ARTICLE

Marine Mammal Science 🐋

Genetic examination of historical North Atlantic right whale (*Eubalaena glacialis*) bone specimens from the eastern North Atlantic: Insights into species history, transoceanic population structure, and genetic diversity

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Abstract

Species monitoring and conservation is increasingly challenging under current climate change scenarios. For the North Atlantic right whale (*Eubalaena glacialis*) this challenge is heightened by the added effects of complicated and uncertain past species demography. Right whales once had a much wider distribution across the North Atlantic Ocean, although the degree to which right whales in the western and eastern North Atlantic were genetically isolated remains unknown. We analyzed DNA from 24 4th–20th century (CE) right whale bone specimens that were collected from 10 historical and archaeological sites in Iceland, the Faroe Islands, and Scotland. Following mtDNA species identification, we obtained 15-locus nuclear microsatellite profiles from a subset of eight specimens and compared these to contemporary data from animals remaining in the western North Atlantic population. While some specimens share mtDNA haplotypes with the contemporary population, several new haplotypes were

found. Moderate mtDNA and nuclear differentiation between the two regions was identified (mtDNA: $F_{ST} = 0.0423$, $\Phi_{ST} = 0.041$; nuclear DNA: $F_{ST} = 0.024$). Interpretation of the relationships between animals in the two regions is not simple, and this research highlights the difficulty in conducting such assessments in species with complex histories of unknown structure prior to extensive exploitation.

KEYWORDS

ancient DNA, archaeology, extirpation, genetic diversity, habitat, North Atlantic right whale, population structure, whaling

1 | INTRODUCTION

The effective conservation of biodiversity is a difficult and complex goal. Today, climate change is adding to this complexity, causing rapid and unpredictable environmental changes and associated shifts in the distributions of marine organisms (e.g., Greene, 2016; Meyer-Gutbrod et al., 2021; Smale et al., 2019). For some species the challenge of monitoring and/or management is heightened even more by the remaining effects of, and uncertainty around, events that occurred in the past. For example, anthropogenic impacts over the past several centuries can confound our understanding and interpretation of species demography, population structure, and history (e.g., Fromentin, 2009). The North Atlantic right whale (*Eubalaena glacialis*) is one species that brings together these characteristics of an uncertain past and shifting habitat use associated with a variable oceanic environment (Greene & Pershing, 2004; Kraus, 2005; Kraus et al., 2016). Unfortunately, recent shifts in habitat usage are also resulting in increasing anthropogenic threats (e.g., Meyer-Gutbrod et al., 2018, 2021). Together, these factors make the task of assessing recovery and designating conservation goals and strategies problematic (reviewed by Lotze et al., 2011).

The North Atlantic right whale is a critically endangered large baleen whale that inhabits North Atlantic waters and currently numbers fewer than 400 individuals (Cooke, 2020; Pettis et al., 2021). Its primary range now spans the eastern coast of the United States and Canada (Figure 1), within an urban coastal environment where it suffers considerable anthropogenic mortality from vessel strikes and entanglement in fishing gear (Corkeron et al., 2018; Knowlton et al., 2012; Knowlton & Kraus, 2001; Moore et al., 2021; Sharp et al., 2019; Van Der Hoop et al., 2013). In addition to these extrinsic factors, two primary intrinsic factors appear to be limiting recovery. First, the species exhibits a low reproductive rate (approximately one-third the rate of its congeneric species, the southern right whale, *E. australis*; Corkeron et al., 2018; Knowlton et al., 1994; Knowlton & Kraus, 2001) and highly variable calving intervals (Knowlton & Kraus, 2001). Second, the species has a low level of genetic diversity both at nuclear and mitochondrial (mtDNA) markers (Malik et al., 2000; Schaeff et al., 1991, 1997; Waldick et al., 2002). This low diversity has commonly been attributed to genetic erosion of alleles or bottleneck events from population reductions due to centuries of whaling. However, attempts to document genetic bottlenecks causing loss of diversity over the past 200–400 years (the period of intensive subsistence or commercial whaling activities) suggest that the low diversity identified in the species predates commercial whaling activities (McLeod et al., 2010; Waldick et al., 2002). These findings raise questions about the North Atlantic right whale's demographic history and abundance prior to the onset of commercial whaling.

In addition to contemporary anthropogenic mortality, it is widely recognized that the current status of the species, with so few remaining individuals, is largely a result of almost a thousand years of commercial whaling activities
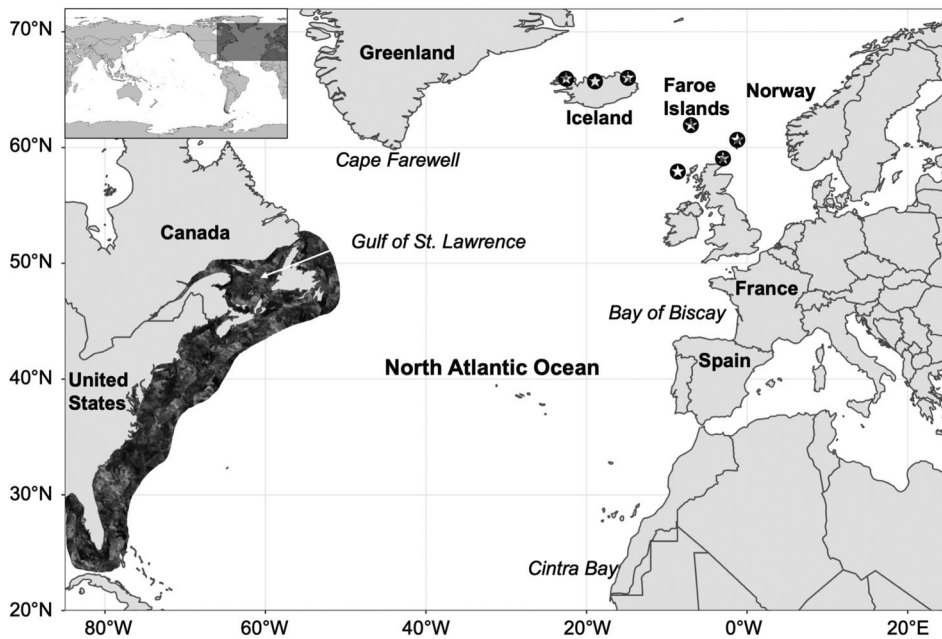
FIGURE 1 Map of the North Atlantic Ocean, including the contemporary distribution (mottled waters) of the North Atlantic right whale and several placenames mentioned in the article. Starred circles indicate sites of origin for historical and archaeological samples.

that occurred throughout the North Atlantic Ocean. While historical evidence from premodern Europe includes specific description of the right whale in behavior, appearance, and migration, and while ancient and medieval fisher-hunters may have used rudimentary hunting techniques of spearing, netting, driving, and calf-hunting to obtain right whales along northern European coasts (Lindqvist, 1994; Szabo, 2008), commercial exploitation began in earnest in the 11th century in the Bay of Biscay off the coasts of France and Spain (Aguilar, 1981, 1986). Whaling activities followed a counterclockwise pattern of expansion around the North Atlantic. Right whales were first commercially hunted by the Basques whose activities expanded northwards in the mid-14th century to the coasts of Ireland and England, and by the 15th and 16th centuries to waters around Iceland, Norway, Greenland, and the shores of Eastern Canada (Barkham, 1984; Mowat, 1985; True, 1904), as several other nations also took up whaling. During the 17th–20th centuries, right whales were also hunted along the shores of New England (Reeves et al., 1999; Reeves & Mitchell, 1986a, 1986b). By the mid-19th–20th centuries they were sporadically hunted around the North Atlantic, until they received international protection in 1935.

Thus, North Atlantic right whales were once present on both sides of the North Atlantic and were exploited first in the eastern Atlantic (along the west coasts of north Africa and Europe) and subsequently in the western Atlantic (along the east coasts of Canada and the United States). Today there is a single remaining population in only a small portion of its former range (i.e., in the western North Atlantic, ranging from the Gulf of St. Lawrence, Canada, in the north to the coast of Florida in the south; Figure 1). A major question regarding the historical status of this species is: How many populations existed prior to whaling? This topic of historical population structure has been widely discussed (reviewed by Reeves & Mitchell, 1986a; True, 1904), but there are few data available on which to base any interpretation, and the question has largely remained unanswered. Understanding the past population structure and interactions among right whales may give insight into whether individuals remaining in the western North Atlantic have the potential to reoccupy historical habitat areas.

Three hypotheses have traditionally been proposed regarding right whale population structure across the North Atlantic (Figure 2). Reeves and Mitchell (1986a) extensively reviewed North Atlantic right whale historical
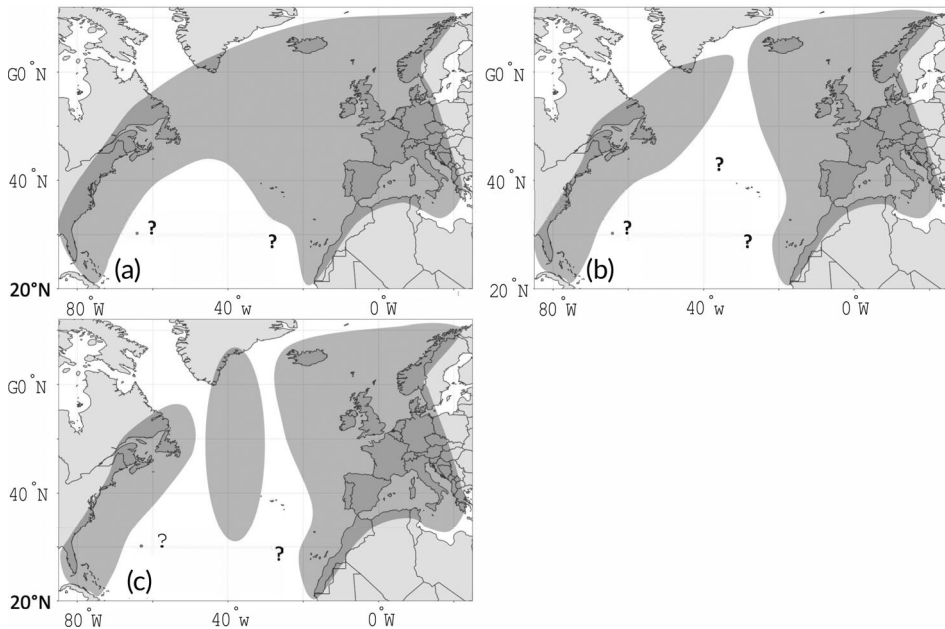
FIGURE 2 Hypothesized distribution scenarios of historical North Atlantic right whale populations. Shown here are approximate distributions under the hypotheses of one (a), two (b), and three (c) populations. Distribution boundaries were determined based on data and textual information from Reeves & Mitchell (1986a), Monsarrat et al. (2015), and COSEWIC (2013).

observations and whaling records, and discussed potential population structure hypotheses. We review and expand upon these briefly here. Hypothesis 1: *Right whales that inhabited the western and eastern regions of the North Atlantic represented a single interbreeding population*. Under this hypothesis, animals from these regions may have had some shared habitat areas but also some temporal isolation (reviewed by Reeves & Mitchell, 1986a). For example, right whales may have had separate calving grounds in the eastern (e.g., waters of the Bay of Biscay south to Cintra Bay) and western North Atlantic (e.g., off the coast of Georgia and Florida), but shared offshore summer feeding areas around Iceland and Cape Farewell (Figure 2). Under this scenario, knowledge or "cultural memory" (Clapham et al., 2008) of these areas may have been lost in the contemporary right whale population due to the historical extirpation of whales that used them, and therefore they have not been reoccupied. Hypothesis 2: *Right whales in the North Atlantic comprised two populations: one in the western and one in the eastern North Atlantic* (e.g., Allen, 1908; Reeves & Mitchell, 1986a). Based on historical observations and known whaling grounds, the eastern population may have spent winters along the coast of northwest Africa to the Bay of Biscay and summers in the waters ranging between Iceland, the North Cape of Norway, and the British Isles. The western population would have wintered between Florida and New England and summered in the coastal waters ranging between the Gulf of Maine and Labrador (Reeves & Mitchell, 1986a). This hypothesis, which is perhaps the most widely presented and accepted in the literature (e.g., Greene & Pershing, 2004; Rosenbaum et al., 2000), has been based primarily on two lines of evidence. First, the eastern North Atlantic region has not been repopulated by individuals from the western North Atlantic over the past century, despite a lack of whaling for almost 100 years and the fact that the western population was growing fairly steadily from 1980 through 2010. Despite occasional sightings of known photo-identified individuals from the western North Atlantic in the eastern North Atlantic (Jacobsen et al., 2004; Knowlton et al., 1992; Martin & Walker, 1997; Silva et al., 2012), there have been no consistent signs of repopulation of the area. Second, contemporary and historical observations of migration patterns of the animals in the western and eastern North Atlantic suggest a primarily north–south migration pattern, with animals from the western North Atlantic migrating between

waters off the coasts of southern Florida to the Gulf of St. Lawrence (Kraus et al., 1986; Winn et al., 1986), and animals from the eastern North Atlantic migrating between the coasts of northwest Africa and Norway (Aguilar, 1986; Figure 2). Further, each of these areas appear to have distinct calving areas in the waters around Florida/Georgia and Cintra Bay, Morocco/Bay of Biscay (Reeves & Mitchell, 1986a). It is still unclear under this scenario of theoretical habitat use which population of whales might have inhabited the Cape Farewell area off southeast Greenland, a possible right whale hunting ground (Reeves & Mitchell, 1986a). Hypothesis 3: Reeves and Mitchell (1986a) suggested that *perhaps in addition to western and eastern populations, there was a third "offshore"/mid-Atlantic population that could have spent winters in the waters around the Azores or Bermuda and summers around Cape Farewell.* To date there is little evidence to support this hypothesis, so we will not discuss it further.

As part of a larger-scale study that focuses on the assessment of historical use of marine mammals throughout the North Atlantic over the past 1,200 years, we genetically identified a set of 24 North Atlantic right whale bone samples. These samples originate from 10 sites (archaeological sites or historical whaling stations) around the North Atlantic, including Iceland ($n = 12$), the Faroe Islands ($n = 5$), and Scotland (Orkney, Shetland, Outer Hebrides) ($n = 7$). Samples range in age from the Late Iron Age (4th century CE) to the Modern Period (early 20th century). These provide a rare representation of the extirpated eastern North Atlantic right whale, and therefore represent a valuable opportunity to better understand the history of this species. Here, we compare ancient DNA (both mtDNA and nuclear DNA [nDNA]) collected from these historical and archaeological bones from around the eastern North Atlantic to DNA data collected from the remaining contemporary population in the western North Atlantic over the past ~40 years. We assess the genetic characteristics of the historical eastern North Atlantic right whale and test hypotheses regarding historical population structure within this species.

2 | METHODS

2.1 | Sample origin and collection

Depending upon the site of origin, the approximate ages of the samples were determined using a variety of methods including the known date of site occupation (e.g., those from 17th–19th century whaling stations), collection dates from museum records (e.g., those from 20th century whaling stations), and archaeological assessment using stratigraphy, tephrochronology, and C^{14} dating (see Table S1).

Bone shavings were collected from samples with a cordless drill. A small area of each bone was first brushed clean and then 5–12 mm depth pilot holes were drilled. Outer shavings were discarded and 0.5–4.0 g of bone shavings were then collected from the bone interior using a clean drill bit. Between specimens, drill bits (varying in size from 3 to 10 mm) were soaked with a 1%–3% sodium hypochlorite solution and rinsed in distilled water to minimize the risk of intersample contamination. Upon arrival at the laboratory, shavings were immediately stored at −20°C. All downstream ancient DNA precautionary handling protocols followed those of McLeod et al. (2008, 2012, 2014).

2.2 | DNA extraction, PCR amplification, mtDNA sequencing, and nuclear genotyping

DNA was extracted from 300 mg of bone shavings after McLeod et al. (2008) and Rastogi et al. (2004). Two regions of the mtDNA were amplified to (1) identify species of origin of each bone sample and (2) to obtain haplotype data for the assessment of mtDNA variation as it compares to the contemporary right whale population in the western North Atlantic. For species identification, we used a two-stage PCR amplification approach to amplify a portion of the mtDNA cytochrome *b* gene. For all samples, we first amplified a ~445 bp fragment using primers designed for this study, CBCet4F (5′ ACA TGG ACT TCA ACC ATG AC 3′) and CBCet5R (5′ CTC AGA ATG ATA TTT GTC CTC

AGG 3′). For any samples that failed to amplify, we then attempted amplification of a shorter region (∼186 bp) (which overlaps the original fragment) using the primers CBCet4F and CBCet1R (5′ GTA TTG CTA GAA ATA GGC C 3′). These primers were designed to amplify across a wide range of odontocete and mysticete species that inhabit North Atlantic waters. For the assessment of mtDNA diversity and for comparison of historical samples to the contemporary population, we also amplified a ∼345 bp fragment of the mtDNA control region using the primers T-Pro and Primer-2 (Yoshida et al., 2001) For samples that failed to amplify at one of the mtDNA regions assessed, we attempted a second DNA extraction and reamplification.

For all mtDNA PCR amplifications, conditions were as follows within a 15 μl reaction volume containing 2 μl DNA extract (of unknown DNA concentration): 1× PCR buffer (Promega), 0.2 mM each dNTP (Invitrogen), 0.3 μg/μl UltraPure BSA (Invitrogen), 1.5 mM $MgCl_2$, 0.3 μM each primer, 0.1 U/μl *Taq* (Promega). PCR cycling conditions were as follows for all mtDNA reactions: an initial 5 min denaturation step at 94°C; 50 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 1 min; and a final extension step at 60°C for 45 min. All PCR products were then evaluated for quantity and quality using agarose gel electrophoresis, prepared for sequencing, cycle sequenced, and size separated and visualized on an ABI 3500xl Genetic Analyzer after (McLeod et al., 2014). All samples were sequenced in both directions (e.g., with both primers of the primer pair used for the initial amplification). Sequences were then examined visually, using 4Peaks v1.8 (https://nucleobytes.com), and edited and aligned in MEGA7 v7.0.26 (Kumar et al., 2016). To assign haplotypes to sequences, we compared cytochrome *b* and control region sequences to all known previously published right whale haplotypes, as well as those identified within our laboratory during continuing genetic assessment of the contemporary population. Any sequences with "new" haplotypes found only once within the sample set were reamplified and sequenced for confirmation.

To examine levels of genetic diversity in the archaeological and historical eastern specimens, as well as genetic similarity of these specimens to the contemporary population in the western North Atlantic, we obtained multilocus genetic profiles. These profiles were based on analysis of 15 nuclear microsatellite loci for eight specimens from within the sample set (Table S1). These samples were chosen based on their success in mtDNA amplification, as well as their initial success in amplification at three trial microsatellite loci. The 15 loci were chosen from a larger array of 35 microsatellite loci used in continuing genetic monitoring of the species (Frasier et al., 2006, 2007). This specific subset of loci was chosen based on both their known levels of genetic variability in the contemporary population (among the highest) and their success in ancient DNA amplification trials, which included optimization of PCR for large quantities of amplified DNA with minimal extraneous amplicons from very low initial quantities of DNA extract (<250 pg).

The microsatellite PCR amplification conditions for the bone samples are shown in Table S3. For all PCR amplifications, conditions were as follows within a 15 μl reaction volume containing 2 μl DNA extract (of unknown DNA concentration): 1× PCR buffer (Promega), 0.2 mM each dNTP (Invitrogen), 0.3 μg/μl UltraPure BSA (Invitrogen), 1.5 mM $MgCl_2$, 0.3 μM each primer, 0.1 U/μl *Taq* (Promega). We used two PCR cycling conditions: The first was a 50-cycle program identical to that used for mtDNA amplification (above) and the second was a 50-cycle "touchdown" program (after McLeod et al., 2010). Annealing temperatures and cycling conditions varied across loci and are indicated in Table S3. All PCR products were then evaluated for quantity and quality using agarose gel electrophoresis. In preparation for capillary electrophoresis on an ABI 3500xL Genetic Analyzer, we combined 2 μl of PCR product (diluted in deionized water as needed), 0.25 μl GeneScan-600 LIZ size standard (ThermoFisher Scientific) and 10 μl HiDi formamide (ThermoFisher Scientific).

Electropherograms were visually examined, and alleles were binned and scored using GeneMarker software 2.0 (SoftGenetics). Because low template DNA profiling is vulnerable to allelic dropout, we followed the protocol of McLeod et al. (2010) to ensure profile reliability. Briefly, each sample was genotyped at each locus up to six times to ensure with 95% confidence that a locus was not falsely identified as homozygous. We required that each allele be observed at least twice to designate a locus as heterozygous, and six times to designate a locus as homozygous. If, after 10 amplification trials at a locus, we did not have a confirmed genotype, the sample was not given a profile score at that particular locus.

Profiling methodology used to obtain comparison data from the contemporary population, which includes nuclear profiles collected from the contemporary population over the past ∼15 years, has been published previously (Frasier et al., 2006, 2007). This methodology is used annually with the arrival of new contemporary specimens as part of ongoing genetic monitoring of the species. However, to make sure that scoring of genetic profiles was consistent across all electrophoresis runs containing historical specimens, we included four contemporary control specimens in each run. With each run, profiles of these samples were compared to the known profiles previously collected from the sample. Each run was then validated before any of the data were scored and accepted. No errors or discrepancies were found in the control samples.

2.3 | Species ID

Species ID was confirmed by constructing a phylogenetic tree of the longer fragment cytochrome *b* haplotypes using the Bayesian inference of phylogeny approach implemented in MrBayes ver. 3.2.7a (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For this portion of the cytochrome *b* gene, two haplotypes have been identified previously in the North Atlantic right whale, both from specimens of the western North Atlantic ("EGLCB1"/EU303335 and "EGLCB2"/EU303336; McLeod et al., 2008). For this analysis we included all haplotypes identified in this study, representative sequences from all mysticete species known to have inhabited the North Atlantic Ocean in the past millennium (Table S2), and an additional recently identified third right whale cytochrome *b* haplotype from the western North Atlantic ("EGLCB3"). We used a generalized time-reversible model of molecular evolution with gamma distributed rate variation across sites and a proportion of invariant sites (GTR + I + G), as indicated by JMODELTEST to be appropriate for our data set (Posada, 2008). Analyses involved running four chains for 5,000,000 steps, with a sample taken every 100 steps, and 25% initial steps discarded as burn-in. The resulting tree was visualized using FigTree ver. 1.4.4 (Rambaut, 2018).

Short sequences that were not long enough to assign to haplotypes (<400 bp) were identified to species by both visual comparison to haplotypes of known species and using BLAST (https://www.ncbi.nlm.nih.gov/blast/; Altschul et al., 1990) with identification confirmed only if criteria of 100% query coverage and 95% maximum identity were confirmed, and by phylogenetic analysis (see below).

2.4 | Population structure and diversity analysis

To visually examine relationships between all known mtDNA control region haplotypes identified to date from both the western and eastern North Atlantic, we constructed a median joining network (Bandelt et al., 1999) using popART v1.7 (Leigh & Bryant, 2015). This network included any previously identified North Atlantic right whale haplotypes and any new haplotypes identified in this study. For this portion of the mtDNA control region, analysis of ∼78% of the contemporary population in the western North Atlantic has identified seven distinct haplotypes: "A–E" (Malik et al., 1999), "G" and "H" (a heteroplasmic haplotype; McLeod & White, 2010), a single historical haplotype from a 16th century Basque whaling site in Red Bay, Labrador, "F" (Rastogi et al., 2004), and a historical haplotype from an 18th century right whale baleen "stomacher" (a decorative clothing accessory that was stiffened with "whalebone" [baleen] stays), of unknown origin that was previously cited as "TCC2674.1," and henceforth "L" (Eastop & McEwing, 2004). Because PopART cannot resolve heteroplasmic sites within haplotype networks, haplotype "H" (a heteroplasmic haplotype) was omitted from this analysis. However, "H" is a combination of haplotypes "A" and "G"; thus, the haplotypes are still accounted for in the analyses. Finally, as a means to compare the genetic diversity between the two sample sets, we used DnaSP ver. 5.10.01 (Rozas et al., 2003) to calculate nucleotide (π) and haplotype (*h*) diversity as well as their standard deviations (*SD*; square root of the variance of each index).

Differentiation of mitochondrial sequences between samples from the western and eastern North Atlantic was quantified and assessed in several different ways. First, differentiation was quantified using the relatively standard AMOVA approach described in Excoffier et al. (1992) using functions from the *ape* and *pegas* packages (Paradis, 2010; Paradis & Schliep, 2019) in R v.4.0.2 (R Core Team, 2020). These analyses were conducted twice: Once using the raw frequencies of each sequence within each data set (to obtain estimates of F_{ST}), and a second time based on the genetic distances between the sequences as calculated using the F84 model of molecular evolution (to obtain estimates of Φ_{ST}). For future comparability across studies, we also quantified F_{ST} using the "standardized" approach described in Meirmans (2006) using the *mmod* R package (Winter, 2012). We were concerned that the large difference in sample sizes between the two data sets could be biasing our estimates (mitochondrial sequences were obtained for 21 eastern samples and 575 western samples). Therefore, to visualize and quantify this effect, we conducted simulations comparing differentiation between the western and eastern samples to that calculated just among the western samples if they were subdivided in the same manner. Briefly, for 1,000 iterations we (1) randomly selected 21 western samples to be used as a reference group (representing the same number of eastern samples for which mitochondrial sequences were obtained), (2) calculated differentiation (F_{ST}) between the remaining western samples and the 21 eastern samples, and (3) calculated differentiation (F_{ST}) between the same remaining western samples and the original 21 reference western samples. These simulations provided distributions of differentiation between the two groups, as well as just within the western samples if they were randomly subdivided as per the sample size for the eastern samples.

We compared historical nuclear DNA profiles from specimens in the eastern North Atlantic to those of the contemporary population in the western North Atlantic, of which ∼78% of the population have been profiled. We quantified differentiation based on the microsatellite data in two different ways. First, we estimated F_{ST} between the two groups using the approach described in Weir & Cockerham (1984) using the *pegas* R package, as well as Hedrick's standardized G'_{ST} (Hedrick, 2005) using the *gstudio* R package (Dyer, 2016). We also conducted simulations in the same manner as described for the analysis of mitochondrial differentiation (but with simulations selecting eight eastern Atlantic samples), to assess within- versus between-group differentiation based on subsampling the western samples to the same sample size as for the eastern samples. For these simulations differentiation was quantified using Weir and Cockerham's analogue of F_{ST}. Finally, to assess whether heterozygosity differs between the western and eastern samples, we compared the observed microsatellite heterozygosity of the eight eastern profiles to the observed heterozygosity values of 1,000 random subsets of eight western samples.

3 | RESULTS

3.1 | Species ID

Mitochondrial cytochrome *b* fragments were amplified from all specimens. Of the 24 right whale archaeological and historical eastern samples, 16 yielded complete cytochrome *b* sequences (400 bp) and eight yielded partial sequences by amplifying a shorter fragment of the cytochrome *b* (Table S1, Genbank MW710993-MW711000). Final trimmed sequence lengths used for haplotype assignment and phylogenetic analysis were 400 bp. All samples were designated as North Atlantic right whale either by (1) using Bayesian phylogenetic analysis of the sequences in MrBayes (Figure 3) or (2) (for sequences <400 bp) through visual assessment followed by an NCBI BLAST search and identification (Table S1). For the sequences that were complete, we identified four cytochrome *b* haplotypes within the sample set (EGLCB1–EGLCB4) (Genbank EU303335, EU30336, MW674936, MW674937). While EGLCB1 and EGLCB2 have been previously identified within the contemporary population, EGLCB3 and EGLCB4 have not. However, extensive sequencing of the cytochrome *b* region for the extant population has previously not been conducted. These haplotypes fall within the Balaenidae taxonomic lineage, along with the bowhead whale (*Balaena mysticetus*), in the phylogenetic tree.
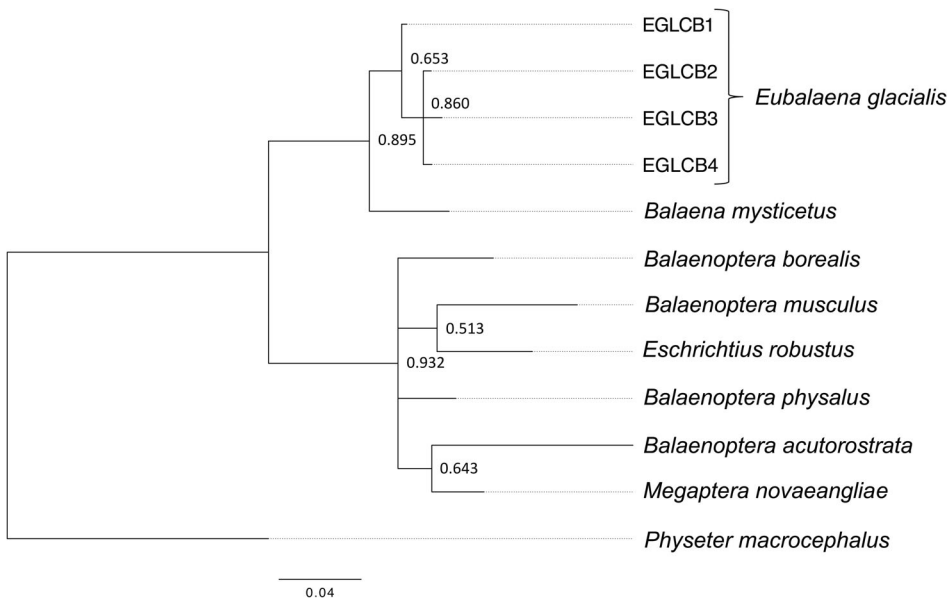
FIGURE 3 Bayesian phylogenetic tree of cytochrome *b* sequences of all North Atlantic baleen whales (Table S2) and those collected from archaeological and historical eastern right whale bone specimens (EGLCB1–EGLCB4). Reference sequence information is shown in Table S2.

3.2 | Population structure and diversity analyses - mtDNA

A consensus length of 312 bp for the control region was used for haplotype assignment and construction of the median joining network. For 18 of the 24 samples, we obtained complete sequences for haplotype determination. For the remaining six samples, amplification failed (Table S1). We identified 11 control region haplotypes, five of which are shared with the contemporary population ("A," "B," "D," "E," and "G") and six of which are previously unidentified, or "new" haplotypes (I–N) (Table S1, Genbank Accession MW684333-MW684338). One haplotype, "L" (MW684336), was found to match the 18th century haplotype previously identified by Eastop and McEwing (2004).

We estimated mtDNA control region nucleotide (π) and haplotype (*h*) diversity for the contemporary (*n* = 6) and the archaeological and historical (*n* = 12) sample sets. While nucleotide diversity is relatively similar between the sample sets (Contemporary: $\pi = 0.00940 \pm 0.00176$; Archaeological/Historical: $\pi = 0.00957 \pm 0.00109$), there was much greater haplotype diversity in the archaeological and historical eastern sample set (*h* = 0.919 ± 0.039) set than in the contemporary sample set (*h* = 0.666 ± 0.012). There were no fixed sequence differences between the sample sets and the average number of nucleotide differences between the sample sets was 2.722.

When examining the median joining network, it is evident that all new haplotypes fall closely within the contemporary star-like haplotype network, without any observable structuring or distinctive patterning of haplotypes by geographical origin (Figure 4). It is notable that when observed alone, the contemporary network exhibits several "missing" intermediate haplotypes amongst those found within the population today, whereas the historical network does not.

Estimates of F_{ST} and Φ_{ST} between the western and eastern mitochondrial sequences were 0.0423 (*p* = .0729) and 0.041 (*p* = .0819), respectively. These were substantially smaller than the "standardized" F_{ST} estimate ($F_{ST} = 0.71$) of Meirmans (2006). The simulations showed that the estimated F_{ST} between the western and eastern samples was larger than that within the western samples, and did not change much regardless of which subsets of the western samples were used for comparison (Figure 5a); the 95% confidence interval for F_{ST} based on these simulations was 0.040–0.045.
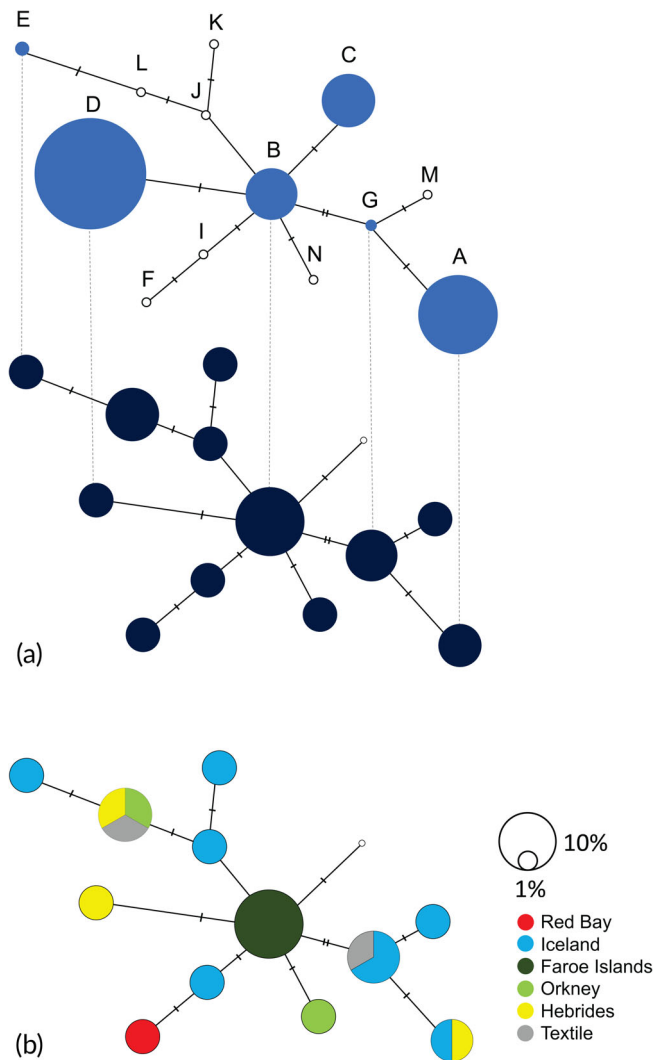
FIGURE 4 (a) Median joining network of all known contemporary (light blue) and historical eastern North Atlantic right whale (dark blue) mtDNA control region haplotypes. Dashed lines link haplotypes that are identified in both regions, while haplotypes not identified within a region are white. In (b), historical eastern North Atlantic right whale haplotypes are shown colored by their region of origin (except for the garment-derived sample of unknown origin, "Textile"). The size of each circle is indicative of the proportion of samples found with particular haplotypes within the total sample size. Hatchmarks on the network indicate the number of haplotype differences between sequences.

3.3 | Population structure and diversity analyses - Nuclear microsatellite DNA

Of the total 24 archaeological and historical eastern North Atlantic right whale samples analyzed, eight were determined to be appropriate for nuclear profiling based on their success in amplification for both mtDNA regions. We were able to obtain complete 15-locus microsatellite profiles for four of the eight samples that were attempted and incomplete profiles for the remaining four samples (which were missing 1–7 loci). We identified 55 alleles across 15 loci (Tables S4 and S5). For most loci, the alleles found also exist within the contemporary western population. However, for one locus (RW4-17) we identified two alleles that have not been found within the contemporary

western population (alleles 119 and 129, Table S5). Allele 119 has, however, been identified in the western North Atlantic region in a skull fragment recovered on Martha's Vineyard, Massachusetts in 2018. The age of this specimen is unknown; however, based on morphological characteristics it is presumed to be very old (e.g., not a recent stranding). The remaining 53 alleles exist in the western population with allele frequencies ranging from 0.002 to 0.775 (Table S5). This included a single "rare" allele (frequency < 0.005). As was found previously in the genetic profiling of a single right whale bone specimen from the coast of Labrador (McLeod et al., 2010), the majority (56%) of the alleles found in the specimens were alleles that are currently present in the extant population at frequencies that are <0.2.

The estimates of F_{ST} and G'_{ST} between western and eastern samples were 0.024 and 0.31, respectively. A plot of genetic differentiation (F_{ST}) within and between the archaeological and historical eastern specimens and contemporary western population, are shown in Figure 5b. The figure shows that the two distributions are quite different, with differentiation being greater between western and eastern samples than within the subsets of the western samples. Based on these simulations, the 95% confidence intervals for within and between differentiation were − 0.015–0.18 and 0.024–0.025, respectively.

The comparison between the observed heterozygosity of the eight archaeological and historical eastern specimens and the distribution of observed heterozygosity values of 1,000 random samples of eight contemporary
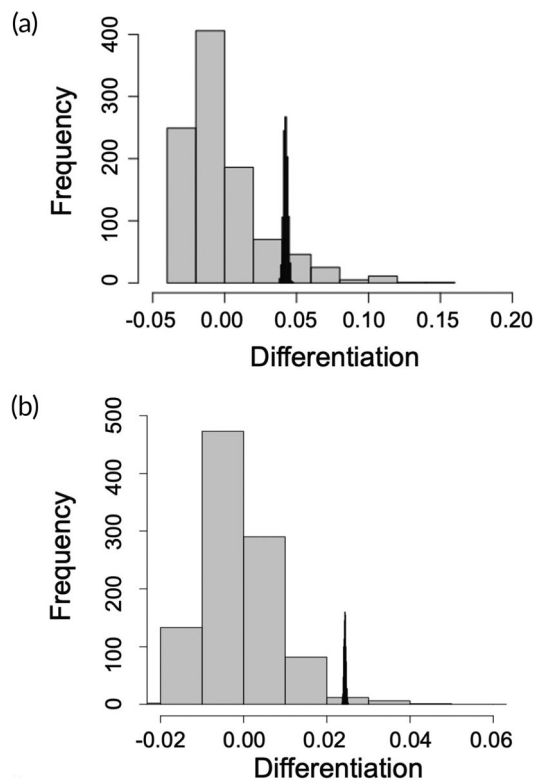
FIGURE 5 (a) mtDNA differentiation and (b) nuclear differentiation (calculated as F_{ST}) between the contemporary western population (light gray) and the archaeological/historical eastern samples (black). Distributions show (1) in light gray, differentiation *within* the western North Atlantic samples based on 1,000 iterations of randomly sampling 21 (mtDNA) or eight (nuclear DNA) individuals and comparing them to the remaining "reference" contemporary samples, and (2) in black, differentiation *between* western and eastern samples by comparing each of the randomized 1,000 "reference" western samples to the archaeological/historical eastern samples.
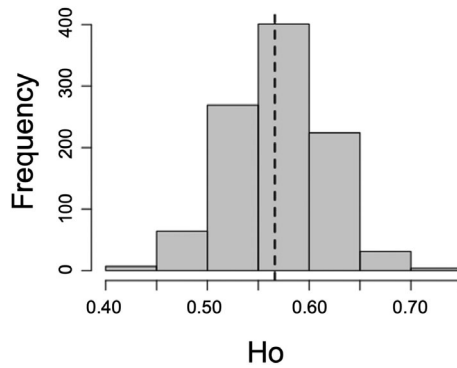
FIGURE 6 Observed heterozygosity (Ho) for the eight eastern samples (black dash) compared to "expected" Ho for 1,000 subsets of eight western contemporary samples (gray histogram).

western specimens are shown in Figure 6. The figure shows that the calculated observed heterozygosity (Ho) for the eastern specimen falls within the center of the distribution of values calculated for the contemporary western population. This suggests that there is no significant difference in heterozygosity between the sample groupings.

4 | DISCUSSION

In the past, there have been very few data with which to estimate historical demographic characteristics of the North Atlantic right whale. These characteristics, particularly historical ocean-wide population structure and historical levels of genetic diversity, are critical in evaluating the potential for contemporary species recovery and subsequent reoccupation of historical habitat areas. Here, we successfully extracted and analyzed mtDNA and nDNA from 24 eastern North Atlantic right whale bone samples ranging in age from the Late Iron Age to the Modern Age (~600–1912 CE) from both archaeological and historical sites in Iceland, the Faroe Islands, and Scotland (Orkney, Shetland, Outer Hebrides).

4.1 | Evaluating hypotheses of population structure of right whales in the North Atlantic

Here, we set out to examine whether right whales in the North Atlantic historically comprised one or two populations. Ideally, we would test hypotheses of historical population structure with the addition of genetic data collected from archaeological and historical specimens from all putative populations/areas of the western and eastern North Atlantic. However, there is only one known sample representing preexploitation western North Atlantic right whales: A bone from a 16th century Basque whaling site in Red Bay, Labrador, Canada (McLeod et al., 2008, 2010; Rastogi et al., 2004). Therefore, we can only compare the available archaeological and historical eastern specimens to genetic data collected from the contemporary western population. Although these data sets would certainly allow us to identify a scenario where western and eastern populations were fully genetically differentiated, they differ temporally as well as spatially, and therefore there are inherent limits to what conclusion(s) can be drawn. For example, genetic differences may be temporal rather than spatial, reflecting the genetic effects of population reductions caused by whaling rather than spatial genetic structuring (e.g., Béland et al., 2020). However, we suggest that this comparison is made less tenuous because data from the single 16th century western sample that dates to precommercial exploitation is genetically very similar to the contemporary population (McLeod et al., 2010).

In their extensive review, Reeves and Mitchell (1986a) concluded that "the available record of nineteenth-century exploitation, incomplete though it is, suggests a degree of independence between eastern and western North Atlantic populations" (p. 242). Indeed, the one-population hypothesis seems unlikely for several reasons: (1) historical observations show evidence of temporal and spatial isolation of right whales in the western and eastern North Atlantic, with some degree of a latitudinal seasonal distribution; (2) contemporary research has found that right whales in the western North Atlantic show site fidelity to their habitat areas in the region; (3) there has been no evidence of reoccupation of the eastern North Atlantic despite an increasing right whale population size over the past 40 years (but see discussion on repopulation of the area below); and (4) the identification within our data set of a relatively large number of new mtDNA haplotypes, as well as some degree of mtDNA and nuclear DNA differentiation between the western and eastern North Atlantic sample sets is not concordant with a single interbreeding population (but see discussion below).

It would be expected that if two populations of right whales existed that were completely genetically isolated over many thousands of years, there would be a clear pattern of genetic differentiation. However, the challenge with species such as the North Atlantic right whale, is that it would take a long time for such differentiation to occur, given that individuals are long-lived and have such long generation times (~20 years; Brown et al., 2009). Thus, it is possible that the populations could be genetically isolated, but not show a clear genetic signal of such differentiation if they were interconnected relatively recently in the past.

We identified some differentiation of both nuclear DNA and mtDNA across the North Atlantic, with slightly greater differentiation in mtDNA, and many shared microsatellite alleles and mtDNA haplotypes across regions. Thus, there was not complete reproductive isolation between the two sides of the ocean. What seems most plausible, at least with the data currently available, is something in between a simple one- and two-population scenario. It is most likely that historically there existed two breeding populations with some degree of limited genetic interchange. Indeed, the existence of complex patterns of population structure, that are not necessarily completely defined by geographic locations, is not uncommon in cetaceans (e.g., Kershaw et al., 2017; Mate et al., 2015). Under scenarios in which the populations were genetically isolated to varying degrees (e.g., incomplete), one would expect that with greater degrees of isolation there would be fewer shared alleles and more novel variants within each population. Our results show that the nuclear and mtDNA profiles of the specimens found in the eastern North Atlantic were genetically similar to those that remain in the western North Atlantic, but with some differentiation. Again, it is difficult to tease apart whether the observed differences within the two sample sets is a result of (1) genetic shifts due to population reductions resulting from centuries of whaling, (2) a signature of historical population differentiation across the ocean basin, or (3) some combination of both factors. Therefore, although we do not know from what this difference results, we can confirm that the animals from the two sides of the ocean basin had at least some degree of gene flow, enough to make them genetically similar. We suggest that the observed slightly greater degree of population mtDNA differentiation is a result of maternally directed site fidelity to summer feeding grounds and/or calving grounds on each side of the Atlantic.

4.2 | Genetic diversity of right whales in the North Atlantic

The control region mtDNA and nuclear microsatellite data have permitted the examination of historical diversity in the species both on a temporal scale and across the Atlantic Ocean. Both mitochondrial and nuclear data indicate some degree of genetic loss over time. Until now, the only previously available data on historical right whale diversity was that from two sources. The first was from a single 16th century right whale bone from Red Bay, Labrador (McLeod et al., 2008, 2010; Rastogi et al., 2004), which carried a previously unidentified haplotype "F" (McLeod et al., 2008), and a microsatellite profile similar to those found in the contemporary population (McLeod et al., 2010). Based on these data, it was concluded that the species had not lost an extensive amount of genetic diversity due to whaling, but that perhaps whaling in northern regions had resulted in loss of matrilines that used these areas for

summer feeding areas. This idea was based on evidence that North Atlantic right whales show significant matrilineal structuring in these areas (Malik et al., 1999). The second source of information on historical right whale genetic diversity was that of Rosenbaum et al. (2000), who examined mtDNA control region sequences collected from late 19th to early 20th century baleen specimens from both the west and eastern North Atlantic. In that study, no new haplotypes were found, but the high diversity of haplotypes found in the small sample set was used to infer that there has been at least some diversity lost between the 19th and 21st centuries.

Indeed, a similar pattern has been found in our data set, where there has been a potential loss of many mtDNA haplotypes but a smaller signature of nuclear DNA differences. Again, we suggest that the loss of many haplotypes for the species may be indicative of a higher degree of historical population structuring that was only reflected in mtDNA and not nuclear DNA; a signature that the species exhibited some degree of mixture in mating areas but site fidelity in northern regions in summer feeding areas. Indeed, all our samples are from northern areas that would have represented summer feeding areas.

While the estimate of nucleotide diversity is very similar between the data sets, the haplotype diversities are quite different, and lower in the contemporary population. The similarity in nucleotide diversity (which represents the average *difference* between to randomly selected samples in a data set) reflects the fact that the haplotypes in both sample sets are very similar to each other, usually differing by only a single nucleotide, resulting in similar degrees of expected differences. In contrast, we suggest that the difference in the haplotype diversity values (which quantifies the probability that two randomly selected samples have different haplotypes) reflects (1) our sampling strategy, with historical samples collected over a long period of time (>1,000 years) from a historically larger population containing a larger number of mtDNA haplotypes while the contemporary sample set is collected from a much smaller time-period and a much smaller extant population; and (2) the fact that the species has undergone severe population reduction as a result of whaling activities.

The results and interpretation above are concordant with the observed historical and contemporary characteristics of other *Eubalaena* species. Observations of both the North Pacific right whale (*E. japonica*) and the southern right whale suggest that they are distributed in populations with relatively isolated latitudinal migratory patterns on either side of the North Pacific (*E. japonica*) (e.g., Gregr, 2011; Josephson et al., 2008; LeDuc et al., 2012) and within the South Atlantic/Indian Oceans (*E. australis*) (e.g., Carroll et al., 2019; IWC, 2001; Patenaude et al., 2007). In addition, matrilineal site fidelity has been observed in right whales globally (Best, 2000; Malik et al., 1999; Payne, 1986; Valenzuela et al., 2009). The southern right whale exhibits a hierarchical pattern of population structure, with one primary genetic subdivision between the South Atlantic and Indian Ocean/South Pacific Ocean basins, but also finer scale substructure with evidence of gene flow within ocean basins (e.g., Carroll et al., 2019). Overall, the factors driving these patterns are complicated and vary by region, but appear to be at least partially driven by maternally inherited site fidelity to both nursery grounds and feeding grounds, with potential mating occurring on migratory routes or in wintering areas (Best, 2000; Burnell, 2001; Carroll et al., 2015; Payne, 1986; Valenzuela et al., 2009; Watson et al., 2021).

4.3 | Implications for recolonization of the eastern North Atlantic

Here, we have identified some nuclear genetic differentiation between western and eastern North Atlantic right whales, which may suggest that repopulation of the eastern Atlantic is not limited by genetic constraints. However, repopulation may be limited by the loss of cultural knowledge of the existence of these areas in the whales that have survived since commercial whaling. Comparatively, all three *Eubalaena* species appear to have habitat areas with populations that have either not recovered, or are showing only limited signs of recovery, following the cessation of whaling activities, e.g., southern right whale populations in the southeast Pacific (Chile and Peru) and southwest Indian Ocean (Madagascar and Mozambique; Cooke & Zerbini, 2018); North Pacific right whale population in the eastern North Pacific (Cooke & Clapham, 2018; Ford et al., 2016). This limited recovery within these areas may be linked to a loss of "cultural memory" that was once held within these populations (Clapham et al. 2008).

Additionally, it should not be ignored that today there may be new ecological and behavioral barriers to repopulation as well as other contemporary factors affecting the western population itself that may be preventing repopulation. For example, it may be the case that contemporary ecological and environmental conditions have created a hostile habitat that the whales are avoiding. It is also possible that the species has not yet recovered to an adequate population size with enough pressures to warrant emigration to reoccupy habitat areas. In addition, if North Atlantic right whale migration is condition-dependent (Gowan et al., 2019) their migratory potential may currently be constrained by limiting factors in the western North Atlantic environment. If the western Atlantic population were able to recover more strongly in the future, we see no reason based on our current knowledge of the species' genetics, why this population could not act as a source for reoccupation of the eastern Atlantic as has been observed in some Southern right whale habitat areas (Carroll et al., 2011, 2014; Groch et al., 2005).

This finding may be important for future management and conservation "forecasting" for the species. For example, shifts in abundance and distribution of the right whales' primary prey species, *Calanus finmarchicus*, over the past decade have resulted in a shift in right whale distribution and a subsequent increase in anthropogenic mortality events within "new" habitats. In 2017, following a shift in summer distribution from the Bay of Fundy into the Gulf of St Lawrence, Canada, the species lost 17 individuals, primarily as a result of combined effects of entanglements in fishing gear and ship strikes (reviewed in Meyer-Gutbrod et al., 2018). This shift, particularly for a species that has had a somewhat predictable pattern of migration and habitat use over the past 40 years, has led to a scrambling effort by managers, fisheries, researchers, and conservationists to adapt conservation activities to protect the species (Meyer-Gutbrod et al., 2018).

4.4 | Conclusion

The complicated history of the North Atlantic right whale, including uncertain demography and centuries of commercial exploitation, has left us with a challenge to define indices of species "recovery," particularly under the challenge of shifting baselines (Lotze & Worm, 2009). The species has suffered a long and complicated history of whaling and is currently struggling to survive in the 21st century under an array of extrinsic and intrinsic threats. Our work presented here illustrates that interpretation of historical population structure is not always straightforward, and sometimes the details are impossible to disentangle. This highlights the need for management strategies to be multifaceted, interdisciplinary, and collaborative, evaluating not only the current condition of a species, but also the status of the species in the past, particularly in light of potential habitat-use shifts with climatic changes to come. Finally, this study highlights the scientific value and great potential resource of archaeological and historical specimens for providing important insights into current species population genetics to support the development of more effective conservation and management strategies.

AUTHOR CONTRIBUTIONS

Leah Springate: Data curation; validation; writing – review and editing. **Timothy Ray Frasier:** Formal analysis; investigation; resources; writing – review and editing. **Seth Brewington:** Data curation; writing – review and editing. **Martin Carruthers:** Data curation; writing – review and editing. **Ragnar Edvardsson:** Data curation; writing – review and editing. **Ramona Harrison:** Data curation; writing – review and editing. **Andrew C. Kitchener:** Data curation; funding

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REFERENCES

Aguilar, A. (1981). The black right whale, *Eubalaena glacialis*, in the Cantabrian Sea. *Reports of the International Whaling Commission*, *31*, 457–459.

Aguilar, A. (1986). A review of old Basque whaling and its effect on the right whales (*Eubalaena glacialis*) of the North Atlantic. *Reports of the International Whaling Commission, Special Issue 10*, 191–199.

Allen, J. A. (1908). The North Atlantic right whale and its near allies. *Bulletin of the American Museum of Natural History*, *24*(18), 66.

Altschul, S. F., Gish, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Bandelt, H. J., Forster, P., & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barkham, S. H. (1984). The Basque whaling establishments in Labrador 1536–1632—A summary. *Arctic*, *37*(4), 515–519. https://doi.org/10.14430/arctic2232

Béland, S. L., Frasier, B. A., Darling, J. D., & Frasier, T. R. (2020). Using pre- and postexploitation samples to assess the impact of commercial whaling on the genetic characteristics of eastern North Pacific gray and humpback whales and to compare methods used to infer historic demography. *Marine Mammal Science*, *36*(2), 398–420. https://doi.org/10.1111/mms.12652

Best, P. B. (2000). Coastal distribution, movements and site fidelity of right whales *Eubalaena australis* off South Africa, 1969–1998. *South African Journal of Marine Science*, *22*(1), 43–55. https://doi.org/10.2989/025776100784125618

Brown, M. W., Fenton, D., Smedbol, R. K., Merriman, C., Robichaud-Leblanc, K., & Conway, J. D. (2009). *Recovery strategy for the North Atlantic right whale* (Eubalaena glacialis) *in Atlantic Canadian waters* (Species at Risk Act Recovery Strategy Series). Fisheries and Oceans Canada.

Burnell, S. R. (2001). Aspects of the reproductive biology, movements and site fidelity of right whales off Australia. *Journal of Cetacean Research and Management, Special Issue 2*, 89–102. https://doi.org/10.47536/jcrm.vi.272

Carroll, E. L., Alderman, R., Bannister, J. L., Bérubé, M., Best, P. B., Boren, L., Baker, C. S., Constantine, R., Findlay, K., Harcourt, R., Lemaire, L., Palsbøll, P. J., Patenaude, N. J., Rowntree, V. J., Seger, J., Steel, D., Valenzuela, L. O., Watson, M., & Gaggiotti, O. E. (2019). Incorporating non-equilibrium dynamics into demographic history inferences of a migratory marine species. *Heredity*, *122*(1), 53–68. https://doi.org/10.1038/s41437-018-0077-y

Carroll, E. L., Baker, C. S., Watson, M., Alderman, R., Bannister, J., Gaggiotti, O. E., Gröcke, D. R., Patenaude, N., & Harcourt, R. (2015). Cultural traditions across a migratory network shape the genetic structure of southern right whales around Australia and New Zealand. *Scientific Reports*, *5*(16182), 1–13. https://doi.org/10.1038/srep16182

Carroll, E. L., Patenaude, N., Alexander, A., Steel, D., Harcourt, R., Childerhouse, S., Smith, S., Bannister, J., Constantine, R., & Baker, C. (2011). Population structure and individual movement of southern right whales around New Zealand and Australia. *Marine Ecology Progress Series*, *432*, 257–268. https://doi.org/10.3354/meps09145

Carroll, E. L., Rayment, W. J., Alexander, A. M., Baker, C. S., Patenaude, N. J., Steel, D., Constantine, R., Cole, R., Boren, L. J., & Childerhouse, S. (2014). Reestablishment of former wintering grounds by New Zealand southern right whales. *Marine Mammal Science*, *30*(1), 206–220. https://doi.org/10.1111/mms.12031

Clapham, P. J., Aguilar, A., & Hatch, L. T. (2008). Determining spatial and temporal scales for management: Lessons from whaling. *Marine Mammal Science*, *24*(1), 183–201. https://doi.org/10.1111/j.1748-7692.2007.00175.x

Cooke, J. G. (2020). *Eubalaena glacialis* (errata version published in 2020). *IUCN Red List of Threatened Species 2020*, e.T41712A178589687. https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T41712A178589687.en.

Cooke, J. G., & Clapham, P. J. (2018). Eubalaena japonica. IUCN Red List of Threatened Species 2018, E.T41711A50380694. https://doi.org/10.2305/IUCN.UK.2018-1.RLTS.T41711A50380694.en

Cooke, J. G., & Zerbini, A. (2018). Eubalaena australis. IUCN Red List of Threatened Species 2018, E.T8153A50354147. https://doi.org/10.2305/IUCN.UK.2018-1.RLTS.T8153A50354147.en.

Corkeron, P., Hamilton, P., Bannister, J., Best, P., Charlton, C., Groch, K. R., Findlay, K., Rowntree, V., Vermeulen, E., & Pace, R. M. (2018). The recovery of North Atlantic right whales, *Eubalaena glacialis*, has been constrained by human-caused mortality. *Royal Society Open Science*, *5*(180892), 1–12. https://doi.org/10.1098/rsos.180892

COSEWIC. (2013). *COSEWIC assessment and status report on the North Atlantic right whale* (Eubalaena glacialis) *in Canada*. Environment Canada, Canadian Wildlife Service, COSEWIC. https://www.registrelep-sararegistry.gc.ca/default_e.cfm

Dyer, R. J. (2016). *Gstudio: Tools related to the spatial analysis of genetic marker data* (R package version 1.5.0) [Computer software]. https://dyerlab.github.io/gstudio/

Eastop, D., & McEwing, R. (2004). Informing textile and wildlife conservation: DNA analysis of baleen from an 18th-century garment found deliberately concealed in a building. *Scientific Analysis of Ancient and Historic Textiles: Informing Preservation, Display and Interpretation: Postprints of the First Conference of the AHRB Research Centre for Textile Conservation and Textile Studies*, 161–167.

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), 479–491. https://doi.org/10.1093/genetics/131.2.479

Ford, J. K. B., Pilkington, J. F., Gisborne, B., Frasier, T. R., Abernethy, R. M., & Ellis, G. M. (2016). Recent observations of critically endangered North Pacific right whales (*Eubalaena japonica*) off the west coast of Canada. *Marine Biodiversity Records*, *9*(50), 1–7. https://doi.org/10.1186/s41200-016-0036-3

Frasier, T. R., Hamilton, P. K., Brown, M. W., Conger, L. A., Knowlton, A. R., Marx, M. K., Slay, C. K., Kraus, S. D., & White, B. N. (2007). Patterns of male reproductive success in a highly promiscuous whale species: The endangered North Atlantic right whale. *Molecular Ecology*, *16*(24), 5277–5293. https://doi.org/10.1111/j.1365-294X.2007.03570.x

Frasier, T. R., Rastogi, T., Brown, M. W., Hamilton, P. K., Kraus, S. D., & White, B. N. (2006). Characterization of tetranucleotide microsatellite loci and development and validation of multiplex reactions for the study of right whale species (genus *Eubalaena*). *Molecular Ecology Notes*, *6*(4), 1025–1029. https://doi.org/10.1111/j.1471-8286.2006.01417.x

Fromentin, J.-M. (2009). Lessons from the past: Investigating historical data from bluefin tuna fisheries. *Fish and Fisheries*, *10*(2), 197–216. https://doi.org/10.1111/j.1467-2979.2008.00311.x

Gowan, T. A., Ortega-Ortiz, J. G., Hostetler, J. A., Hamilton, P. K., Knowlton, A. R., Jackson, K. A., George, R. C., Taylor, C. R., & Naessig, P. J. (2019). Temporal and demographic variation in partial migration of the North Atlantic right whale. *Scientific Reports*, *9*(353), 1–11. https://doi.org/10.1038/s41598-018-36723-3

Greene, C. H. (2016). North America's iconic marine species at risk due to unprecedented ocean warming. *Oceanography*, *29*(3), 14–17. https://doi.org/10.5670/oceanog.2016.67

Greene, C. H., & Pershing, A. J. (2004). Climate and the conservation biology of North Atlantic right whales: The right whale at the wrong time? *Frontiers in Ecology and the Environment*, *2*(1), 29–34. https://doi.org/10.1890/1540-9295(2004)002[0029:CATCBO]2.0.CO;2

Gregr, E. (2011). Insights into North Pacific right whale *Eubalaena japonica* habitat from historic whaling records. *Endangered Species Research*, *15*(3), 223–239. https://doi.org/10.3354/esr00381

Groch, K. R., Palazzo, J. T., Jr., Flores, P. A. C., Adler, F. R., & Fabian, M. E. (2005). Recent rapid increases in the right whale (*Eubalaena australis*) population off southern Brazil. *Latin American Journal of Aquatic Mammals*, *4*(1), 41–47. https://doi.org/10.5597/lajam00068

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution*, *59*(8), 1633–1638. https://doi.org/10.1111/j.0014-3820.2005.tb01814.x

Huelsenbeck, J. P., & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

IWC. (2001). Report of the workshop on the comprehensive assessment of right whales: A worldwide comparison. *Journal of Cetacean Research and Management*, 1–35. https://doi.org/10.47536/jcrm.vi.270

Jacobsen, K.-O., Marx, M., & Oien, N. (2004). Two-way trans-Atlantic migration of a North Atlantic right whale (*Eubalaena glacialis*). *Marine Mammal Science*, *20*(1), 161–166. https://doi.org/10.1111/J.1748-7692.2004.TB01147.X

Josephson, E., Smith, T. D., & Reeves, R. R. (2008). Historical distribution of right whales in the North Pacific: Right whales in the North Pacific. *Fish and Fisheries*, *9*(2), 155–168. https://doi.org/10.1111/j.1467-2979.2008.00275.x

Kershaw, F., Carvalho, I., Loo, J., Pomilla, C., Best, P. B., Findlay, K. P., Cerchio, S., Collins, T., Engel, M. H., Minton, G., Ersts, P., Barendse, J., Kotze, P. G. H., Razafindrakoto, Y., Ngouessono, S., Meÿer, M., Thornton, M., & Rosenbaum, H. C. (2017). Multiple processes drive genetic structure of humpback whale (*Megaptera novaeangliae*) populations across spatial scales. *Molecular Ecology*, *26*(4), 977–994. https://doi.org/10.1111/mec.13943

Knowlton, A. R., Hamilton, P. K., Marx, M. M., Pettis, H. M., & Kraus, S. D. (2012). Monitoring North Atlantic right whale *Eubalaena glacialis* entanglement rates: A 30 yr retrospective. *Marine Ecology Progress Series*, *466*, 293–302. https://doi.org/10.3354/meps09923

Knowlton, A. R., & Kraus, S. D. (2001). Mortality and serious injury of northern right whales (*Eubalaena glacialis*) in the western North Atlantic Ocean. *Journal of Cetacean Research and Management*, Special Issue 2, 193–208. https://doi.org/10.47536/jcrm.vi.288

Knowlton, A. R., Kraus, S. D., & Kenney, R. D. (1994). Reproduction in North Atlantic right whales (*Eubalaena glacialis*). *Canadian Journal of Zoology*, 72(7), 1297–1305. https://doi.org/10.1139/z94-173

Knowlton, A. R., Sigukjósson, J., Ciano, J. N., & Kraus, S. D. (1992). Long-distance movements of North Atlantic right whales (*Eubalaena glacialis*). *Marine Mammal Science*, 8(4), 397–405. https://doi.org/10.1111/j.1748-7692.1992.tb00054.x

Kraus, S. D. (2005). North Atlantic right whales in crisis. *Science*, 309(5734), 561–562. https://doi.org/10.1126/science.1111200

Kraus, S. D., Kenney, R. D., Mayo, C. A., McLellan, W. A., Moore, M. J., & Nowacek, D. P. (2016). Recent scientific publications cast doubt on North Atlantic right whale future. *Frontiers in Marine Science*, 3(137), 1–3. https://doi.org/10.3389/fmars.2016.00137

Kraus, S. D., Prescott, J. H., Knowlton, A. R., & Stone, G. S. (1986). Migration and calving of right whales (*Eubalaena glacialis*) in the Western North Atlantic. *Reports of the International Whaling Commission, Special Issue 10*, 139–144.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

LeDuc, R., Taylor, B., Martien, K., Robertson, K., Pitman, R., Salinas, J., Burdin, A., Kennedy, A., Wade, P., Clapham, P., & Brownell, R., Jr. (2012). Genetic analysis of right whales in the eastern North Pacific confirms severe extirpation risk. *Endangered Species Research*, 18(2), 163–167. https://doi.org/10.3354/esr00440

Leigh, J. W., & Bryant, D. (2015). POPART: full feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410

Lindqvist, O. (1994). *Whales, dolphins and porpoises in the economy and culture of peasant fishermen in Norway, Orkney, Shetland, Faroe Islands and Iceland, ca. 900–1900 A.D., and Norse Greenland, ca. 1000–1500 A.D.* (Vol. 1). [Doctoral dissertation]. University of St. Andrews.

Lotze, H. K., Coll, M., Magera, A. M., Ward-Paige, C., & Airoldi, L. (2011). Recovery of marine animal populations and ecosystems. *Trends in Ecology & Evolution*, 26(11), 595–605. https://doi.org/10.1016/j.tree.2011.07.008

Lotze, H. K., & Worm, B. (2009). Historical baselines for large marine animals. *Trends in Ecology & Evolution*, 24(5), 254–262. https://doi.org/10.1016/j.tree.2008.12.004

Malik, S., Brown, M. W., Kraus, S. D., Knowlton, A. R., Hamilton, P. K., & White, B. N. (1999). Assessment of mitochondrial DNA structuring and nursery use in the North Atlantic right whale (*Eubalaena glacialis*). *Canadian Journal of Zoology*, 77(8), 1217–1222. https://doi.org/10.1139/z99-073

Malik, S., Brown, M. W., Kraus, S. D., & White, B. N. (2000). Analysis of mitochondrial DNA diversity within and between North and South Atlantic right whales. *Marine Mammal Science*, 16(3), 545–558. https://doi.org/10.1111/j.1748-7692.2000.tb00950.x

Martin, A. R., & Walker, F. J. (1997). Sighting of a right whale (*Eubalaena glacialis*) with calf off S.W. Portugal. *Marine Mammal Science*, 13(1), 139–140. https://doi.org/10.1111/j.1748-7692.1997.tb00617.x

Mate, B. R., Ilyashenko, V. Yu., Bradford, A. L., Vertyankin, V. V., Tsidulko, G. A., Rozhnov, V. V., & Irvine, L. M. (2015). Critically endangered western gray whales migrate to the eastern North Pacific. *Biology Letters*, 11(20150071), 1–4. https://doi.org/10.1098/rsbl.2015.0071

McLeod, B. A., Brown, M. W., Frasier, T. R., & White, B. N. (2010). DNA profile of a sixteenth century western North Atlantic right whale (*Eubalaena glacialis*). *Conservation Genetics*, 11(1), 339–345. https://doi.org/10.1007/s10592-009-9811-6

McLeod, B. A., Brown, M. W., Moore, M. J., Stevens, W., Barkham, S. H., Barkham, M., & White, B. N. (2008). Bowhead whales, and not right whales, were the primary target of 16th- to 17th-century Basque whalers in the western North Atlantic. *Arctic*, 61(1), 61–75. https://doi.org/10.14430/arctic7

McLeod, B. A., Frasier, T. R., Dyke, A. S., Savelle, J. M., & White, B. N. (2012). Examination of ten thousand years of mitochondrial DNA diversity and population demographics in bowhead whales (*Balaena mysticetus*) of the Central Canadian Arctic. *Marine Mammal Science*, 28(4), E426–E443. https://doi.org/10.1111/j.1748-7692.2011.00551.x

McLeod, B. A., Frasier, T. R., & Lucas, Z. (2014). Assessment of the extirpated Maritimes walrus using morphological and ancient DNA analysis. *PLoS ONE*, 9(6), e99569. https://doi.org/10.1371/journal.pone.0099569

McLeod, B. A., & White, B. N. (2010). Tracking mtDNA heteroplasmy through multiple generations in the North Atlantic right whale (*Eubalaena glacialis*). *Journal of Heredity*, 101(2), 235–239. https://doi.org/10.1093/jhered/esp098

Meirmans, P. G. (2006). Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution*, 60(11), 2399–2402. https://doi.org/10.1111/j.0014-3820.2006.tb01874.x

Meyer-Gutbrod, E., Greene, C. H., & Davies, K. (2018). Marine species range shifts necessitate advanced policy planning: The case of the North Atlantic right whale. *Oceanography*, 31(2), 19–23. https://doi.org/10.5670/oceanog.2018.209

Meyer-Gutbrod, E., Greene, C., Davies, K., & Johns, D. (2021). Ocean regime shift is driving collapse of the North Atlantic right whale population. *Oceanography*, 34(3), 22–31. https://doi.org/10.5670/oceanog.2021.308

Monsarrat, S., Pennino, M. G., Smith, T. D., Reeves, R. R., Meynard, C. N., Kaplan, D. M., & Rodrigues, A. S. L. (2015). Historical summer distribution of the endangered North Atlantic right whale (*Eubalaena glacialis*): A hypothesis based on environmental preferences of a congeneric species. *Diversity and Distributions*, 21(8), 925–937. https://doi.org/10.1111/ddi.12314

Moore, M., Rowles, T., Fauquier, D., Baker, J., Biedron, I., Durban, J., Hamilton, P., Henry, A., Knowlton, A., McLellan, W., Miller, C., Pace, R., Pettis, H., Raverty, S., Rolland, R., Schick, R., Sharp, S., Smith, C., Thomas, L., ... Ziccardi, M. (2021). REVIEW Assessing North Atlantic right whale health: Threats, and development of tools critical for conservation of the species. *Diseases of Aquatic Organisms*, *143*, 205–226. https://doi.org/10.3354/dao03578

Mowat, F. (1985). *Sea of slaughter*. McClelland and Stewart-Bantam.

Paradis, E. (2010). pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics*, *26*(3), 419–420. https://doi.org/10.1093/bioinformatics/btp696

Paradis, E., & Schliep, K. (2019). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*(3), 526–528. https://doi.org/10.1093/bioinformatics/bty633

Patenaude, N. J., Portway, V. A., Schaeff, C. M., Bannister, J. L., Best, P. B., Payne, R. S., Rowntree, V. J., Rivarola, M., & Baker, C. S. (2007). Mitochondrial DNA diversity and population structure among southern right whales (*Eubalaena australis*). *Journal of Heredity*, *98*(2), 147–157. https://doi.org/10.1093/jhered/esm005

Payne, R. (1986). Long term behavioural studies of the southern right whale (*Eubalaena australis*). *Reports of the International Whaling Commission*, *Special Issue 10*, 161–167.

Pettis, H. M., Pace, R. M. I., & Hamilton, P. K. (2021). North Atlantic right whale consortium 2020 annual report card. *Report to the North Atlantic Right Whale Consortium*. https://www.narwc.org/uploads/1/1/6/6/116623219/2019reportfinal.pdf

Posada, D. (2008). Jmodeltest: Phylogenetic model averaging. *Molecular Biology and Evolution*, *25*(7), 1253–1256. https://doi.org/10.1093/molbev/msn083

R Core Team. (2020). *R: A language and environment for statistical computing* [Computer software]. R Foundation of Statistical Computing.

Rambaut, A. (2018). *Figtree Ver. 1.4.4.* [Computer software]. https://github.com/rambaut/figtree/releases

Rastogi, T., Brown, M. W., McLeod, B. A., Frasier, T. R., Grenier, R., Cumbaa, S. L., Nadarajah, J., & White, B. N. (2004). Genetic analysis of 16th-century whale bones prompts a revision of the impact of Basque whaling on right and bowhead whales in the western North Atlantic. *Canadian Journal of Zoology*, *82*(10), 1647–1654. https://doi.org/10.1139/z04-146

Reeves, R. R., Breiwick, J. M., & Mitchell, E. D. (1999). History of whaling and estimated kill of right whales, *Balaena glacialis*, in the northeastern United States, 1620–1924. *Marine Fisheries Review*, *61*(3), 1–36.

Reeves, R. R., & Mitchell, E. D. (1986a). American pelagic whaling for right whales in the North Atlantic. *Reports of the International Whaling Commission*, *Special Issue 10*, 221–254.

Reeves, R. R., & Mitchell, E. D. (1986b). The Long Island, New York right whale fishery: 1650-1924. *Reports of the International Whaling Commission*, *Special Issue 10*, 201–220.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*(12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rosenbaum, H. C., Egan, M. G., Clapham, P. J., Jr., R. L. B., Malik, S., Brown, M. W., White, B. N., Walsh, P., & Desalle, R. (2000). Utility of North Atlantic right whale museum specimens for assessing changes in genetic diversity. *Conservation Biology*, *14*(6), 1837–1842. https://doi.org/10.1111/j.1523-1739.2000.99310.x

Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, *19*(18), 2496–2497. https://doi.org/10.1093/bioinformatics/btg359

Schaeff, C. M., Kraus, S. D., Brown, M. W., Perkins, J., & Payne, R. (1991). Preliminary analysis of mitochondrial DNA variation within and between the right whale species *Eubalaena glacialis* and *Eubalaena australis*. *Reports of the International Whaling Commission*, *Special Issue 13*, 217–223.

Schaeff, C. M., Kraus, S. D., Brown, M. W., Perkins, J. S., Payne, R., & White, B. N. (1997). Comparison of genetic variability of North and South Atlantic right whales (*Eubalaena*), using DNA fingerprinting. *Canadian Journal of Zoology*, *75*(7), 1073–1080. https://doi.org/10.1139/z97-129

Sharp, S., McLellan, W., Rotstein, D., Costidis, A., Barco, S., Durham, K., Pitchford, T., Jackson, K., Daoust, P., Wimmer, T., Couture, E., Bourque, L., Frasier, T., Frasier, B., Fauquier, D., Rowles, T., Hamilton, P., Pettis, H., & Moore, M. (2019). Gross and histopathologic diagnoses from North Atlantic right whale *Eubalaena glacialis* mortalities between 2003 and 2018. *Diseases of Aquatic Organisms*, *135*(1), 1–31. https://doi.org/10.3354/dao03376

Silva, M. A., Steiner, L., Cascão, I., Cruz, M. J., Prieto, R., Cole, T., Hamilton, P. K., & Baumgartner, M. (2012). Winter sighting of a known western North Atlantic right whale in the Azores. *Journal of Cetacean Research and Management*, *12*(1), 65–69.

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., Burrows, M. T., Alexander, L. V., Benthuysen, J. A., Donat, M. G., Feng, M., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Sen Gupta, A., Payne, B. L., & Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, *9*(4), 306–312. https://doi.org/10.1038/s41558-019-0412-1

Szabo, V. E. (2008). Monstrous fishes and the mead-dark sea. *Brill*. https://doi.org/10.1163/ej.9789004163980.i-326

True, F. W. (1904). *The whalebone whales of the western North Atlantic compared with those occurring in European waters with some observations on the species of the Pacific* (Vol. 33). Smithsonian Institution Press. https://doi.org/10.5962/bhl.title.15879

Valenzuela, L. O., Sironi, M., Rowntree, V. J., & Seger, J. (2009). Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*). *Molecular Ecology*, *18*(5), 782–791. https://doi.org/10.1111/j.1365-294X.2008.04069.x

Van Der Hoop, J. M., Moore, M. J., Barco, S. G., Cole, T. V. N., Daoust, P.-Y., Henry, A. G., McAlpine, D. F., Mclellan, W. A., Wimmer, T., & Solow, A. R. (2013). Assessment of management to mitigate anthropogenic effects on large whales: Mitigation of human-whale interactions. *Conservation Biology*, *27*(1), 121–133. https://doi.org/10.1111/j.1523-1739.2012.01934.x

Waldick, R. C., Kraus, S. D., Brown, M., & White, B. N. (2002). Evaluating the effects of historic bottleneck events: An assessment of microsatellite variability in the endangered, North Atlantic right whale. *Molecular Ecology*, *11*(11), 2241–2249. https://doi.org/10.1046/j.1365-294X.2002.01605.x

Watson, M., Stamation, K., Charlton, C., & Bannister, J. (2021). Calving intervals, long-range movements and site fidelity of southern right whales (*Eubalaena australis*) in south-eastern Australia. *Journal of Cetacean Research and Management*, *22*, 17–28. https://doi.org/10.47536/jcrm.v22i1.210

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Winn, H. E., Price, C. A., & Sorensen, P. W. (1986). The distributional biology of the right whale (*Eubalaena glacialis*) in the western North Atlantic. *Reports of the International Whaling Commission, Special Issue 10*, 129–138.

Winter, D. J. (2012). MMOD: an R library for the calculation of population differentiation statistics. *Molecular Ecology Resources*, *12*(6), 1158–1160. https://doi.org/10.1111/j.1755-0998.2012.03174.x

Yoshida, H., Yoshioka, M., Shirakihara, M., & Chow, S. (2001). Population structure of finless porpoises (*Neophocoena phocaenoides*) in coastal waters of Japan based on mitochondrial DNA sequences. *Journal of Mammalogy*, *82*(1), 123–130. https://doi.org/10.1644/1545-1542(2001)082<0123:PSOFPN>2.0.CO;2

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