editors. Encyclopedia of Marine Mammals (Second Edition). Academic Press, London.

Meek P, Ballard G, Fleming P, Falzon G. 2016. Are we getting the full picture? Animal responses to camera traps and implications for predator studies. Ecology and Evolution **6**:3216-3225.

Meek PD, Ballard GA, Fleming PJS, Schaefer M, Williams W, Falzon G. 2014a. Camera traps can be heard and seen by animals. Plos One **9**.

Meek PD, Ballard GA, Fleming PJS, Schaefer M, Williams W, Falzon G. 2014b. Camera traps can be heard and seen by animals. Plos One **9(10): e110832**.

Monteiro-Riviere NA, Inman AO, Mak V, Wertz P, Riviere JE. 2001. Effect of Selective Lipid Extraction from Different Body Regions on Epidermal Barrier Function. Pharmaceutical Research **18**:992-998.

Morin PA, et al. 2017. Genetic structure of the beaked whale genus Berardius in the North Pacific, with genetic evidence for a new species. Marine Mammal Science **33**:96-111.

Mulero-Pazmany M, Jenni-Eiermann S, Strebel N, Sattler T, Negro JJ, Tablado Z. 2017. Unmanned aircraft systems as a new source of disturbance for wildlife: A systematic review. Plos One **12**.

Newbold T. 2010. Applications and limitations of museum data for conservation and ecology, with particular attention to species distribution models. Progress in Physical Geography: Earth and Environment **34**:3-22.

Newsome SD, Clementz MT, Koch PL. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science **26**:509-572.

Newsome SD, del Rio CM, Bearhop S, Phillips DL. 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment **5**:429-436.

Newsome SD, Koch PL, Etnier MA, Aurioles-Gamboa D. 2006. Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Marine Mammal Science **22**:556-572.

Olsen KC, White CD, Longstaffe FJ, von Heyking K, McGlynn G, Grupe G, Ruhli FJ. 2014. Intraskeletal Isotopic Compositions (δ13C, δ15N) of Bone Collagen: Nonpathological and Pathological Variation. American Journal of Physical Anthropology **153**:598-604.

Pereira JN, et al. 2011. Diet of mid-Atlantic Sowerby's beaked whales *Mesoplondon bidens*. Deep-Sea Research Part I-Oceanographic Research Papers **58**:1084-1090.

Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Phillips DL, Eldridge PM. 2006. Estimating the timing of diet shifts using stable isotopes. Oecologia **147**:195-203.

Pietz PJ, Krapu GL, Greenwood RJ, Lokemoen JT. 1993. Effects of harness transmitters on behavior and reproduction of wild mallards. Journal of Wildlife Management **57**:696-703.

Piggott MP, Taylor AC. 2003. Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. Wildlife Research **30**:1-13.

Post DM. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**:703-718.

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG. 2007. Getting to the Fat of the Matter: Models, Methods and Assumptions for Dealing with Lipids in Stable Isotope Analyses. Oecologia **152**:179-189.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rachlow JL, Peter RM, Shipley LA, Johnson TR. 2014. Sub-lethal effects of capture and collaring on wildlife: Experimental and field evidence. Wildlife Society Bulletin **38**:458-465.

Reverdin G, Niiler PP, Valdimarsson H. 2003. North Atlantic Ocean surface currents. Journal of Geophysical Research: Oceans **108**:2-1-2-21.

Riofrío-Lazo M, Aurioles-Gamboa D. 2013. Timing of isotopic integration in marine mammal skull: Comparative study between calcified tissues. Rapid Communications in Mass Spectrometry : RCM **27**:1076-1082.

Robbirt KM, Davy AJ, Hutchings MJ, Roberts DL. 2011. Validation of biological collections as a source of phenological data for use in climate change studies: a case study with the orchid Ophrys sphegodes. Journal of Ecology **99**:235-241.

Roberts DL, Taylor L, Joppa LN. 2016. Threatened or data deficient: assessing the conservation status of poorly known species. Diversity and Distributions **22**:558-565.

RStudio Team. 2016. RStudio: integrated development for R. RStudio Inc, Boston, Massachusetts.

Rugh DJ, Ferrero RC, Dahlheim ME. 1990. Inter-observer count discrepancies in a shore-based census of gray whales (*Eschrichtius robustus*). Marine Mammal Science **6**:109-120.

Ryan C, McHugh B, Trueman CN, Harrod C, Berrow SD, O'Connor I. 2012. Accounting for the effects of lipids in stable isotope (δ13C and δ15N values) analysis of skin and blubber of balaenopterid whales. Rapid Communications in Mass Spectrometry **26**:2745-2754.

Sanchez JA, Berta A. 2010. Comparative anatomy and evolution of the odontocete forelimb. Marine Mammal Science **26**:140-160.

Sarakinos HC, Johnson ML, Zanden MJV. 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. Canadian Journal of Zoology **80**:381-387.

SAS. 2019. JMP®. SAS Institute Inc., Cary, NC.

Schimmelmann A, Albertino A, Sauer PE, Qi H, Molinie R, Mesnard F. 2009. Nicotine, acetanilide and urea multi-level 2H-, 13C- and 15N-abundance reference materials for continuous-flow isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry **23**:3513-3521.

Schwartz ALW, Shilling FM, Perkins SE. 2020. The value of monitoring wildlife roadkill. European Journal of Wildlife Research **66**:12.

Shadwick RE, Goldbogen JA, Pyenson ND, Whale JCA. 2017. Structure and Function in the Lunge Feeding Apparatus: Mechanical Properties of the Fin Whale Mandible. The Anatomical Record **300**:1953-1962.

Skinner MM, Martin AA, Moore BC. 2016. Is lipid correction necessary in the stable isotope analysis of fish tissues? Rapid Communications in Mass Spectrometry **30**:881-889.

Soulé ME. 1985. What is Conservation Biology? A new synthetic discipline addresses the dynamics and problems of perturbed species, communities, and ecosystems. BioScience **35**:727-734.

Sowerby J 1806. The British miscellany, or, Coloured figures of new, rare, or little known animal subjects : many not before ascertained to be inhabitants of the British Isles : and chiefly in the possession of the author, James Sowerby. Printed by R. Taylor & Co., and sold by the author, J. Sowerby by White, Johnson, Symonds, and all other booksellers, London.

Spitz J, Cherel Y, Bertin S, Kiszka J, Dewez A, Ridoux V. 2011. Prey preferences among the community of deep-diving odontocetes from the Bay of Biscay, Northeast Atlantic. Deep Sea Research Part I: Oceanographic Research Papers **58**:273-282.

Stanistreet JE, Nowacek DP, Baumann-Pickering S, Bell JT, Cholewiak DM, Hildebrand JA, Hodge LEW, Moors-Murphy HB, Van Parijs SM, Read AJ. 2017. Using passive acoustic monitoring to document the distribution of beaked whale species in the western North Atlantic Ocean. Canadian Journal of Fisheries and Aquatic Sciences **74**:2098-2109.

Stanistreet JE, Nowacek DP, Read AJ, Baumann-Pickering S, Moors-Murphy HB, Van Parijs SM. 2016. Effects of duty-cycled passive acoustic recordings on detecting the presence of beaked whales in the northwest Atlantic. Journal of the Acoustical Society of America **140**:EL31-EL37.

Sweeting CJ, Polunin NVC, Jennings S. 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Communications in Mass Spectrometry **20**:595-601.

Tatsch ACC, Secchi ER, Botta S. 2016. Effects of acidification, lipid removal and mathematical normalization on carbon and nitrogen stable isotope compositions in beaked whale (Ziphiidae) bone. Rapid Communications in Mass Spectrometry **30**:460-466.

Taylor BL, Baird R, Barlow J, Dawson SM, Ford J, Mead JG, Notarbartolo di Sciara G, Wade P, Pitman RL. 2008. *Mesoplodon bidens* The IUCN Red List of Threatened Species

Taylor GC, Hill JM, Jackson MC, Peel RA, Weyl OLF. 2017. Estimating δ15N fractionation and adjusting the lipid correction equation using Southern African freshwater fishes. PLOS ONE **12**:e0178047.

Thompson DR, Bury SJ, Hobson KA, Wassenaar LI, Shannon JP. 2005. Stable isotopes in ecological studies. Oecologia **144**:517-519.

Tomaszewicz CNT, Seminoff JA, Ramirez MD, Kurle CM. 2015. Effects of demineralization on the stable isotope analysis of bone samples. Rapid communications in mass spectrometry : RCM **29**:1879-1888.

Trueman CN, St John Glew K. 2019. Isotopic tracking of marine animal movement. Pages 137-172 in Hobson KA, and Wassenaar LI, editors. Tracking Animal Migration with Stable Isotopes (Second Edition). Academic Press.

van Helden AL, Baker AN, Dalebout ML, Reyes JC, Waerebeek KV, Baker CS. 2002. Resurrection of *Mesoplodon Traversii* (Gray, 1874), senior synonym of *M. Bahamondi* Reyes, Van Waerebeek, Cárdenas and Yáñez, 1995 (Cetacea: Ziphiidae). Marine Mammal Science **18**:609-621.

Vander Zanden HB, Nelson DM, Wunder MB, Conkling TJ, Katzner T. 2018. Application of isoscapes to determine geographic origin of terrestrial wildlife for conservation and management. Biological Conservation **228**:268-280.

Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC. 2015. Stable isotope turnover and half-life in animal tissues: A literature synthesis. Plos One **10**.

Visser F. 2012. Diving behavior of Sowerby's beaked whale in the Azores.

Wahl SA, Dauner M, Wiechert W. 2004. New tools for mass isotopomer data evaluation in 13C flux analysis: Mass isotope correction, data consistency checking, and precursor relationships. Biotechnology and Bioengineering **85**:259-268.

Waller GNH. 2013. Note on James Sowerby and the discovery of Sowerby's beaked whale, *Mesoplodon bidens*. Archives of Natural History **40**:270-276.

Webb EC, Stewart A, Miller B, Tarlton J, Evershed RP. 2016. Age effects and the influence of varying proportions of terrestrial and marine dietary protein on the stable nitrogen-isotope compositions of pig bone collagen and soft tissues from a controlled feeding experiment. Science and Technology of Archaeological Research **2**:54-66.

Weinrich MT, Belt CR, Morin D. 2001. Behavior and ecology of the Atlantic white-sided dolphin (*Lagenorhynchus acutus*) in coastal New England waters. Marine Mammal Science **17**:231-248.

Wenzel FW, Polloni PT, Craddock JE, Gannon DP, Nicolas JR, Read AJ, Rosel PE. 2013. Food habits of Sowerby's beaked whales (*Mesoplodon bidens*) taken in the pelagic drift gillnet fishery of the western North Atlantic. Fishery Bulletin **44**:381-389.

West JB, Bowen GJ, Cerling TE, Ehleringer JR. 2006. Stable isotopes as one of nature's ecological recorders. Trends in Ecology & Evolution **21**:408-414.

Williams TM, Dunkin RC, Yochem PK, Mcbain JW, Fox-Dobbs K, Mostman-Liwanag H, Maresh J. 2008. Assessing stable isotope signature variation in cetaceans : an evaluation of skin sampling techniques and correlations with diet for bottlenose dolphins and killer whales.

Wright R, Stein M. 2005. Snowball sampling. Pages 495–500 in Kempf-Leonard K, editor. Encyclopedia of social measurement Elsevier, Amsterdam, The Netherlands.

Yamada TK, Kitamura S, Abe S, Tajima Y, Matsuda A, Mead JG, Matsuishi TF. 2019. Description of a new species of beaked whale (Berardius) found in the North Pacific. Scientific Reports **9**:14.

Yurkowski DJ, Hussey NE, Semeniuk C, Ferguson SH, Fisk AT. 2015. Effects of lipid extraction and the utility of lipid normalization models on δ13C and δ15N values in Arctic marine mammal tissues. Polar Biology **38**:131-143.

Appendix

Appendix Table 1.1 List of Sowerby's beaked whale specimens we located in museum and research institutions throughout North America and Europe.

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Biological Museum, Lund University	L938/5004	Unknown	Unknown	8	9	1938	56.24	12.81	Complete skeleton	-	-	-	-	-	-	-	-	
Canadian Museum of Nature	26484	Male	Adult	-	-	1952	49.64	-55.90	Damaged skull and damaged mandibles	71.6	31.1	13.7	43.2	44.3	23.2	21.1	-	
Canadian Museum of Nature	1987-143 2	Male	Adult	1	9	1986	49.40	-54.29	Complete skeleton	61.4	31.1	13.6	32.1	55.4	43.2	12.2	-	
Canadian Museum of Nature	1987-143 1	Male	Adult	1	9	1986	49.40	-54.29	Skull and mandibles	80.3	31.8	14.4	47.6	71.0	46.8	24.2	-	
Canadian Museum of Nature	1987-143 3	Male	Adult	1	9	1986	49.40	-54.29	Skull and mandibles	79.6	32.1	14.5	50.1	72.4	48.5	23.9	-	
Canadian Museum of Nature	26483	Unknown	Subadult	-	9	1953	49.64	-55.90	Damaged skull and damaged mandibles	52.2	27.2	12.6	32.6	51.7	32.6	19.1	-	
Ciência e Recursos Naturais	MMF 2674	Female	Unknown	14	8	1941	32.72	-16.76	Skull and mandibles; partial postcranial	-	-	-	-	-	-	-	-	
Department of Fisheries and Oceans Canada	Mb2006-01	Female	Unknown	23	6	2006	48.80	-64.21	Soft tissue only	-	-	-	-	-	-	-	-	
Department of Fisheries and Oceans Canada	Mb2018-01	Unknown	Unknown	17	10	2018	50.27	-64.77	Soft tissue only	-	-	-	-	-	-	-	-	
Gothenburg Museum of Natural History	Coll. an.-8234	Female	Adult	7	9	1956	57.59	11.86	Skull and mandibles; partial postcranial	79.8	32.7	13.9	49.8	69.6	42.4	27.2	-	Stranded with Coll. an.-8236
Gothenburg Museum of Natural History	Coll. an.-2696	Male	Adult	30	10	1881	59.36	18.24	Skull and mandibles; partial postcranial	72.7	28.2	12.2	44.2	63.5	42.4	21.1	-	

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Gothenburg Museum of Natural History	Coll. an.-459	Male	Adult	15	6	1869	59.32	4.87	Skull and mandibles; partial postcranial	71.2	30.1	12.3	42.3	65.1	47.9	17.2	-	
Gothenburg Museum of Natural History	Coll. an -18527	Male	Unknown	1	6	2010	58.72	11.17	Partial postcranial	-	-	-	-	-	-	-	-	
Gothenburg Museum of Natural History	Coll. an.-8236	Unknown	Juvenile	7	9	1956	57.59	11.86	Skull and mandibles; partial postcranial	46.5	22.8	10.2	25.9	41.1	31.4	9.7	-	Stranded with Coll. an.-8234
Group for Research and Education on Marine Mammals	2013069	Male	Unknown	27	7	2013	48.11	-69.32	Skull and mandibles; partial postcranial	-	-	-	-	-	-	-	-	
Husavik Whale Museum	A	Female	Adult	-	-	-	-	-	Skull and right mandible	77.7	30.3	12.9	48.5	71.2	44.3	26.9	-	
Husavik Whale Museum	B	Female	Unknown	-	1	2009	64.90	-23.73	Complete skeleton	-	-	-	-	-	-	-	-	On display
Icelandic Institute of Natural History	IINH15734	Unknown	Adult	-	7	2004	63.99	-22.75	Skull without mandibles; vertebrae	67.6	34.9	13.1	36.5	-	-	-	-	
Museum National d'Histoire Naturelle	MNHN-ZM-AC-1975-172	Female	Adult	4	9	1975	49.37	-0.86	Skull and mandibles	67.3	27.3	11.7	43.4	58.7	41.5	17.2	-	
Museum National d'Histoire Naturelle	MNHN-ZM-AC-A3541	Female	Adult	-	-	1825	49.49	0.09	Skull and mandibles	76.3	31.2	13.6	51.2	64.5	43.9	20.6	-	
Museum National d'Histoire Naturelle	MNHN-ZM-AC-1975-111	Female	Subadult	4	9	1975	49.36	-0.84	Skull and mandibles; partial postcranial	62.1	24.4	11.1	37.7	53.9	38.6	15.3	-	
Museum National d'Histoire Naturelle	MNHN-ZM-A14519	Male	Adult	-	-	1909	49.59	-1.26	Skull and vertebrae	-	-	-	-	-	-	-	-	On display; also listed as MNHN-ZM-1909-81
Museum National d'Histoire Naturelle	MNHN-ZM-AC-1963-259	Unknown	Adult	-	-	1963	43.48	-1.57	Damaged skull and without mandibles; hyoids	-	29.1	11.3	-	-	-	-	-	

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Museum of Comparative Zoology, Harvard	51084	Unknown	Unknown	-	-	1898	42.66	-70.68	Complete skeleton	-	-	-	-	-	-	-	-	
Museum of Comparative Zoology, Harvard	BOM-1727	Unknown	Unknown	-	-	1867	41.29	-70.12	Skull	-	-	-	-	-	-	-	-	
National Museum of Natural History (USNM)	572954	Female	Adult	2	10	2003	43.34	-70.52	Skull and mandible	70.5	33.1	13.8	43.9	67.1	43.2	23.9	440.0	Also listed as USNM ID 594602; pregnant with USNM 572963
National Museum of Natural History (USNM)	550204	Female	Adult	9	9	1982	41.27	-71.20	Skull and mandibles	78.6	27.6	13.4	52.8	71.0	45.6	25.4	448.0	Trawl net entanglement
National Museum of Natural History (USNM)	572009	Female	Adult	4	7	1996	40.02	-68.46	Complete skeleton	78.0	29.5	14.2	53.9	71.3	44.4	26.9	462.0	Drift net entanglement; North East Fisheries Science Center ID DO3070
National Museum of Natural History (USNM)	594220	Female	Adult	24	6	2015	41.96	-70.63	Postcranial only	-	-	-	-	-	-	-	426.8	Weight: 785.5 kg
National Museum of Natural History (USNM)	572996	Female	Subadult	29	7	2004	31.63	-81.13	Skull and damaged mandibles	73.2	25.7	11.6	41.1	-	-	26.3	380.0	
National Museum of Natural History (USNM)	572007	Female	Unknown	4	7	1996	40.03	-68.43	Postcranial only	-	-	-	-	-	-	-	471.0	Drift net entanglement, North East Fisheries Science Center ID DO3458
National Museum of Natural History (USNM)	594130	Male	Adult	8	11	2009	37.06	-76.28	Complete skeleton	69.6	29.9	12.1	43.6	61.1	40.3	20.8	430.0	Weight: 397.0 kg
National Museum of Natural History (USNM)	550414	Male	Adult	17	10	1984	29.77	-85.40	Complete skeleton	80.1	28.9	14.2	53.4	70.1	44.7	25.4	457.0	Weight: 649.0 kg

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
National Museum of Natural History (USNM)	572008	Male	Adult	6	7	1996	40.35	-67.35	Complete skeleton	80.7	29.8	13.8	42.2	74.9	48.8	26.1	473.0	Drift net entanglement; North East Fisheries Science Center ID DO3202
National Museum of Natural History (USNM)	572963	Male	Fetus	2	10	2003	43.34	-70.52	Whole fetus	-	-	-	-	-	-	-	47.0	Weight: 1.7 kg
National Museum of Natural History (USNM)	593438	Male	Subadult	22	1	2009	37.22	-76.02	Skull and mandibles	58.9	23.2	10.7	32.8	49.5	35.3	14.2	322.0	
National Museum of Natural History (USNM)	572377	Unknown	Adult	24	6	1995	40.01	-68.49	Partial skull and complete mandibles	-	-	14.6	50.9	70.3	43.7	26.6	-	Drift net entanglement, North East Fisheries Science Center ID DO0341
National Museum of Natural History (USNM)	504146	Unknown	Adult	-	9	1973	54.17	-58.58	Skull and mandibles; partial postcranial	75.9	28.9	15.5	49.1	70.7	44.5	26.2	-	
National Museum of Natural History (USNM)	572378	Unknown	Calf	-	-	1999	-	-	Postcranial only	-	-	-	-	-	-	-	-	
National Museum of Natural History (USNM)	572376	Unknown	Juvenile	9	8	1999	40.47	-66.34	Partial left mandible	-	-	-	-	-	-	-	-	Drift net entanglement, North East Fisheries Science Center ID DO6061
National Museums Scotland	Z.1986.45	Female	Adult	17	10	1986	55.97	-5.83	Damaged skull with mandibles; partial postcranial	-	29.1	13.3	-	57.2	45.4	11.8	-	-
National Museums Scotland	Z.1956.36.47	Male	Adult	-	4	1895	55.95	-3.01	Skull and mandibles	-	-	12.8	48.7	-	-	21.9	-	Also listed as Z.1981.057.527
National Museums Scotland	No ID	Male	Adult	12	6	2018	57.69	-3.99	Rib and scapula	-	-	-	-	-	-	-	370.0	SMASS stranding ID M325/18
National Museums Scotland	No ID	Unknown	Unknown	-	-	2016	57.83	-8.58	Skull and mandibles; partial postcranial	-	-	-	-	-	-	-	-	-

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
National Museums Scotland	Z.1902.25.1	Female	Adult	-	9	1899	57.59	-3.85	Damaged skull with mandibles; partial postcranial	72.6	29.7	14.2	45.3	67.5	46.2	21.3	-	Stranded with Z.1902.25.2
National Museums Scotland	Z.1902.25.2	Unknown	Juvenile	-	9	1899	57.59	-3.85	Skull and mandibles	45.7	21.2	9.4	26.6	41.0	30.8	10.2	-	Stranded with Z.1902.25.1
National Museums Scotland	Z.1954.27	Male	Adult	27	10	1954	56.16	-3.06	Mandibular frgaments	-	-	-	-	-	-	-	-	-
National Museums Scotland	Z.1954.27b	Male	Adult	27	10	1954	56.16	-3.06	Partial left mandible and tooth	-	-	-	-	-	-	38.9	-	-
National Museums Scotland	Z.1956.36.44	Male	Adult	25	5	1885	60.41	-1.35	Skull and mandibles; partial postcranial	71.3	30.2	11.8	46.1	60.1	44.3	15.8	-	Also listed as Z.1981.057.526 and Z.1981.57.453
National Museums Scotland	Z.1956.36.45	Male	Adult	-	10	1888	56.03	-3.35	Skull with mandibles; partial postcranial; preserved caudal fin	76.0	34.0	13.1	46.6	64.4	47.9	16.5	-	Also listed as Z.1981.57.528
National Museums Scotland	Z.1956.36.46	Male	Adult	-	4	1881	60.49	-1.46	Skull and mandibles; partial postcranial	59.4	27.6	12.3	29.4	63.7	45.4	18.3	-	Also listed as Z.1981.57.570
National Museums Scotland	Z.1960.11	Female	Adult	-	-	1872	57.37	-1.87	Skull and mandibles	71.2	28.8	13.9	49.4	61.3	43.2	18.1	-	-
National Museums Scotland	Z.1961.18.1	Female	Adult	19	6	1961	59.13	-3.32	Partial skull; mandible tips	-	30.2	12.6	-	-	-	27.2	-	Stranded with Z.1961.18.2
National Museums Scotland	Z.1961.18.2	Female	Subadult	19	6	1961	59.13	-3.32	Damaged skull and damaged mandibles; partial postcranial	46.2	22.8	11.1	-	-	-	-	-	Stranded with Z.1961.18.1
National Museums Scotland	Z.1990.3.3	Female	Adult	24	10	1967	57.52	-1.78	Skull and mandibles; partial postcranial	71.4	29.1	14.4	43.9	64.9	45.6	19.3	-	-
National Museums Scotland	Z.1990.3.4	Male	Adult	24	3	1968	60.35	-1.27	Damaged skull and damaged mandibles	-	32.5	14.8	53.2	74.4	50.1	24.3	-	-

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
National Museums Scotland	Z.1990.3.5	Male	Subadult	5	10	1981	55.99	-3.46	Skull and mandibles; scapula	55.1	26.7	11.2	31.4	49.6	35.7	13.9	-	-
National Museums Scotland	Z.1990.3.6	Female	Adult	28	5	1908	56.34	-2.78	Skull and mandibles; clavicles	77.3	30.9	13.9	58.0	72.0	47.1	24.9	-	-
National Museums Scotland	Z.1992.151.4	Female	Adult	19	8	1992	57.75	-3.91	Skull and mandibles; partial postcranial	75.5	31.2	14.1	45.3	68.3	49.4	-	504.0	SMASS stranding ID M1880/9
National Museums Scotland	Z.1992.151.5	Female	Adult	28	7	1992	57.44	-6.58	Skull and mandibles; scapula	76.2	29.3	13.7	47.8	68.3	44.1	24.2	490.0	SMASS stranding ID M1681/92
National Museums Scotland	Z.1992.62.7	Male	Subadult	15	6	1992	58.21	-6.28	Skull and mandibles; scapula	56.7	25.2	11.6	32.1	49.3	37.0	12.3	-	Stranded with Z.1992.62.8; SMASS stranding ID M1309/92B
National Museums Scotland	Z.1992.62.8	Male	Subadult	15	6	1992	58.21	-6.28	Skull and mandibles; scapula	-	23.1	10.3		43.2	34.9	8.3	-	Stranded with Z.1992.62.7; SMASS stranding ID M1309/92A
National Museums Scotland	Z.1992.62.9	Female	Juvenile	15	6	1992	58.21	-6.28	Skull and mandibles; scapula	47.6	20.3	9.9	27.4	42.9	34.1	8.8	298.0	SMASS stranding ID M1309/92C
National Museums Scotland	Z.1994.100	Male	Adult	5	8	1994	57.69	-3.49	Skull and mandibles	74.6	29.4	14.5	43.9	64.1	43.7	20.4	472.0	SMASS stranding ID M1622/94
National Museums Scotland	Z.1994.55.26	Unknown	Unknown	24	4	1994	55.42	-4.75	Scapula only	-	-	-	-	-	-	-	-	
National Museums Scotland	Z.1994.61	Female	Subadult	30	4	1994	56.69	-6.08	Damaged skull and damaged mandibles; partial postcranial	-	-	10.8	29.1	-	-	-	-	SMASS stranding ID M808/94
National Museums Scotland	Z.1997.19	Male	Juvenile	10	6	1996	55.99	-3.38	Complete skeleton	46.4	20.9	8.9	24.4	40.0	30.9	9.1	269.0	SMASS stranding ID M1878/96
National Museums Scotland	Z.1997.20	Female	Adult	5	10	1996	56.06	-3.22	Skull and mandibles; partial postcranial	74.1	28.6	12.7	42.8	67.9	46.8	21.1	459.0	SMASS stranding ID M1877/96
National Museums Scotland	Z.1999.119	Unknown	Subadult	29	9	1994	57.80	-8.59	Skull and mandibles; partial postcranial	-	24.1	11.9	-	-	37.1	-	385.0	SMASS stranding ID M2070/94

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
National Museums Scotland	Z.2000.25.15	Female	Subadult	7	1	2000	56.11	-3.15	Damaged skull and damaged mandibles; partial postcranial	-	27.1	12.4	-	-	-	-	414.0	SMASS stranding ID M3/00
National Museums Scotland	Z.2000.372	Unknown	Adult	22	3	2000	59.35	-2.91	Damaged skull and damaged mandibles; partial postcranial	-	28.1	-	-	-	-	-	-	SMASS stranding ID M56/00
National Museums Scotland	Z.2001.109.2	Female	Adult	14	4	2001	58.01	-3.84	Skull and mandibles; partial postcranial	74.4	29.9	13.5	45.4	67.3	43.9	23.4	450.0	SMASS stranding ID M41/01
National Museums Scotland	Z.2002.35.1	Male	Adult	18	9	2001	57.59	-4.11	Skull and post cranial	-	30.3	14.9	-	70.9	46.3	24.6	459.0	SMASS stranding ID M138/01
National Museums Scotland	Z.2004.143.120	Female	Unknown	4	8	2004	57.58	-4.11	Skull and mandibles; partial postcranial	-	-	-	-	-	-	-	378.0	SMASS stranding ID M165/04
National Museums Scotland	Z.2004.143.96	Female	Adult	4	7	2003	58.58	-4.75	Damaged mandibles; scapula	-	-	-	-	-	-	-	473.0	SMASS stranding ID M211/03
National Museums Scotland	Z.2007.7.153	Unknown	Subadult	26	2	2006	57.60	-3.84	Skull and mandibles; partial postcranial	63.4	24.9	11.8	38.4	54.6	37.2	17.4	-	SMASS stranding ID M045/06
National Museums Scotland	Z.2011.41.171	Female	Subadult	18	8	2007	57.50	-7.34	Partial skull with mandibles	-	26.5	12.3	-	57.3	40.7	16.6	371.0	SMASS stranding ID M165/07
National Museums Scotland	Z.2011.41.226	Unknown	Unknown	-	-	-	-	-	Partial postcranial	-	-	-	-	-	-	-	-	
National Museums Scotland	Z.2011.97.116	Male	Subadult	11	2	2008	57.43	-7.39	Damaged skull and damaged mandibles; scapula	-	25.4	12.4	-	-	38.9	-	358.0	SMASS stranding ID M35/08
National Museums Scotland	Z.2011.97.117	Female	Adult	9	4	2008	58.19	-6.91	Damaged skull and damaged mandibles; partial postcranial	-	30.8	15.4	-	-	47.5	-	483.0	SMASS stranding ID M88/08

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
National Museums Scotland	Z.2011.97.187	Female	Adult	26	1	2007	57.81	-5.67	Damaged skull and damaged mandibles; partial postcranial	-	31.9	14.9	-	-	-	-	492.0	Pregnant with Z.2014.21.42; SMASS stranding ID M18/07
National Museums Scotland	Z.2011.97.188	Female	Subadult	6	8	2006	56.43	-6.34	Damaged skull and damaged mandibles	-	23.6	11.4	-	-	36.9	-		SMASS stranding ID M202/06
National Museums Scotland	Z.2011.97.189	Female	Adult	4	12	1994	59.97	-1.34	Damaged skull and damaged mandibles; clavicles	-	29.7	13.8	-	-	-	-	488.0	SMASS stranding ID M2556/94
National Museums Scotland	Z.2014.21.39	Female	Subadult	4	8	2012	56.05	-3.63	Damaged skull with mandibles; partial postcranial	-	22.2	9.1	-	44.8	32.2	12.6	281.0	Stranded with Z.2014.21.40; SMASS stranding ID M256.1/12
National Museums Scotland	Z.2014.21.40	Female	Subadult	16	8	2012	56.02	-3.61	Damaged skull with mandibles; partial postcranial	67.7	26.4	13.3	41.8	59.7	42.6	17.1	372.0	Stranded with Z.2014.21.39; SMASS stranding ID M256.2/12
National Museums Scotland	Z.2014.21.41	Female	Subadult	13	6	2013	58.46	-5.06	Damaged skull and damaged mandibles; partial postcranial	-	25.6	12.1	-	-	-	-	372.0	SMASS stranding ID M199/13
National Museums Scotland	Z.2014.21.42	Unknown	Neonate	26	1	2007	57.50	-7.34	Skull and mandibles; partial postcranial	-	-	-	-	23.5	18.6	4.9	-	Fetus of Z.2011.97.187; SMASS stranding ID M018/07
National Museums Scotland	Z.2015.142.79	Female	Adult	30	9	2014	58.58	-4.77	Damaged skull with mandibles; one scapula	73.1	28.7	12.7	48.2	67.6	44.1	23.5	460.0	Stranded with Z.2015.142.80; SMASS stranding ID M282.1/14
National Museums Scotland	Z.2015.142.80	Male	Juvenile	30	9	2014	58.58	-4.70	Damaged skull with mandibles; scapula	47.5	20.3	9.8	26.3	44.1	30.9	10.2	290.0	Stranded with Z.2015.142.79; SMASS stranding ID M282.2/14
National Museums Scotland	Z.2017.29.1	Female	Adult	22	6	2015	58.49	-6.23	Damaged skull and damaged mandibles	-	-	-	-	-	-	-	345.0	SMASS stranding ID M187/15
National Museums Scotland	Z.2017.29.2	Male	Adult	30	11	2016	58.62	-3.35	Damaged skull and damaged mandibles	-	-	-	-	-	-	-	462.0	SMASS stranding ID M565/16

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
National Museums Scotland	Z.2019.18.15	Male	Adult	22	4	2018	55.88	-2.09	Partial postcranial	-	-	-	-	-	-	-	362.0	SMASS stranding ID M256/18
National Museums Scotland	No Data A	Unknown	Subadult	-	-	-	-	-	Partial skull; mandibles; scapula	-	27.4	11.9	40.6	61.2	42.9	18.3	-	-
National Museums Scotland	Z.2019.18.16	Unknown	Juvenile	-	-	-	-	-	Partial skull with mandibles; partial postcranial	51.7	21.8	12.6	29.2	44.1	33.7	10.4	-	-
Natural History Museum of Denmark	C.N. 5	Female	Adult	8	10	1900	56.52	10.45	Complete skeleton	76.2	30.3	14.1	50.4	73.3	48.2	25.1	-	
Natural History Museum of Denmark	C.N. 4	Female	Subadult	20	12	1899	57.05	8.49	Complete skeleton	71.3	27.1	12.4	46.6	63.0	41.6	21.4	-	
Natural History Museum of Denmark	C.N. 7	Male	Adult	-	-	1932	62.04	-6.94	Skull and mandibles	70.3	32.1	13.7	42.5	62.6	44.7	17.9	-	
Natural History Museum of Denmark	M1677	Male	Juvenile	7	11	2016	56.53	8.11	Frozen head	-	-	-	-	-	-	-	271.0	
Natural History Museum of Denmark	C.N. 8	Male	Subadult	26	11	1957	55.90	10.64	Skull and mandibles	71.7	26.9	12.1	43.8	62.6	45.2	17.4	-	
Natural History Museum of Denmark	C.N. 901	Unknown	Subadult	21	10	1992	56.74	10.50	Skull and mandibles	63.8	26.3	11.9	37.4	54.3	39.4	14.9	-	
Natural History Museum of Denmark	C.N. 3	Unknown	Adult	13	2	1894	55.56	8.07	Mandibles	-	-	-	-	59.7	43.6	16.1	-	
Natural History Museum Rotterdam	NMR999000000450	Unknown	Juvenile	-	-	1994	54.27	2.57	Left mandible	-	-	-	-	44.5	34.1	10.4	-	Recovered in dredging operation
Natural History Museum Rotterdam	NMR999100012066	Unknown	Unknown	-	-	1995	53.08	1.99	Right mandible	-	-	-	-	-	-	-	-	Recovered in dredging operation

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Natural History Museum Rotterdam	NMR999000002861	Unknown	Unknown	-	-	-	-	-	Ulna	-	-	-	-	-	-	-	-	
Natural History Museum Rotterdam	NMR999000002218	Unknown	Unknown	-	-	2003	52.26	2.94	Vertebrae	-	-	-	-	-	-	-	-	Recovered in dredging operation
Natural History Museum Rotterdam	NMR999000002217	Unknown	Unknown	-	-	1994	54.08	2.93	Vertebrae	-	-	-	-	-	-	-	-	Recovered in dredging operation
Natural History Museum Rotterdam	NMR999000002216	Unknown	Unknown	-	-	1994	54.08	2.93	Vertebrae	-	-	-	-	-	-	-	-	Recovered in dredging operation
Natural History Museum Rotterdam	NMR999000002860	Unknown	Unknown	-	-	-	-	-	Vertebrae	-	-	-	-	-	-	-	-	
Natural History Museum, London	C.1920.12.20.1	Female	Adult	12	9	1916	53.14	0.35	Skull and mandibles	70.4	29.8	12.8	47.2	66.5	48.6	17.9	448.6	Also listed as SW.1916.21
Natural History Museum, London	C.1964.6.3.3	Female	Adult	20	7	1939	51.53	0.69	Skull and mandibles; partial postcranial	79.3	27.9	13.4	51.7	67.9	44.2	23.7	452.1	Also listed as SW1938/18
Natural History Museum, London	C.1964.6.3.6	Female	Adult	-	-	1932	57.49	-4.26	Complete skeleton	73.2	28.9	14.4	49.4	68.0	42.3	25.7	472.4	Also listed a SW.1932:28
Natural History Museum, London	C.1964.6.3.7	Female	Adult	-	-	1926	53.05	-9.54	Skull and damaged mandibles	75.9	30.4	14.6	49.8	71.1	47.2	23.9	-	Also listed as SW.1926.2
Natural History Museum, London	C.1914.9.28.1	Female	Subadult	21	9	1914	52.25	-6.35	Skull and mandibles	51.4	24.9	11.1	32.1	48.7	38.6	10.1	362.7	
Natural History Museum, London	C.1964.6.3.11	Female	Adult	13	8	1915	57.49	-4.23	Damaged mandibles	-	-	-	-	48.5	32.4	16.1	365.8	Also listed as SW.1915.17
Natural History Museum, London	ZD.1980.519	Female	Adult		10	1978	52.94	0.09	Mandibles	-	-	-	-	68.2	44.1	24.1	-	Also listed as SW1978/30

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Natural History Museum, London	ZD.1980.518	Female	Adult	-	-	1980	-	-	Mandibles	-	-	-	-	72.7	45.9	26.8	-	
Natural History Museum, London	C.1964.6.3.13	Female	Adult	-	-	1959	51.50	0.45	Tooth; partial postcranial	-	-	-	-	-	-	-	-	Also listed as SW.1959.26
Natural History Museum, London	ZD.1980.521	Male	Adult	-	-	1980	50.36	-4.39	Skull and mandibles	69.2	30.4	13.7	42.9	59.9	44.8	15.1	-	Also listed as SW.1975.28
Natural History Museum, London	C.1908.2.28.1	Male	Adult	-	-	1908	57.70	-2.00	Skull and mandibles	67.1	28.4	12.2	42.6	64.2	43.9	20.3	-	
Natural History Museum, London	C.1964.6.3.5	Male	Adult	-	-	1928	53.34	0.27	Skull and mandibles	74.3	28.2	12.4	49.3	64.6	43.7	20.9	442.0	Also listed as SW.1928.4
Natural History Museum, London	C.1954.9.9.1	Male	Adult	-	-	1954	59.38	-2.38	Damaged skull and damaged mandibles	78.3	30.6	13.1	50.1	66.0	46.3	19.7	472.4	Also listed as SW.1954.5
Natural History Museum, London	C.1964.6.3.8	Male	Adult	17	7	1933	58.81	-2.99	Damaged skull and mandibles; partial postcranial	-	29.7	13.2	-	68.0	45.9	22.1	487.7	Also listed as SW.1933.21
Natural History Museum, London	C.1964.6.3.4	Male	Adult	26	7	1940	54.68	-1.20	Damaged skull and damaged mandibles; partial postcranial	66.1	27.8	12.6	41.2	-	44.3	-	462.3	Also listed as SW.1940-9
Natural History Museum, London	SW.1967.28	Male	Adult	24	10	1967	53.48	0.17	Skull without mandibles	82.8	31.9	14.9	55.3	-	-	-	518.2	
Natural History Museum, London	C.1964.6.3.9	Male	Subadult	18	11	1949	60.27	-1.21	Skull and mandibles	48.1	26.1	12.4	23.9	54.7	37.4	17.3	370.8	
Natural History Museum, London	C.1964.6.3.1	Male	Subadult	-	-	1938	51.58	-4.00	Complete skeleton	63.8	24.4	10.9	39.2	-	-	-	365.8	Also listed as SW1938.31

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Natural History Museum, London	C.1964.6.3.10	Unknown	Juvenile	-	-	1952	52.83	1.54	Skull and mandibles	40.4	19.9	8.1	25.3	34.9	25.8	9.1	320.0	Also listed as SW1952/13
Natural History Museum, London	C.1948.5.1.3	Unknown	Neonate	-	-	1948	-	-	Complete skeleton	30.3	12.4	5.6	17.3	24.5	19.8	4.7	-	
Natural History Museum, London	C.1964.6.3.12	Unknown	Subadult	-	-	1948	50.22	-5.49	Mandibles	-	-	-	-	49.8	36.4	13.4	353.0	Also listed as SW.1948.7
Natural History Museum, London	C.1964.6.3.2	Unknown	Unknown	-	-	1949	50.36	-4.13	Damaged skull and damaged mandibles	-	-	-	-	-	-	-	442.0	Also listed as SW.1949.26
Naturalis Biodiversity Center	ZMA.MAM.1383	Female	Adult	11	7	1952	52.55	4.60	Skull and mandibles; partial postcranial	63.1	28.4	12.8	35.9	56.7	42.1	14.6	-	
Naturalis Biodiversity Center	RMNH.MAM.7512	Female	Adult	16	7	1947	53.09	4.75	Skull and mandibles; partial postcranial	83.6	30.4	13.6	48.1	72.6	46.2	26.4	-	
Naturalis Biodiversity Center	RMNH.MAM.41153	Female	Adult	1	9	2002	53.49	6.13	Partial postcranial	-	-	-	-	-	-	-	350.0	
Naturalis Biodiversity Center	RMNH.MAM.38259	Female	Subadult	5	8	1992	51.41	3.77	Skull and mandibles; partial postcranial	68.1	26.4	11.7	44.1	59.0	38.7	20.3	415.0	
Naturalis Biodiversity Center	RMNH.MAM.9404	Male	Adult	20	9	1949	51.50	3.46	Skull and mandibles; partial postcranial	71.9	28.5	13.1	46.8	63.9	43.5	20.4	-	
Naturalis Biodiversity Center	ZMA.MAM.2721	Unknown	Subadult	22	9	1952	52.62	4.62	Skull and mandibles; partial postcranial	56.2	24.2	10.7	33.1	48.4	37.8	10.6	-	
Naturalis Biodiversity Center	RMNH 2114	Unknown	Subadult	14	9	1932	51.42	3.92	Damaged skull and damaged mandibles; partial postcranial	-	-	-	-	53.5	45.2	8.3	-	

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Naturalis Biodiversity Center	RMNH.MAM.59145	Unknown	Unknown	-	9	1896	51.50	4.18	Damaged skull and damaged mandibles; partial postcranial	-	-	-	-	-	-	-	-	
New Brunswick Museum	NBM- 011488	Female	Adult	15	6	2004	47.89	-53.08	Complete skeleton	55.9	29.9	14.9	28.3	70.6	48.4	22.2	-	
New Brunswick Museum	NBM- 016536	Female	Adult	25	10	2016	46.21	-60.20	Complete skeleton	78.2	30.3	13.9	50.2	73.8	50.4	23.4	-	
New Brunswick Museum	NBM- 18027	Female	Adult	20	6	1997	43.93	-59.95	Soft tissue only	-	-	-	-	-	-	-	-	
New Brunswick Museum	NBM- 012438	Male	Adult	5	6	2013	46.50	-63.35	Complete skeleton	77.6	30.5	13.2	50.1	68.9	46.2	22.7	-	
New Brunswick Museum	NBM- 016530	Male	Adult	14	6	2016	48.01	-66.63	Complete skeleton	77.4	31.9	14.4	52.6	71.3	44.4	26.9	-	
New Brunswick Museum	NBM- 011494	Unknown	Juvenile	-	-	2007	48.93	-54.88	Complete skeleton	59.8	22.1	10.4	36.1	50.2	36.1	14.1	-	
Northeast Fisheries Science Center	FO0190	Female	Adult	28	8	1989	41.02	-66.52	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO3486	Female	Adult	6	7	1994	40.56	-66.24	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	FO0120	Male	Adult	26	8	1989	40.52	-66.29	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO1369	Male	Adult	10	6	1994	40.52	-66.26	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	No ID	Male	Adult	26	8	1989	40.57	-66.29	Mandibles and soft tissue	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO3685	Unknown	Subadult	6	8	1998	40.47	-66.39	Soft tissue only	-	-	-	-	-	-	-	-	

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Northeast Fisheries Science Center	DO0247	Unknown	Subadult	11	8	1998	40.34	-66.54	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	FO3720	Unknown	Subadult	23	8	1989	40.52	-66.27	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	FO3730	Unknown	Subadult	23	8	1989	40.51	-66.27	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	No ID	Unknown	Subadult	27	7	1990	40.30	-67.06	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO6009	Unknown	Subadult	7	8	1998	40.44	-66.35	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO6013	Unknown	Subadult	8	8	1998	40.43	-66.40	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO0052	Unknown	Subadult	4	10	1989	40.53	-66.22	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO0253	Unknown	Subadult	10	10	1989	40.14	-67.54	Soft tissue only	-	-	-	-	-	-	-	-	
Northeast Fisheries Science Center	DO1380	Unknown	Subadult	3	6	1994	40.58	-66.19	Mandibles and soft tissue	-	-	-	-	-	-	-	-	
Oxford University Museum of Natural History	ZC-06998	Male	Adult	-	-	1800	57.66	-3.72	Damaged skull and damaged mandibles	-	-	-	-	-	-	-	456.0	
OZEANEUM Stralsund	B14/09	Male	Adult	9	7	2009	54.13	8.83	Skull and mandibles; partial postcranial	70.3	27.7	12.2	45.4	62.3	42.7	19.6	406.0	
Scottish Marine Animal Stranding Scheme	M151/07	Female	Adult	3	8	2007	56.69	-2.44	Soft tissue only	-	-	-	-	-	-	-	-	

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Scottish Marine Animal Stranding Scheme	M228/08	Female	Adult	10	11	2008	57.65	-3.70	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M299.1/13	Female	Adult	12	9	2013	57.81	-8.57	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M344/17	Female	Adult	9	8	2017	57.65	-7.37	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M14/08	Male	Adult	22	1	2008	57.57	-4.10	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M43/10	Male	Adult	4	3	2010	59.32	-2.56	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M391/18	Male	Adult	25	7	2018	58.36	-6.57	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M402/18	Male	Adult	29	7	2018	56.01	-2.57	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M1325/96	Unknown	Subadult	29	7	1996	57.20	-7.43	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M122/09	Unknown	Subadult	23	8	2009	57.46	-7.40	Soft tissue only	-	-	-	-	-	-	-	-	
Scottish Marine Animal Stranding Scheme	M126/17	Unknown	Subadult	27	2	2017	57.11	-7.28	Soft tissue only	-	-	-	-	-	-	-	-	

Institution	Specimen ID	Sex	Age class	Collection date and location					Specimen condition	Total skull length (cm)	Braincase width (cm)	Proximal beak width (cm)	Beak length (cm)	Total mandibular length (cm)	Mandibular skull to symphysis length (cm)	Mandibular symphysis to distal end length (cm)	Total body length (cm)	Additional notes
				Day	Month	Year	Lat.	Long.										
Scottish Marine Animal Stranding Scheme	M1325/96	Unknown	Subadult	29	7	1996	57.20	-7.42	Soft tissue only	-	-	-	-	-	-	-	-	
Society for the Study of Cetacean in the Canary Archipelago	No ID	Male	Adult	4	16	2007	28.85	-13.79	Complete skeleton	-	-	-	-	-	-	-	-	
University Museum of Bergen	B.2776	Male	Adult	14	11	1901	61.90	5.37	Damaged skull and damaged mandibles; postcranial	-	34.1	12.6	-	-	-	-	-	
University Museum of Bergen	B.1097	Unknown	Juvenile	-	-	1895	59.36	5.24	Complete skeleton	-	-	-	-	52.8	38.4	14.4	-	
University Museum of Bergen	B.1102	Unknown	Subadult	-	-	1895	59.41	5.23	Complete skeleton	60.7	22.9	10.1	37.8	52.9	37.1	15.8	-	
University Museum of Bergen	B.9095	Unknown	Subadult	-	6	2011	60.69	4.93	Left mandible	-	-	-	-	54.9	37.3	17.6	-	
University of Oslo	M4287	Unknown	Adult	30	11	1859	58.09	6.81	Damaged skull and damaged mandibles; partial postcranial	-	-	-	-	-	-	-	-	
University of Oslo	M7191	Unknown	Adult	5	9	1907	63.47	8.02	Damaged skull and damaged mandibles; partial postcranial	-	-	-	-	-	-	-	-	

Appendix Table 2.1 List of specimens and skeletal elements sampled for Chapter 2 held in the collections of the National Museums Scotland, Edinburgh.

Species	NMS accession no.	Age class	Skeletal element sampled							
			Occipital condyle	Mandibular ramus	Thoracic vertebral body	Thoracic vertebral spinous process	Proximal rib	Distal rib	Scapula	Humeral head
Balaenoptera acutorostrata	NMS.Z.1965.49	Adult	X	X	X	X	X	X	*	X
Balaenoptera acutorostrata	NMS.Z.1993.4	Juvenile	X	X	X	X	X	X	X	X
Balaenoptera acutorostrata	NMS.Z.2000.386.1	Juvenile	X	X	X	X	X	X	X	X
Delphinus delphis	NMS.Z.2011.41.60	Adult	X	X	X	X	X	X	X	X
Delphinus delphis	NMS.Z.2011.41.62	Adult	X	X	X	X	X	X	X	X
Delphinus delphis	NMS.Z.2011.41.63	Adult	X	X	X	X	X	X	X	X
Delphinus delphis	NMS.Z.2011.41.64	Adult	X	X	X	X	X	X	X	X
Delphinus delphis	NMS.Z.2011.41.66	Adult	X	X	X	X	X	X	X	X
Globicephala melas	NMS.Z.1997.116.42	Adult	X	X	X	X	X	X	X	X
Globicephala melas	NMS.Z.2000.25.9	Subadult	X	X	X	X	X	X	X	X
Globicephala melas	NMS.Z.2011.41.198	Juvenile	X	X	X	X	X	X	X	X
Globicephala melas	NMS.Z.2014.21.17	Subadult	X	X	X	X	X	X	X	X
Grampus griseus	NMS.Z.1993.77	Subadult	X	X	X	X	X	X	X	X
Grampus griseus	NMS.Z.1995.93.1	Subadult	X	X	X	X	X	X	X	X
Grampus griseus	NMS.Z.2011.41.9	Adult	X	X	X	X	X	X	X	X

Species	NMS accession no.	Age class	Skeletal element sampled							
			Occipital condyle	Mandibular ramus	Thoracic vertebral body	Thoracic vertebral spinous process	Proximal rib	Distal rib	Scapula	Humeral head
Grampus griseus	NMS.Z.2011.41.12	Juvenile	X	X	X	X	X	X	X	X
Grampus griseus	NMS.Z.2015.142.16	Juvenile	X	X	X	X	X	X	X	X
Hyperoodon ampullatus	NMS.Z.1989.59.1	Juvenile	X	X	X	X	X	X	X	X
Hyperoodon ampullatus	NMS.Z.2001.166	Adult	X	X	X	X	X	X	X	X
Hyperoodon ampullatus	NMS.Z.2003.6	Juvenile	X	X	X	X	X	X	X	X
Hyperoodon ampullatus	NMS.Z.2007.7.151	Juvenile	X	X	X	X	X	X	X	X
Hyperoodon ampullatus	NMS.Z.2011.97.138	Subadult	X	X	X	X	X	X	X	X
Kogia breviceps	NMS.Z.1999.264.1	Adult	X	X	X	X	X	X	X	X
Kogia breviceps	NMS.Z.1999.264.2	Juvenile	X	X	X	X	X	X	X	X
Kogia breviceps	NMS.Z.2011.97.192	Subadult	X	X	X	X	X	X	X	X
Lagenorhynchus acutus	NMS.Z.2000.386.5	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus acutus	NMS.Z.2000.386.7	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus acutus	NMS.Z.2011.41.109	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus acutus	NMS.Z.2011.41.114	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus acutus	NMS.Z.2011.41.115	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus albirostris	NMS.Z.2011.41.81	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus albirostris	NMS.Z.2011.41.82	Adult	X	X	X	X	X	X	X	X

Species	NMS accession no.	Age class	Skeletal element sampled							
			Occipital condyle	Mandibular ramus	Thoracic vertebral body	Thoracic vertebral spinous process	Proximal rib	Distal rib	Scapula	Humeral head
Lagenorhynchus albirostris	NMS.Z.2014.21.33	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus albirostris	NMS.Z.2014.21.34	Adult	X	X	X	X	X	X	X	X
Lagenorhynchus albirostris	NMS.Z.2014.21.35	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.2019.18.15	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.2019.18.16	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.1992.151.4	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.1994.61	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.1994.100	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.1997.20	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.1999.119	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.2000.25.15	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.2001.109.2	Adult	X	X	X	X	X	X	X	X
Mesoplodon bidens	NMS.Z.2002.35.1	Adult	X	X	X	X	X	X	X	X
Orcinus orca	NMS.Z.1876.11	Adult	X	X	X	X	X	X	X	X
Orcinus orca	NMS.Z.2001.7	Adult	X	X	X	X	X	X	X	X
Orcinus orca	NMS.Z.2015.179	Adult	X	X	X	X	X	X	X	X
Orcinus orca	NMS.Z.2016.118	Adult	X	X	X	X	X	X	X	X

Species	NMS accession no.	Age class	Skeletal element sampled							
			Occipital condyle	Mandibular ramus	Thoracic vertebral body	Thoracic vertebral spinous process	Proximal rib	Distal rib	Scapula	Humeral head
Phocoena phocoena	NMS.Z.1989.23.2	Adult	X	X	X	X	X	X	X	X
Phocoena phocoena	NMS.Z.2011.41.141	Adult	X	X	X	X	X	X	X	X
Phocoena phocoena	NMS.Z.2011.41.151	Subadult	X	X	X	X	X	X	X	X
Phocoena phocoena	NMS.Z.2011.41.155	Adult	X	X	X	X	X	X	X	X
Phocoena phocoena	NMS.Z.2011.41.156	Adult	X	X	X	X	X	X	X	X
Stenella coeruleoalba	NMS.Z.1995.93.3	Subadult	X	X	X	X	X	X	X	X
Stenella coeruleoalba	NMS.Z.2011.41.20	Subadult	X	X	X	X	X	X	X	X
Stenella coeruleoalba	NMS.Z.2011.41.21	Adult	X	X	X	X	X	X	X	X
Stenella coeruleoalba	NMS.Z.2011.41.23	Juvenile	X	X	X	X	X	X	X	X
Stenella coeruleoalba	NMS.Z.2011.41.24	Subadult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.1993.36.4	Subadult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.1993.36.5	Adult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.1994.13.33	Adult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.1998.40	Adult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.2001.109.1	Adult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.2001.109.21	Adult	X	X	X	X	X	X	X	X

Species	NMS accession no.	Age class	Skeletal element sampled							
			Occipital condyle	Mandibular ramus	Thoracic vertebral body	Thoracic vertebral spinous process	Proximal rib	Distal rib	Scapula	Humeral head
Tursiops truncatus	NMS.Z.2011.41.16	Adult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.2011.41.17	Adult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.2011.41.18	Adult	X	X	X	X	X	X	X	X
Tursiops truncatus	NMS.Z.2017.29.20	Adult	X	*	X	X	X	X	X	X
Ziphius cavirostris	NMS.Z.1999.121.1	Adult	X	X	X	X	X	X	X	X
Ziphius cavirostris	NMS.Z.2011.41.168	Juvenile	X	X	X	X	X	X	X	X
Ziphius cavirostris	NMS.Z.2011.41.169	Juvenile	X	X	X	X	X	X	X	X

Curriculum Vita

Kerri Smith received her B.S. in Marine Biology from Texas A&M University Galveston (Galveston, TX) in 2008 and her M.S. in Marine Biology from Texas A&M University (College Station, TX) in 2012. While a student at UTEP, Kerri was a Teaching Assistant for Human Anatomy and Physiology, received the Dodson Research grant (2017 and 2018), Graduate Student Travel Grant (2016, 2017, 2018), the Department of Biological Science travel grant (2016, 2017, 2018), and the Graduate School Dissertation Completion Fellowship (2020). Kerri spent a large portion of her time as a graduate student working with museums to collect samples and data from Sowerby's beaked whale specimens, visiting 26 museums and research institutions. She received an NSF-funded ITCE Research-in-Residence Grant to support the research conducted in Chapter 2 of this dissertation, and she received the Smithsonian National Museum of Natural History Peter Buck Research Fellowship in 2018.

Contact Information: SmithKerriJ@gmail.com