Shadows of the past,
a life through fisheries and climate change

Historical patterns in the evolution, demography and distribution of Atlantic cod (*Gadus morhua*)

Lourdes Martínez García

Dissertation presented for the degree of
Philosophiae Doctor (PhD)
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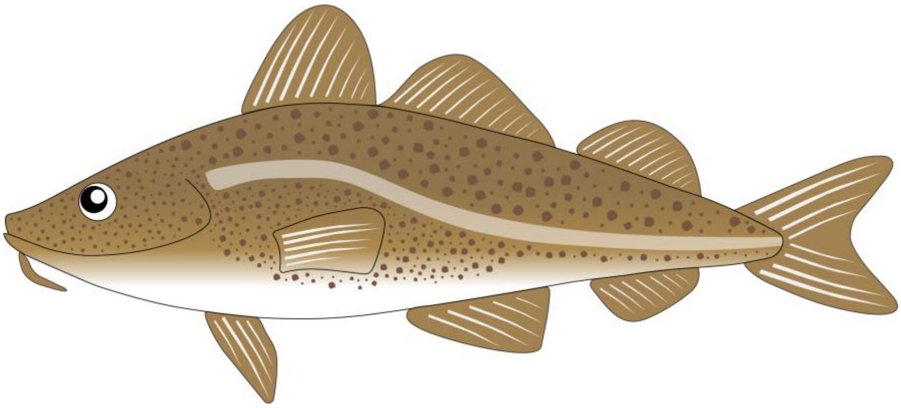

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Para Martin, quién me impulsa a seguir soñando

Para Sara Lilia y Rogelio, mis más grandes inspiraciones

Contents

Reina del Mar

Mónica Gutiérrez de Kiy

(La Paz, Baja California Sur, México)

Desde la tierra donde nace la Paz
gobierna en silencio la Reina del Mar
protege su acuario -tesoro inmortal-
su oasis marino de alga y de sal
y acoge en sus manos al pez que vendrá.

La Reina del Mar observa pasar
los barcos que llegan los barcos que van
los besos furtivos de espina y marea
y en completo silencio dicta una ley nueva:
que nadie corrompa su mansión de ballenas
que nadie se atreva a envenenar sirenas
y que a partir de mañana recuerde la tierra
que se repite el milagro si la vida se crea.

Preface

When I first finished reading the first (zoo)archaeological report in my hands, I thought, *“what am I doing here?”*. I had to translate the meaning of more than ten words, and I remember thinking, *“am I supposed to know where that bone is?”*.

I will not lie and say that that thought never came to my mind again in the three years of my PhD. However, as time went by, less words needed translation, and it was easier to know which bone people were describing without looking at pictures (though I am not an expert). I became eager to join an excavation and experience all the amazing things described in literature… But I never got the opportunity.

If someone would invite me to an excavation right now, I would definetely jump into that adventure. But hey, working with ancient DNA, and dressing as an astronaut inside the ancient DNA lab is already a big adventure by itself, which is something that I will not forget.

It is funny to think about how I got here in the first place. Considering I always complain about cold weather and non-spicy food, anybody would have thought I would leave the Nordics and go to warmer latitudes. But here I am, three (and five in total) years later, ready to finish this PhD and move even further north, where I will most certainly still complain about the cold. The more I think about it, the more I think I may be like Atlantic cod. The cold is good for me, but also is the warmth.

Speaking of Atlantic cod, I never thought of myself as a fish person. But here I am, extremely happy to have had the opportunity to work with such an interesting fish that, in spite of the public’s opinion, is quite beautiful. I did not know much about the history of Atlantic cod before I started this PhD. But the more I read about it, the more fascinating its history seemed. It might not be the most colourful or the most charming fish, but it is absolutely one of the most curious and frankly coolest fish I know.

If you would ask me, *“will you work with Atlantic cod again?”* I would not doubt it even for a second: Yes, I would.

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I began this (PhD) journey in October 2019, and in these three years I have met a large number of people to whom I must thank for their guidance, support and encouragement.

Bastiaan, thank you for giving me the opportunity to learn by your side these years. You have a wonderful and inspiring ability for scientific thinking and scientific writing. Thank you for pushing me to always give the best of myself and for believing that my spelling mistakes in english would improve :). Talking to you was always refreshing. You always made me laugh with your spontaneous questions about Mexican food and the Spanish language. It made me very happy that you have adopted "*Hola*" as part of your daily vocabulary :). Thank you very much for that epic spicy sauce you prepared back in October 2021. I am still waiting for the recipe. You are an excellent researcher, Bastiaan. You truly are. Thank you for everything.

James, thank you very much for guiding me through the archaeological world and the poetic narrative of my scientific findings. Your puffin story is something I will always remember.

Kjetill and Sissel, thank you for always being on top of the up-to-date findings in the Cod world. You are both insightful researchers with whom I wish I had spent more time sharing Cod thoughts. Thank you for all your valuable comments and suggestions in my projects, the outcome would not have been the same without your input.

All my co-authors, thank you for being such a welcoming network of researchers, for all your hard work, and for guiding me through additional concepts and novel literature in the evolutionary and archaeological world.

The Archaeogenomics group (previous and current members that I was lucky enough to meet). First, my dear office mates: **Anneke, Oliver and Lane.** You guys are the best, I really enjoyed your company :). **Anneke**, you are such an amazing and talented person. I just love your laugh. I was always happy when I got to spend some time with you. **Oliver**, you are such a science rockstar. I admire you. Thank you for always making the time to answer my never ending bioinformatic questions until the very end. It was lovely to see you become a dad. **Lane**, thank you for so so many laughs :). Read more below. **Giada**, thank you for answering all my questions, even if I asked you the same five times in a row. I have to admit I failed with the yellow sweater. But the yarn is still with me and believe me that the day I finish it, you will be the first one to get a picture, so, stay tuned. **Angélica María**, colombiana querida, la única persona tan mañanera como yo en este grupo. Gracias por hacer del trabajo en el laboratorio una aventura más agradable. Nuestras pláticas me han llenado con un sentimiento de hogar al hablar español. Muchas gracias por compartir conmigo tus sabios consejos en ciencia. And finally, to the members of our group with whom I did not spent as much time as I wish I had, **Álbina, Emma, Lauren and Lydia**, thank you for the conversations and laughs we shared together.

Lane, a big thank you to you. It made me very happy to share this journey with you. I will miss those confidential looks we will give to each other in special situations (:)). You should know that I will always be thankful for the infinite patience you showed to me when my frustration with the computer tasks became too obvious (it did happen often). I know there is such a bright future in front of you, and you deserve it. Thanks for the long walks, the spicy food and the drinks we have shared, and once again, all the laughs shared in Norway and around the world.

Rachel, my dear Racheli, it was a pleasure to be your friend and to have worked with you. You are such a sweet person and such a good teacher. Everything I know about fish morphology is because of you. Thank you for making our lessons so fun and for trusting I would learn to identify fish. It was truly a lovely time the one I spent working with you and next to you. My time spent in Trondheim was even more special because you and Youri were there (Thank you for everything, **Youri**). Thank you for all the early and late chats. Thank you for constantly feeding me every time I was forgetting my snacks. Thank you for letting me keep the room warm even when you were burning hot. Thank you for teaching me fish are cool. I will truly miss you.

The **SeaChanges PhD crew**. What a journey guys! It was a pleasure to join this ITN and have the opportunity to meet such talented and lovely people as all of you: **Adam, Emily, Fabri, Giulia**, **Katrien, Lane, Laura**, **Liz**, **Lucía**, **Magie**, **Marie, Rachel W**, **Rachel B** and **Willemien**. It has been my pleasure to share this journey with you all. Thank you for the wonderful experiences during our time together. Many of you made me laugh so much, even if you didn't notice :). The scientific world is and will be lucky to have scientists like you in it.

A special thank you goes to **Fabricio and Emily**. Fabri, você tem uma das personalidades mais bonitas que tenho a sorte de ter encontrado. Você é uma pessoa maravilhosa e já um pesquisador incrível. Emily, eres un sol, amiga mía. Que nada en esta vida te detenga, sigue adelante y no pares hasta encontrar aquello que te haga sonreír cada día.

Nell and David, thank you so much for always being there when needed. I think you have both done (and did) an amazing job with the SeaChanges network.

Ryan (Easterday) you literally made my days happier by sharing your chili seeds with me. Thank you so much. Because of those seeds, my food in Norway became better, and that my friend, is something I'm deeply thankful about.

Franzi, my very first friend in Norway and now a lifetime friend. My dear Franzi, what are the odds that it was a hockey match what made us became friends? You have always been there for me, sharing the exciting and complicated news from my life and my research. Is lovely to have amazing friends like you by my side. You are a sunshine, Franzi.

My friends in Lund, **Sachin and Mèmè**, thank you for always being present even if 1000+ kilometres were in between us. **Ana, Pierre, Ainara, Dimitri, Rodrigo, Daniel, Katja, and all of those special Lund people** with whom I spent long talks, barbecues, fikas and dances with, thank you for so many laughs and delicious food. A special thank you goes to **Raphael**; thank you for letting me work for long periods in your desk, I truly appreciated it. The whole **Aquatic Ecology group at Lund University**, thank you for always give me a smile while I was around, just like the very old times. And of course, **Lund** itself, this little town in Sweden was more my home than Oslo for the first two years of my PhD. My time there was truly like being at home.

Julia del Carmen. Carmelita, mi Juli querida, fue (y es) un honor y un placer vivir contigo. Muchas gracias por estar 24/7 junto a mí, aún cuando nos encontrabamos en distintos países y en distintos horarios. Ha sido una maravillosa coincidencia venir a encontrar tan hermosa persona en estos lares escandinavos. Te quiero mucho mi Juli.

Franquita, Guille y Guidaí, mis uruguayos favoritos. Son unas de las personas más maravillosas que he tenido el placer de encontrar en mi vida. No saben cuanto los quiero y cuán feliz me hace verlos y escucharlos.

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Karen, amiga mía, siempre que me preguntan ¿quién es tu autor preferido? Eres tú en quién pienso. Gracias por todo tu apoyo incondicional y por tus regaños necesarios e inecesarios. Eres y siempre serás mi mejor amiga. Te amo.

Mi querida familia. **Sara Lilia**, no hay día en que no desee un abrazo tuyo. **Rogelio**, es tu voz la que resuena en mi mente cuando más ánimos necesito. Ustedes dos son mi tesoro más grande y me siguen inspirando cada día. Los amo. **Lourdes**. Abuelita, releer tus cartas siempre me dan ánimos, y eran mi apoyo principal cuando más lo necesitaba. Extraño tanto salir de casa y verte todos los días. **Liliana,** es imposible expresar únicamente con palabras mi amor por tí. No existe persona alguna que me haga reír como tú. Te amo. **José Manuel**, uno de los retos más grandes que he experimentando ha sido verte crecer a distancia. Tú, tu sonrisa y tus dibujos han sido una inspiración inmensa en mi vida cotidiana. Una de mis actividades favoritas es y siempre será platicar contigo. Gracias por hacerme sonreír y por no dejarme olvidar que éste mundo está lleno de sorpresas, aventuras y sonrisas. Te amo. **Lili,** tus ojos enormes y tu sonrisa traviesa iluminan mis días. Conocerte, abrazarte y sobretodo escucharte decir mi nombre antes que cualquier otro (:)) son mis más preciados recuerdos hoy y siempre. Te amo. Mis queridos sobrinos, ustedes fueron (y son) una de mis principales inspiraciones en mi vida. Mirar sus fotos y videos entre juntas y días largos de trabajo, eran (y son) mi principal fuente de sonrisas. Los amo y los extraño a cada momento.

Martin, no podría haber deseado mejor compañero en esta vida que tú. Cada mañana iluminas mis días y compartes conmigo la magia necesaria para hacerme sonreír sin ninguna dificultad. Gracias por tus continuos abrazos a lo largo de este trayecto y en nuestra vida cotidiana. Gracias por tus palabras de ánimo cuando más lo necesité. Gracias por apoyar siempre cada una de mis decisiones y por siempre empujarme a ser mejor. Eres el café de mi pancito. Te amo Martin.

A single dream is more powerful than a thousand realities.
J.R.R. Tolkien

Abstract

Large-scale fishing activities and the ongoing ocean warming are of substantial concern for marine ecosystems. They can have severe consequences for marine biodiversity, including the depletion of genetic diversity and population collapses leading to extinction of marine species. One such species, the Atlantic cod (*Gadus morhua*), is an economically important marine fish in the North Atlantic which has experienced extended periods of fishing exploitation. Cod fisheries increased the wealth of coastal communities and promoted the growth of urban centres across Europe during the last millennia. Although historically abundant, a changing climate and continuous fishing pressures led to dramatic population declines and collapses in Atlantic cod populations by the late twentieth century. It however remains unclear to what extent have Atlantic cod populations been harvested across time and what consequences have the population declines had on the genomic diversity of Atlantic cod. In this thesis, I used a multidisciplinary approach using ancient DNA (aDNA) and stable isotope methods to provide novel insights into the long-term population dynamics of Atlantic cod under different climatic and harvest scenarios over time. aDNA can provide an additional inference of demographic histories and the estimation of genetic fluctuations over time from heavily exploited species, while stable isotopes can elucidate their source and their movement patterns. Using aDNA (**Paper III** and **Paper IV**) I found that the dramatic population declines in Atlantic cod appear to have been preceded by a larger extension of long-distance cod trading since the eleventh century across northern Europe. In addition, aDNA (**Paper III** and **Paper IV**) and isotope ratios (**Paper IV**) revealed that northern Norway, and possibly Iceland, acted as population sources of Atlantic cod already before the actual expansion of cod trade on a broader international scale. By using mitogenomic (**Paper I**) and genome-wide data (**Paper V**), I found large effective population sizes of Atlantic cod across time, which have been greatly influenced by historical climatic events. Nonetheless, a decrease in heterozygosity levels in some Atlantic cod populations suggests an influence not only of climatic conditions but also of anthropogenic pressures. Lastly, the importance of climatic conditions in the long-term dynamics of Atlantic cod (**Paper II**) was supported by a distributional shift found during the late Holocene, showing the complexity behind the population dynamics in this species. Together, this thesis provides a long-term genomic perspective for Atlantic cod that could eventually have potential implications for sustainable fishing strategies and management measures towards the demographic recovery of this marine fish species.

Resumen

Las actividades pesqueras a gran escala y el aumento en las temperaturas del océano, representan un inmenso problema para los ecosistemas marinos; dentro de las consecuencias que estas actividades y condiciones climáticas pueden tener para la biodiversidad marina se incluyen, la pérdida de la diversidad genética y una disminución poblacional extensiva que puede conducir a la extinción de una especie. El bacalao (*Gadus morhua*), es una especie de pescado marino de gran importancia económica que se distribuye en la región del Océano Atlántico Norte y ha experimentado largos periodos de sobreexplotación; su pesca aumentó la riqueza de las comunidades costeras y promovió el crecimiento de los centros urbanos en toda Europa durante los últimos milenios. Durante siglos, la abundancia del bacalao era extraordinaria; sin embargo, los constantes cambios climáticos y su sobrepesca, lo llevaron a una disminución dramática y al colapso de varias de sus poblaciones a finales del siglo XX; a pesar de ello, no está clara la extensión ni la intensidad pesquera con que se capturaron las poblaciones de éste en el pasado; así como, las consecuencias que dichas pérdidas han provocado en la diversidad genómica y la demografía del mismo. En esta tesis, he utilizado un enfoque multidisciplinario, utilizando métodos de ADN antiguo (aDNA), e isótopos estables, para proporcionar conocimientos novedosos y esenciales acerca de la dinámica poblacional del bacalao a largo plazo, de esta manera, y considerando los diferentes escenarios de cambio climático, como de sobrepesca. Metodologías como el aDNA, pueden proporcionar información acerca de la historia demográfica y las fluctuaciones genéticas de especies severamente explotadas a lo largo del tiempo; mientras que los isótopos estables, pueden esclarecer el origen de dicha especie y sus patrones de movimiento. Por lo tanto, utilizando aDNA (**Paper III** and **Paper IV**), descubrí que estos colapsos dramáticos de la población de bacalao parecen haber sido precedidos por una extensión más amplia de su comercio a larga distancia, desde el siglo XI, en el norte de Europa. Con el uso de aDNA (**Paper III** and **Paper IV**) y valores isotópicos (**Paper IV**), encontré que es posible que el Norte de Noruega e Islandia, fueran las principales fuentes de bacalao, mucho antes de que la expansión del comercio de esta especie alcanzara una escala internacional. Asimismo, mediante el uso de datos mitogenómicos (**Paper I**) y del genoma nuclear (**Paper V**), encontré amplios tamaños poblacionales efectivos de bacalao a través del tiempo; los cuales, se han visto altamente influenciados a través de la historia por eventos climáticos. No obstante, una disminución en

los niveles de heterocigosidad en algunas poblaciones de bacalao, sugiere una influencia, no sólo de las condiciones climáticas, sino también de actividades pesqueras. Por último, la importancia de las condiciones climáticas en las dinámicas poblacionales del bacalao (**Paper II**), se ve respaldada por un cambio en la distribución encontrado durante el Holoceno tardío. En conclusión, esta tesis proporciona una perspectiva genómica a largo plazo para el bacalao, que eventualmente, podría tener implicaciones potenciales para las estrategias de pesca sostenible y para las medidas de gestión enfocadas en la recuperación demográfica de esta especie.

List of Papers

This thesis is composed of three published papers, and two manuscripts:

I. **Martínez-García L.**, Ferrari G., Oosting T., Ballantyne R., van der Jagt I., Ystgaard I., Harland J., Nicholson R., Hamilton-Dyer S., Baalsrud H.T., Brieuc M.S.O., Atmore L.M., Burns F., Schmölcke U., Jakobsen K.S., Jentoft S., Orton D., Hufthammer A.K., Barrett J.H., Star B. (2021). Historical demographic processes dominate genetic variation in ancient Atlantic cod mitogenomes. *Frontiers Ecology and Evolution* 9: doi: 10.3389/fevo.2021.671281

II. **Martínez-García L.,** Ferrari, G., Hufthammer A.K., Jakobsen K.S., Jentoft, S., Barrett J.H., Star B. (2022). Ancient DNA reveals a southern presence of the Northeast Arctic cod during the Holocene. *Biology Letters* 18: 20220021. doi:10.1098/rsbl.2022.0021

III. **Martínez-García L.,** Ferrari G., Cuevas A., Atmore L.M., López-Arias B., Culling M., Llorente-Rodríguez L., Morales-Muñiz A., Roselló-Izquierdo E., Quirós J.A., Marlasca-Martín R., Hänfling B., Hutchinson W.F., Jakobsen K.J., Jentoft S., Orton D., Star B., Barrett J.H. (2022). Ancient DNA evidence for the ecological globalization of cod fishing in medieval and post-medieval Europe. *Proceedings of the Royal Society B 289: 20221107*. doi.org/10.1098/rspb.2022.1107

IV. **Martínez-García L.*,** Cuevas A.*, Ferrari G. Hufthammer A.K., Vedeler M., Hirons A., Kneale C., Barrett J.H., Star B. (*manuscript*). Tracing 600 years of long-distance Atlantic cod trade in medieval and post-medieval Oslo using stable isotopes and ancient DNA.
*Shared first authorship

V. **Martínez-García L.,** Cuevas A., Ferrari G., Jakobsen K.S., Jentoft S., Blevis R., Harland J., Küchelmann H.C., Gotfredsen A.B., Hufthammer A.K., Vedeler M., Barrett J.H, Star B. (*manuscript*). Long-term population dynamics of Atlantic cod revealed by ancient DNA and genomics.

Little by little, one travels far.
J.R.R. Tolkien

Introduction

Background

Marine ecosystems provide ecological stability and mitigate climate change through the conservation of biodiversity and carbon sequestration (Roberts et al., 2017). Nonetheless, it is long known that increasing anthropogenic activities and climate change result in biodiversity loss and continuous fragmentation of marine habitats (Jackson et al., 2001). Human activities have altered the ecosystem composition by overexploiting marine resources (e.g., fish) and polluting coastal waters; while a changing climate is constantly altering the ecology, biology and chemistry of the ocean (Planque et al., 2010; Doney et al., 2012).

In the past, marine resources were thought to be inexhaustible (Caddy and Cochrane, 2001). Nevertheless, at present, several fish stocks have collapsed or have been severely reduced after years and years of overexploitation (Jackson et al., 2001). Failing to acknowledge the effect of such dramatic depletions can lead to a total loss of economically important marine species (e.g., large or local scale population extinction), and it can lead to a reduction of genetic diversity and/or limited connectivity between populations (De Kort et al., 2021).

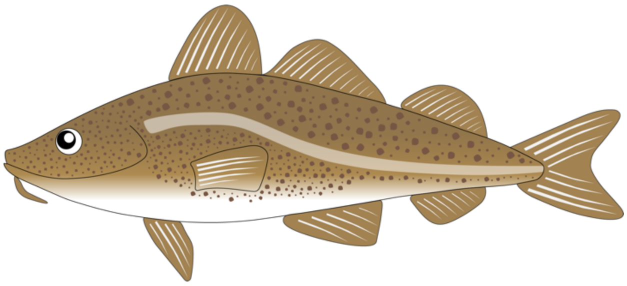
Through the combined use of archaeological, ecological and evolutionary approaches, one can examine altered species distributions, changes in demographic scenarios, trophic transitions over a temporal scale and the adaptive potential of a species to overcome eventual habitat or climate disturbances. Such knowledge, can be obtained to plan optimal sustainable management of marine resources (Hughes et al., 2005). Nevertheless, evolutionary and ecological consequences of historical fisheries and current climate change on marine ecosystems are still largely unknown.

Thus, the aim of this thesis is to provide a long-term genomic perspective of Atlantic cod and interpret this in light of commercial fisheries and climate change. Atlantic cod (*Gadus morhua*) is a marine fish of great economic and ecological importance. It is, therefore, a prime example of a species that has experienced heavy exploitation. In the first part of this thesis, I briefly present the background of the research project including the biological, ecological, and economic history of Atlantic cod where I introduce a general overview of the approaches to study evolutionary changes on a spatial and temporal scale. In the second part of this thesis, I describe in detail the research questions I address in this work. Further on, I expand on the theory (methodologies) of the research projects, followed by the main results and a general discussion addressing the new insights obtained on the current status of Atlantic cod.

Atlantic cod: biology and distribution

Atlantic cod has long been one of the most widely studied marine fish species and is characterized by a long history of fishing exploitation (Drinkwater, 2005). It is a key component in the marine ecosystem by acting as an apex predator across its entire distributional range, extending through North America, the continental shelves of Greenland, Iceland and the Faroe Islands, and across northern Europe as far as Svalbard and the White Sea (Mieszkowska et al., 2009; Lait, 2016; Lait et al., 2018).

Atlantic cod is the largest fish in the Gadidae family with a weight of up to ~100 kilograms in mass and length of up to ~2 meters (O'Brien et al., 1993; Mieszkowska et al., 2009). This fish keeps growing throughout its lifespan which can be more than ~20 years (NOAA, 2022), and feeds on any type of abundant and accessible prey (e.g., capelin (*Mallotus villosus*), herring (*Clupea harengus*), crustaceans, zooplankton) (Smith et al., 2007). Atlantic cod are usually found in a wide variety of depths (shallow to ~500-800 meters) and temperatures (between ~-1°C to ~21°C; Mieszkowska et al., 2009). This fish can reach maturity between ~2 to 9 years old, and females tend to release a large number of planktonic eggs (1-10 million) during spawning season (Hjort, 1914; Jørgensen, 1990; Thorsen et al., 2010).



Atlantic cod populations are generally characterized by large effective population sizes (*Ne*) and high levels of gene flow (Hemmer-Hansen et al., 2013b). Therefore, we could expect to find low genetic structuring between its populations. Nevertheless, there are large- and local-scale variations in their genetic differentiation and distribution. For instance, genetic differentiation (even if limited) between marine regions (e.g., the North Sea and the Baltic Sea) has been associated with temperature and salinity clines as well as with migratory behaviours (Poulsen et al., 2006; Hemmer-Hansen et al., 2013a; Berg et al., 2015; Fairweather et al., 2018). In particular, previous studies have reported ecological ecotypes (i.e., within the western Atlantic, Norway, and Iceland) that undertake different migrating distances from wintering grounds to inshore feeding grounds (Pampoulie et al., 2008; Mieszkowska et al., 2009; Hemmer-Hansen et al., 2013a; Berg et al., 2017; Sinclair-Waters et al., 2018). Stationary ecotypes will spawn along coastal areas and are generally characterised by sedentary behaviour with a limited migration. Migratory ecotypes, on the other hand, will migrate every year during the winter-spring months from their feeding grounds toward coastal regions.

Present day population structure of Atlantic cod (and of other species) can be potentially driven by historical and contemporaneous factors and/or processes like environmental clines (e.g., temperature, salinity) (Stanley et al., 2018), physical barriers (e.g., oceanic distances) (Berg et al., 2016; Sodeland et al., 2016; Barth et al., 2017), and climatic (e.g., last glacial maximum, Little Ice Age) (Lait et al., 2018) or geological events (e.g., development of the Baltic Sea) (Ojaveer et al., 2010; Wenne et al., 2020). The consequences of historical pressures under a changing climate can therefore offer significant knowledge on present Atlantic cod dynamics.

Climate change: a threat to Atlantic cod and the marine ecosystem

Fluctuations in oxygen levels and an increase in the sea temperature or ocean acidification are examples of a changing climate that can have direct and indirect consequences for marine ecosystems (Doney et al., 2012). For example, ecosystems at high latitudes in the Arctic Sea can be threaten by ocean acidification as a result of the melting sea-ice and high primary productivity (Frommel et al., 2012). Temperature oscillations across the North Atlantic Ocean promote a poleward movement of species (e.g., primary and secondary producers), life-history variations, and re-structuring of communities (Hastings et al., 2020).

Climate needs for Atlantic cod and other marine species can differ according to the age and the local distribution of individuals. Climate patterns, for example, can have different seasonal or multidecadal patterns that potentially influence the distribution and development of fish (Brander, 2019). For instance, increasing salinity levels in the Baltic Sea correlate with a rise in

Atlantic cod abundance since low levels of salinity threaten the physiological status of this fish (MacKenzie et al., 2007). Likewise, increasing levels of CO_2 can have drastic consequences on the Atlantic cod larval survival by causing tissue damage on internal organs (Frommel et al., 2012). Previous studies have shown that the influence of temperature in the life history of Atlantic cod is of great importance (Sandø et al., 2020). A broad range of acceptable temperature thresholds of this species –with an optimum temperature for growth between 11°C and 16°C– may buffer the short-term effects of climate change. Nevertheless, temperature fluctuations (e.g., late twentieth to early twenty-first century) can have a negative impact on the locomotory performance, metabolic activity, and physiological characteristics of Atlantic cod, and additionally impacts the phenology of basal ecosystem components like phytoplankton and zooplankton (Lilly et al., 2013), which together, influence the distribution of this and other marine fish species (Mieszkowska et al., 2009; Pinsky et al., 2013; Freitas et al., 2015).

Over time, a changing climate is unavoidable. Thus, understanding the impact of climate change on the abundance of Atlantic cod throughout its distributional range is important. At the same time, increasing concern has been given to continuous anthropogenic activities, such as fisheries, which have grown in magnitude over the last millennia (Figure 1). Is it possible for a species to surmount the negative consequences driven by simultaneous climate change and human overexploitation? Indeed, intense fishing pressures have threatened Atlantic cod abundances (Opdal, 2010). However, a combination of climate changes and overfishing are threating the marine ecosystems in a probable irreversible direction.

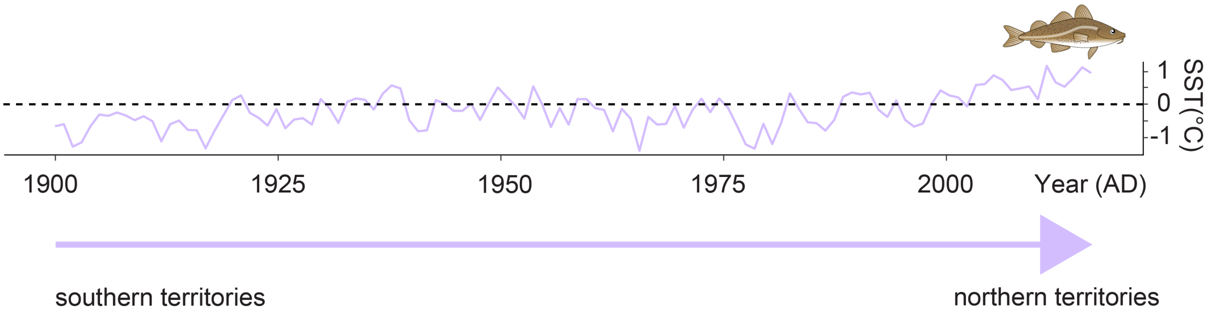
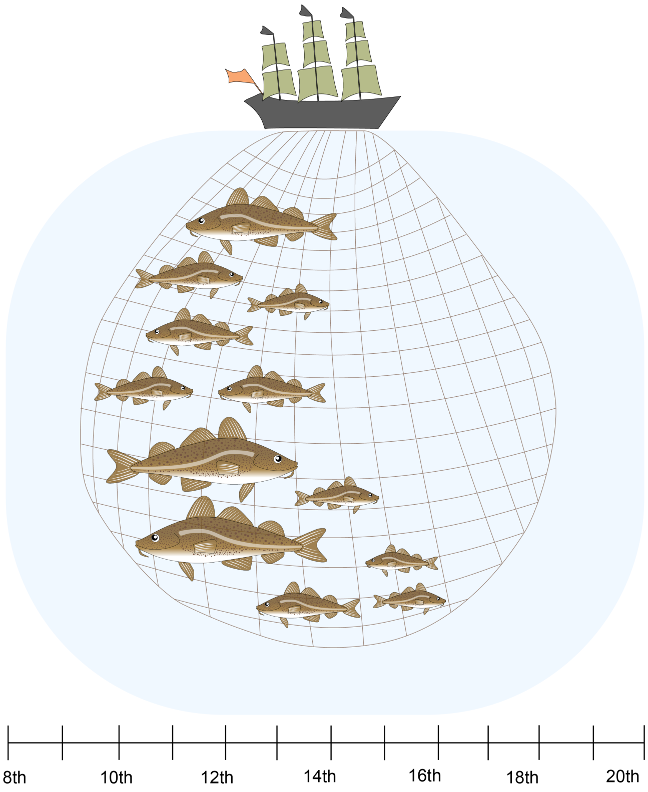


Figure 1. Increasing anomaly sea surface temperatures (SST) during the last twentieth and early twentieth first century at the Russian Kola section (see **Paper II**). Anomalies are calculated with respect to the long-term 1981-2010 average. Temperature estimates at the Russian Kola

section are used as a suitable proxy for temperature across the entire (Norwegian) spawning region of Atlantic cod (Sundby and Nakken, 2008). Kola SST series were obtained from Bochkov (2005) and the Polar Research Institute of Marine Fisheries and Oceanography (PINRO) website (http://www.pinro.ru).

Cod fisheries: a fish that made history

Fisheries have played an important role in the development of coastal communities and afterwards of urban centres. In particular, marine fish (e.g., Atlantic cod, ling (*Molva molva*), herring, flounder (Pleuronectoidei), haddock (*Melanogrammus aeglefinus*)) have been locally consumed since the Stone Age across different places like Denmark, northern of Scotland and the eastern Baltic (Enghoff, 1994; Barrett et al., 1999; Enghoff, 1999; Orton et al., 2011). Only until *ca.* 1050 CE long-distance fishing activities started to extend across central Europe using Norwegian *stockfish* as one of the main trading merchandises (Barrett and Orton, 2016; Star et al., 2017).



Stockfish would generally be air-dried Atlantic cod produced in the cold territories of the Lofoten and Vesterålen islands in Norway, where almost freezing temperatures and strong winds would be common for months at a time (Perdikaris, 1999). Some of the earliest archaeological evidence of *stockfish* production, directed to a local consumption, goes back to the early Iron Age (Perdikaris, 1996; 1998). Large-scale production of *stockfish* expanded through central Europe by the thirteenth to fourteenth century, when the Hanseatic League monopolised the distribution of *stockfish*, from northern Norway to central Europe (Barrett et al., 2011; Orton et al., 2014). By the late fifteenth to the early sixteenth century, the arrival to Newfoundland changed completely the direction and development of cod-fisheries, where salt was the key for successful fish-preservation during long-distance journeys (Pope, 2003; Holm et al., 2019). Vessels would sail across the Atlantic from France (Normandy and Brittany), the Basque country, Portugal, and later on, from England to pursue a transatlantic cod-trade. For instance, the French would mostly fish in the south coast of the Newfoundland Island and the Strait of Belle Isle, the Portuguese mainly fished on the southern shore of the Avalon Peninsula, and Basque fishermen would acquire fish by *ca.* 1520 from southern (i.e., Placentia Bay) to western Newfoundland (i.e., Gulf of St. Lawrence) (Pope, 2008; Candow, 2009). The earliest known English fish-import from Newfoundland to Europe (i.e., Bristol) was in 1502 (Pope, 2004), however, they only participated in the Newfoundland's fisheries until *ca.* 1570s (Candow, 2009).

By the eighteenth to the nineteenth century, Newfoundland already had extensive (and continuous) exportation of Atlantic cod to Europe (Lear, 1998). Improved fishing technology (e.g., longlines, trawling) in the late nineteenth to the early twentieth century provided even more efficient fishing and greater landings (Lear, 1998; Thurstan et al., 2010). Therefore, by the end of the twentieth century, high landings of Atlantic cod were caught across the North Atlantic (i.e., 1.9 million tons in 1968 in western Atlantic, a mean of 850 thousand tons in 1950 in the Northeast Arctic and more than 300 thousand in the North Sea after 1970), only to be followed by severe catch reductions (Hislop, 1996; Lear, 1998; Aglen et al., 2004). For instance, landings in the Northeast Arctic registered *ca.* 350 thousand tons in 1980 (Aglen et al., 2004), while the North Sea and Canada landings declined to *ca.* 100 thousand tons around the same year (Daan et al., 1994; Hutchings and Myers, 1994).

Consequently, Atlantic cod was listed as a vulnerable species in the IUCN red list since 1996 (Sobel, 1996), leading to specific conservation planning for each geographical region (see Box 1). These sustainable measures resulted in an increase of Atlantic cod abundance in some areas (e.g., Northeast Arctic; Hylen et al., 2008; Rose et al., 2019). However, some depletions have not been overcome (e.g., Canadian stocks) resulting in the long absence of Atlantic cod across an extensive distributional area for more than 60 years (Castañeda et al., 2020). There is no doubt that Atlantic cod populations across the North Atlantic and adjacent seas have been intensively fished (Hutchings, 2000; Caddy and Cochrane, 2001). Nonetheless, knowledge of the actual consequences of past harvesting events on the genomic diversity of the species remains uncertain.

Box1. Preserving the legacy of Atlantic cod

The long history of overfishing that characterizes Atlantic cod, requires strong sustainable plans and efforts to recover numbers and production levels that once endured all cod stocks.

There are several organizations that provide fisheries advice-evaluation for a sustainable management of cod stocks (and other economically important marine species). Continuous monitoring in the western North Atlantic region is accomplished by the Northwest Atlantic Fisheries Organization (NAFO) and the Committee of the Status of Endangered Wildlife in Canada (COSEWIC). On the other hand, the International Council for Exploration of the Sea (ICES), the Northeast Atlantic Fisheries Commission (NEAFC), the General Fisheries Commission for the Mediterranean (GFCM) and the Scientific, Technical and Economic Committee for Fisheries (STECF) are examples of organizations in charge of the eastern North Atlantic region.

According to the ICES, there are 14 subareas for protection with specific divisions and subdivisions. These areas are considered as “one” during management planning. Such specific management plans rely on the evaluation of the recruitment and fish mortality of each location, providing a threshold until which a species can be taken (i.e., total allowable catch (TAC)). Regulations based on the spawning-stock biomass (SSB) and the maximum sustainable yield are key to ensure a production increase and avoid unsustainable fisheries (Aglen et al., 2004).

Considering that a stock is a portion of one fish population with specific characteristics within the same reproductive process (Booke, 1999), knowledge of the differences on a large and local scale is of great importance. Genetic inference, therefore offers an opportunity to select optimal and sustainable fish stock units.

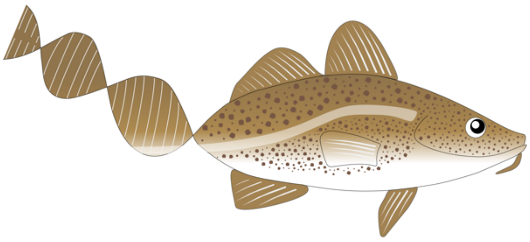
Evolutionary and ecological changes in the ocean: a fish perspective

Within a molecular world: excavating for ancient DNA

Recent developments in molecular methods allow for an accurate assessment of diversity, speciation, adaptation, and functionality (Ahmad et al., 2021). Therefore, genetics, the study of gene heredity and species variation, are a key tool to provide novel and relevant insights in different scientific fields, including those where the role of natural and anthropogenic pressures may untangle increasing concerns.

In particular, recent developments in ancient DNA (aDNA; see Box 2) can estimate long-term fluctuations in population diversity, sizes, and distributions (Welch et al., 2012; Thomas et al., 2019), describe changes in the selection of suitable habitats for different species under a changing climate (Dalén et al., 2007; Foote et al., 2013), and determine the impact of management strategies, especially when diverse anthropogenic scenarios are present (Hansen, 2002; Pinsky et al., 2021). Recently, aDNA has also been used to investigate the influence of climate events on the extinction of different species (e.g., *Thylacinus cynocephalus*) (White et al., 2018), and has successfully revealed the dramatic declines of wildlife biodiversity driven by the arrival of human colonizers (Seersholm et al., 2018). For this reason, aDNA methods are a powerful interdisciplinary tool that provides a unique opportunity to determine the role of fishing expansions and climate events on the decline of Atlantic cod populations.

Atlantic cod has been the subject of several (modern) genetic studies. This species is characterised by a large- and local-scale population structure (Jónsdóttir et al., 2003; Nielsen et al., 2005; Skarstein et al., 2007; Hemmer-Hansen et al., 2013a; Jørgensen et al., 2018; Johansen et al., 2020), and a signature of ecological adaptation in several locations (Jorde et al., 2007; Sodeland et al., 2016; Clucas et al., 2019; Wenne et al., 2020). From a historical perspective, a limited number of studies have contributed to the temporal evolutionary history of Atlantic cod across its distributional range providing examples of increased genetic drift, population replacement (Hutchinson et al., 2003), demographic



stability, complex (regional) population structure (Poulsen et al., 2006; Therkildsen et al., 2013b), loss of haplotypic variation (Ólafsdóttir et al., 2014) and recent population collapses (Bonanomi et al., 2015). More recently, aDNA has been successfully used to elucidate the –large scale– origin of Atlantic cod bones distributed in different areas (Hutchinson et al., 2015; Star et al., 2017; Conrad et al., 2021) and to describe the genomic changes including different fishing scenarios (Pinsky et al., 2021). Although these studies provide some baselines to understand the genomic consequences of historical events on Atlantic cod, detailed studies considering the long exploitation history of the species and the different climate events over longer periods –over 1500 years– have not yet been performed.

Box2. A quick dip into the aDNA world

Ancient DNA (aDNA) can be defined as plant or animal DNA from old (> 100 years) biological material (Leonard, 2008; Oosting et al., 2019), which is usually obtained from zooarchaeological, historical and/or paleontological remains (Leonard, 2008). One of the main characteristics of aDNA is that as it has long been deteriorated, it is highly prone to contamination and post-mortem deamination (i.e., C > T and G > A substitutions) which may complicate its down-stream analyses (Orlando and Cooper, 2014; Hofman et al., 2015). Nonetheless, a constant development of high-throughput methods allows a confident analysis of poor-quality bone remains and low-coverage sequence data (e.g., Ferrari et al., 2021).

An isotopic world: the revelation of life processes

Stable isotopes can elucidate the origin and/or movement patterns of different species by acting as natural tags that distinguish between recent migrants or long-term settlers (Peterson and Fry, 1987). There are several isotopes present in nature, which will differ in the number of neutrons when compared to other isotopes of the same element. The isotopic signature is generally expressed as delta (δ), which represents the variation between isotope ratios (Wiederhold, 2015). In particular, stable isotope analysis can untangle food webs by assigning species to certain trophic levels (Estrada et al., 2003; Roeder and Kaspari, 2017). They can also describe habitat characteristics and phenotype variations of species (Zbinden et al., 2011), identify migratory behaviours (Guiry et al., 2020), and reconstruct their diet preferences (Hückstädt et al., 2007; Alter et al., 2012; Braje et al., 2017; Conrad et al., 2018; Peters et al., 2020). Therefore, stable isotopes are a powerful tool in understanding the ecology, demography, evolution, and conservation of different species (Boecklen et al., 2011), including those in the marine and freshwater ecosystems.

The life-history strategies of Atlantic cod (i.e., spawning distribution) are indirectly (and sometimes directly) shaped by climate variables (i.e., spawning distributions) (Drinkwater, 2005; Martínez-García et al., 2022b). Thus, several stable isotope studies have used this fish species as a study model to untangle historical fishing activities, including trading routes (Barrett et al., 2011; Orton et al., 2014; Hutchinson et al., 2015), or diet reconstructions (Ólafsdóttir et al., 2021). Furthermore, stable isotope evidence has provided descriptions of the location and time of expansion of cod-fishing activities (Hufthammer et al., 2010; Orton et al., 2011; Ritchie et al., 2013; Glykou et al., 2021), distributional shifts caused by temperature fluctuations (Jones and Campana, 2009; Edvardsson et al., 2019), and alterations of trophic states after intense fisheries (Ólafsdóttir et al., 2021).

Overall, a combined approach using aDNA and stable isotope analysis is a powerful tool to infer both (or either) human and climate-driven historical patterns in Atlantic cod.

Aims of the thesis

The primary aim of this thesis is to provide a long-term genomic perspective of Atlantic cod and interpret the role that fisheries and climate change may have had on the evolution, demography, and ecology of Atlantic cod. The particular research questions I address in this thesis are:

Can we find temporal changes in the genomic structure of Atlantic cod that can be related to fisheries or climate change? (**Paper I and V**). Here, I investigated temporal patterns of mitogenome and genome-wide genetic variation and differentiation in Atlantic cod populations. Comparisons between ancient, (historical), and modern specimens allow the identification of temporal differences and their evolutionary, ecological or demographic role in the history of the species.

Has the distributional range of Atlantic cod ecotypes shifted over time? (**Paper II**). Here, I identified genomic regions (i.e., chromosomal inversions) that differ between populations and can be correlated with specific environmental variables to described the historical presence/absence of Atlantic cod ecotypes during climate conditions different to the present-day ones.

Can aDNA and/or stable isotope methods provide new evidence for the understanding of the globalization of marine fisheries? Can we genetically identify the targeted Atlantic cod populations by intensive fishing activities during the earlier described expansions of cod-fisheries? (**Paper III and IV**). Here, I used aDNA methods and stable isotope analysis to investigate the extension of European trading routes from early medieval to the post-medieval centuries and indirectly highlight the patterns of the exploitation of Atlantic cod.

The details on methodological approaches for each paper are described below.

Main methodological approaches

To answer the particular research questions in this thesis, I used a combination of DNA methods (aDNA and genomics) and stable isotope analysis. In **Paper I** and **Paper V**, I compared ancient, historical, and modern specimens to provide a long-term genomic perspective of Atlantic cod populations and interpret this in light of commercial fisheries and climate change. Specifically, in **Paper I** I compared 48 ancient (dated up to 6,500 BCE) and 496 modern Atlantic cod samples. Here, analyses were based on mitochondrial DNA (mtDNA), a haploid and maternally-inherited DNA with a smaller effective population size (*Ne*) in comparison to nuclear genomes. Although this is a single locus, mtDNA is often more easily obtained from ancient specimens compared to nuclear whole genome data, which makes it a good tool to observe consequences of, for example, bottlenecks or other events that may threaten to reduce a population (Marko and Hart, 2012). Ancient mtDNA has previously been used successfully to describe the long-term genomic consequences (if any) of human activities (i.e., hunting; Keighley et al., 2019) and climate change (Louis et al., 2020) in other species that have been heavily exploited or are sensitive to climate changes (sea ice loss).

Expanding on the mitogenomic data, in **Paper V**, I compared 33 ancient (dated up to *ca*. 700 CE), 22 historical (dated up to *ca.* 1900 CE) and 179 modern Atlantic cod specimens using nuclear whole-genome sequencing data (WGS) data. Using WGS allows the examination of a greater number of loci –neutral and under selection– and increases the power and accuracy when estimating genetic divergence among populations (Allendorf et al., 2010). In both, **Paper I** and **Paper V**, I reconstructed the *Ne* of Atlantic cod populations to determine the demographic fluctuations that Atlantic cod populations have experienced across time. *Ne* calculates the rate of evolutionary change caused after a genetic drift event(s) which makes it an ideal concept for evaluating the current genomic potential of a (marine) species (Nadachowska-Brzyska et al., 2022). The use of *Ne* has been increasing and it has proven to be useful to identify the interaction between genetic loss and human pressures (Robin et al., 2022), and even the reduction in the efficacy of selection on specific genomic variation (i.e., marine-freshwater adaptation; Kirch et al., 2021).

In **Paper II** and **Paper III,** we determined the biological origin of ancient Atlantic cod specimens using nuclear whole-genome data. Specifically, in **Paper III**, I assigned 37 Atlantic cod specimens (*ca.* 1050 to 1950 CE) to a specific population in the eastern or western Atlantic Ocean, describing the extension of the globalization of marine fisheries across medieval and postmedieval Europe. In **Paper II**, I expanded our knowledge about the historical distribution of Atlantic cod, specifically of the *skrei* or migratory Norwegian ecotype: the Northeast Arctic cod (NEAC). This ecotype, in contrast to the Norwegian Coastal cod (NCC) who performs relatively short migrations within the Norwegian coast, is characterized by long distance migrations from colder feeding grounds in the Barents Sea to spawning grounds along the Norwegian coast (Finnmark, Troms, Lofoten and Møre; Sundby and Nakken, 2008). In this study, I used five ancient specimens of Atlantic cod ^{14}C dated to *ca.* 4322–2092 years BP to describe the extension of the Northeast Arctic cod spawning grounds during the late Holocene (*ca.* 5900 years BP to present). Analysis in both **Paper II** and **Paper III**, relied on the identification of the individual haplotypes of the four large chromosomal inversions characteristic of Atlantic cod (e.g., LG1, LG02, LG07 and LG12). Chromosomal inversions tend to play an important role on the (local) adaptation and speciation of a species as their distribution and expression variates across environmental gradients (Faria et al., 2019). The four chromosomal inversions of Atlantic cod have been associated with migratory behaviour (LG1) and temperature, salinity and oxygen level preferences (LG2, LG7, LG12) (Bradbury et al., 2010; Berg et al., 2015; Berg et al., 2016; Sodeland et al., 2016). These inversions provide elevated levels of divergence between populations across the North Atlantic Ocean (Berg et al., 2016), therefore, their individual haplotypes provide an opportunity to determine the biological origin of Atlantic cod specimens in specific parts of its range.

In **Paper IV**, I used a similar approach than **Paper II** and **Paper III** while investigating the biological source of Atlantic cod specimens excavated during medieval and post-medieval periods in Oslo, Norway. In addition, we used stable isotope analysis (δ^2H, δ^{15}N, δ^{13}C, and δ^{34}S) to investigate the emergence and extension of Norwegian fisheries through *ca.* 600 years of history (early eleventh to seventeenth centuries). As previously described, stable isotopes can be used in ecological and/or environmental assessment analyses. Nitrogen (δ^{15}N) provides estimates of the trophic position of a species, while carbon (δ^{13}C) offers a great insight in diet components (i.e., flow of organic matter to an individual through food webs) as it is greatly affected by climate variables such as lower temperatures or sea-ice coverage (Post, 2002; Edvardsson et al., 2019). Hydrogen (δ^2H) shows signatures of trophic levels through geographic climate variations (e.g., on precipitation and/or latitude/longitude) (Britzke et al., 2012), while sulphur ($\delta^{34}S$) gives information about salinity clines (Fry and Chumchal, 2011). Therefore, all of these isotope ratios will reflect the signature of the nutrients available and the properties of the ecosystem where a species has constantly feed and/or lived. This particular methodological approach provides essential information to reconstruct the extension of fisheries from and to medieval Oslo.

Note on the supplementary information

Supplementary information consisting of tables and raw analyses are not included in this thesis because of their large size. The online access to these supplementary materials is given at the end of each study.

Main results and Discussion

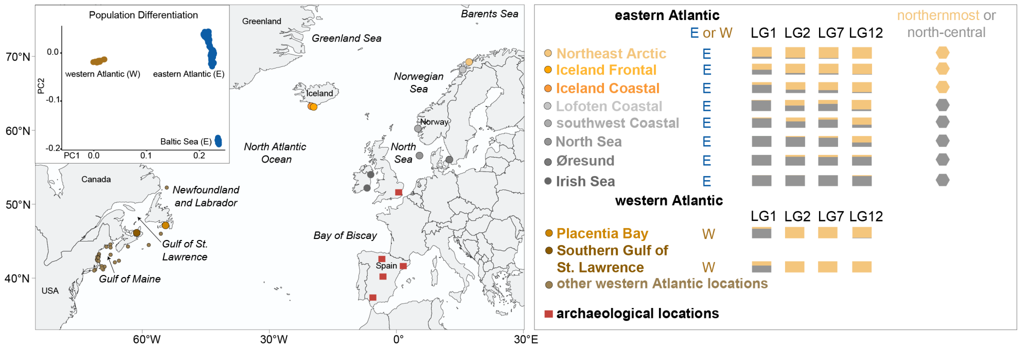
In light of the current ocean warming and the increasing activities disrupting the dynamics of natural ecosystems, the results of this thesis shed interesting findings on the current status of Atlantic cod populations. The discussion is divided in three sections: “*The archaeological past”* covers **Paper III** and **Paper IV** and highlights the extension and possible (present) consequences of cod-fisheries during the medieval and postmedieval periods. “*The genomics in the past*” covers **Paper I** and **Paper V** and focusses in the possible impacts of fisheries on the genomic diversity of Atlantic cod. Lastly, “*The climate in the past*” covers **Paper I**, **Paper II** and **Paper V** and emphasises the ecological conditions and interactions that may drive population changes.

The archaeological past

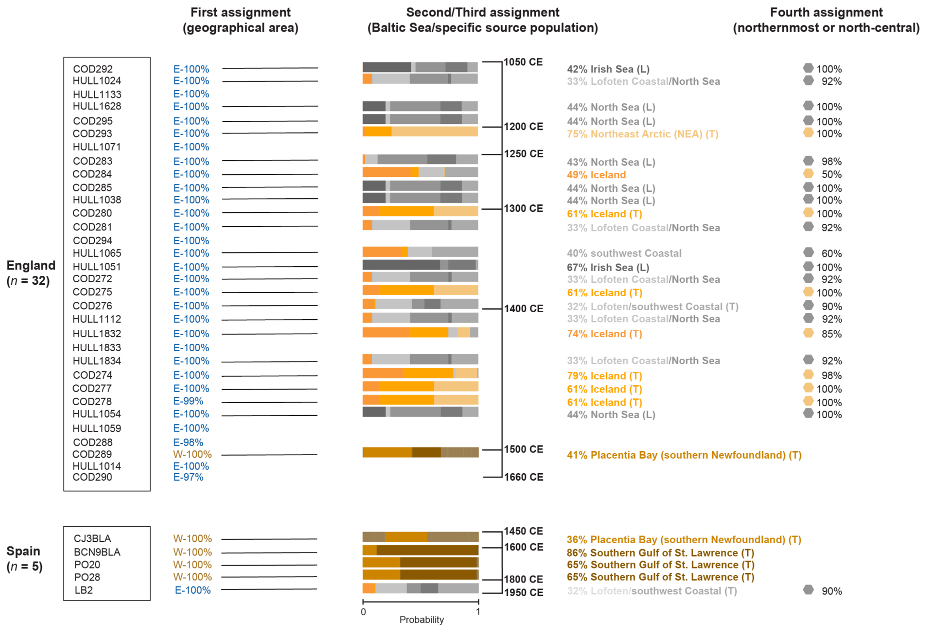
Anecdotal and historical sources mention the extensive abundance of Atlantic cod throughout history (Kurlansky, 1999). According to the findings in **Paper III** and **IV**, this fish (prepared as *stockfish* after the Northeast Arctic cod) was the preferred target of fishing activities for long-distance already by the eleventh century (Figure 2). Such early examples of cod-trading are consistent with previous trading activities in Haithabu as early as *ca.* 1066 CE (Star et al., 2017). Of note, most *stockfish* shipments would be brought from northern Norway to central Europe (i.e., London, see **Paper III**) and sporadically during the fourteenth to fifteenth century, from Iceland (Hoffmann, 2001; McGhee, 2003; Perdikaris and McGovern, 2008). The results in **Paper III and IV** highlight an earlier Icelandic exportation of cod-fish towards London and Oslo, which suggests a more important fish-resource area than we have previously thought. This observation is reflected in the isotopic data also included in **Paper IV**, where different isotope ratios (i.e., carbon ($\delta^{13}C$)) support a diverse origin (probably from the Northeast Arctic or Iceland) of Atlantic cod (Figure 3).

Earlier fishing activities in Iceland and the known early fisheries in northern Norway (Lofoten and Vesterålen) may translate in an earlier “disturbance” of the natural ecosystem. According to Holm et al. (2021), periods of accelerated marine extractions (i.e., 1540-1620 CE and 1750-1790 CE) may entangle more negative consequences in the population dynamics of marine species than we know. Was *stockfish* traded to every town in Europe consistently in time and quantity? Results in **Paper IV** are contrasting to those obtained in **Paper III** as Oslo showed a continuous, not sporadic (as in London), presence of long-distance trade since the eleventh century (Figure 2). Furthermore, the results in **Paper IV** indicate that significant changes of isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$) across time could denote changes in the selected fishing grounds from where Atlantic cod was first obtained. Such changes are simultaneous to the transition from local to long-distance fishing activities between mid-twelfth to late fourteenth centuries (Barrett et al., 2011; Orton et al., 2014; Martínez-García et al., 2022a). Cod-fisheries may have had a slowly increase but it was a constant growth (see **Paper III** and **IV**). Future research should include (and extend in number) archaeological material across time from other European towns and (if possible) from the western Atlantic. A limitation specifically in **Paper III** is the number of specimens from earlier periods. One is left wondering if the local-trading pattern would be stronger with a larger number of samples. With that in mind, I am sure that interesting historical patterns are yet to be discovered. For instance, would fish-remains have the same isotopic signatures coming from sites that are known to have had local fishing activities (i.e., Quoygrew, Scotland) across time? Can we expect any changes in the trophic levels (e.g., a possible reduction of primary producers) during periods of accelerated marine extractions? Modern isotope data from the same (archaeological) sites as the ancient samples would allow for a temporal comparison, offering an opportunity to identify biological changes across the trophic networks.

a) **Modern inversion frequencies**



b) **English and Spanish medieval and postmedieval trading**



c) **Medieval Oslo trading**

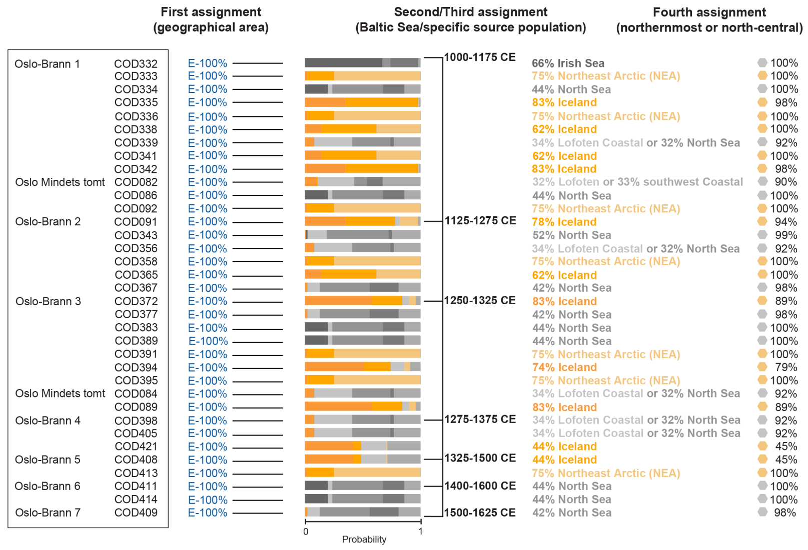
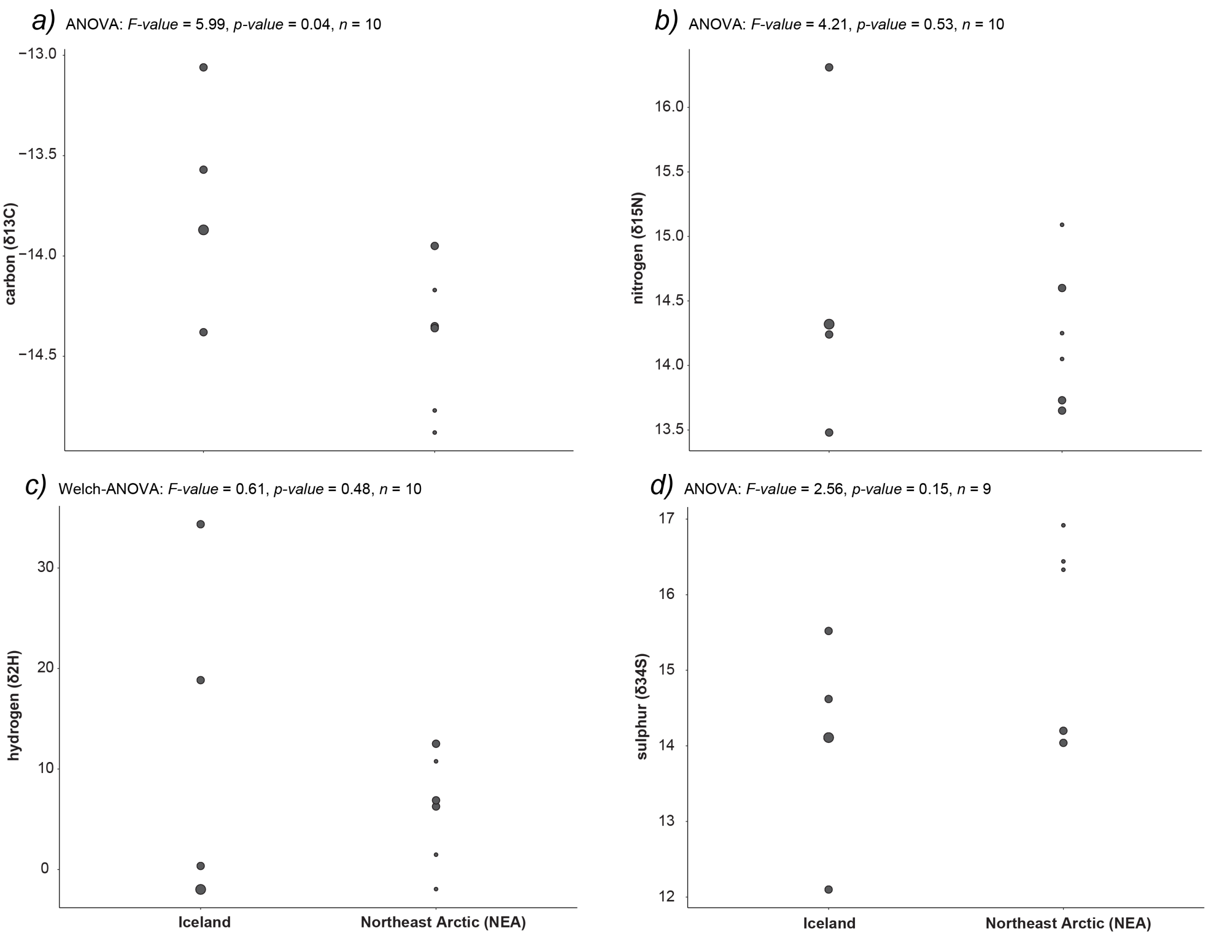


Figure 2. Genomic assignments of archaeological Atlantic cod samples used in **Paper III** and **Paper IV**. ***(a)*** Geographical distribution of inversion frequencies of chromosomal inversions in Atlantic cod (LG1, LG2, LG7 and LG12) across the North Atlantic Ocean. ***(b)*** Genomic assignments for London and Spain (see **Paper III** for specific details). A local (north-central distribution in grey) is mainly observed until the thirteenth century. Thereafter, a trading signature (northernmost distribution in orange) increased. ***(c)*** Genomic assignments for medieval Oslo (see **Paper IV** for specific details). A trading signature (northernmost distribution in orange) can be appreciated throughout the eleventh to the sixteenth to seventeenth centuries.



a) ANOVA: *F-value* = 5.99, *p-value* = 0.04, *n* = 10

b) ANOVA: *F-value* = 4.21, *p-value* = 0.53, *n* = 10

c) Welch-ANOVA: *F-value* = 0.61, *p-value* = 0.48, *n* = 10

d) ANOVA: *F-value* = 2.56, *p-value* = 0.15, *n* = 9

Figure 3. Differences in the isotopic signatures between specimens with a putatively Icelandic or Northeast Arctic (NEA) origin: ***(a)*** carbon ($\delta^{13}C$; significant interaction), ***(b)*** nitrogen ($\delta^{15}N$), ***(c)*** non-exchangeable hydrogen ($\delta^{15}N$) and ***(d)*** sulphur ($\delta^{34}S$). Only specimens with a 70% or higher probability of being assigned to either origin were used (see **Paper IV** for specific details).

The genomics in the past

Paper I (using whole mitogenomes) and **Paper V** (using nuclear whole genome data) are the first studies to ever provide mitogenome and whole genome analyses for archaeological marine fish up to 6500 BCE and *ca.* 700 CE, respectively. Both studies found that archaeological specimens of Atlantic cod have a consistent grouping to their presumed modern origin (geographical region or population), which revealed, in agreement with previous studies, the well-known strong Trans-Atlantic differentiation, and the significant population differences between the eastern Atlantic populations and the Eastern Baltic Sea (Jørgensen et al., 2018; Barth et al., 2019). Such differences highlight that some Atlantic cod populations have evolved differently from one to another as a result of either isolation, or adaptive traits (Berg et al., 2016; Wenne et al., 2020). Considering that the ecological and oceanographic conditions can have such a strong effect on some populations, can we expect local differences between the consequences that fisheries could have on Atlantic cod populations?

Although severe reductions in population size have been reported for Atlantic cod across the North Atlantic Ocean (Daan et al., 1994; Hutchings and Myers, 1994; Hislop, 1996; Lear, 1998; Aglen et al., 2004), few genomic losses have been previously reported. For instance, Therkildsen et al. (2010) found a large *Ne* and no genomic changes over 80 years –using microsatellites– in the Atlantic cod population from the Southern Gulf of St. Lawrence, Canada. This specific population is known historically (Candow, 2009; Fitzhugh et al., 2011), and genomically (see **Paper III**), to have been a source of Atlantic cod since the early sixteenth century to different locations in the Eastern Atlantic (i.e., Spain; see **Paper III**). On the other hand, Ólafsdóttir et al. (2014) found loss of haplotypic variation between the fifteenth to the sixteenth centuries –using CytB sequence data– in an Icelandic population. As previously mentioned, Iceland has been an important provider of *stockfish* since possibly the eleventh century (see **Paper IV**). Results in **Paper I** and **Paper V** describe high estimated population sizes (*Ne*) of Atlantic cod in agreement with previous studies (Hardie et al., 2006; Therkildsen et al., 2010; Pinsky et al., 2021). However, in **Paper I**, there was no evidence of mitogenomic loss across time. In fact, high levels of mitogenomic variation in Atlantic cod were confirmed as expected from a widely distributed fish species with extensive gene flow between regions (Barth et al., 2017; Figure 4). Nevertheless, in **Paper V**, results revealed a significant decline in the individual heterozygosity (*He*) of Atlantic cod specimens between the 800-1250 CE to the *ca.* ~1900 CE (Figure 5). Such decline in *He* was found in the Northeast Arctic cod population and, although I can only speculate about the actual causes of a *He* decline in this population, it may be related to an increase in fishing pressures. This genomic decline coincides with earlier fishing activities in northern Norway and the development of long-distance fisheries (see **Paper III** and **IV**). It is feasible to assume then that such early fishing events presented in **Paper III** and **Paper IV** could have a larger impact in the natural stocks of Atlantic cod, the Northeast Arctic in particular, than what it is currently known.

The loss of genetic diversity due to population bottlenecks can take a significant time to lead to any genomic consequence considering that the *He* retained is higher than the *He* lost after a major disturbance in a population or ecosystem (Kuussaari et al., 2009; Welch et al., 2012). Therefore, loss of genetic diversity can be a long-term process, especially if the species has shown high resilience to a changing climate, as it's the case of Atlantic cod (Therkildsen et al., 2010; Therkildsen et al., 2013a). Future research should include temporal analyses of populations like Canada and the eastern Baltic Sea, could we expect similar results to the Northeast Arctic population? Or constant levels of *He* may arise? In addition, it would be an interesting follow-up to this thesis to include a through description of the outliers (F_{ST}; population differentiation) driving differences within populations (i.e., Northeast Arctic), and a gene ontology set enrichment analysis (GSEA) to associate biological and common gene functions to such outliers.

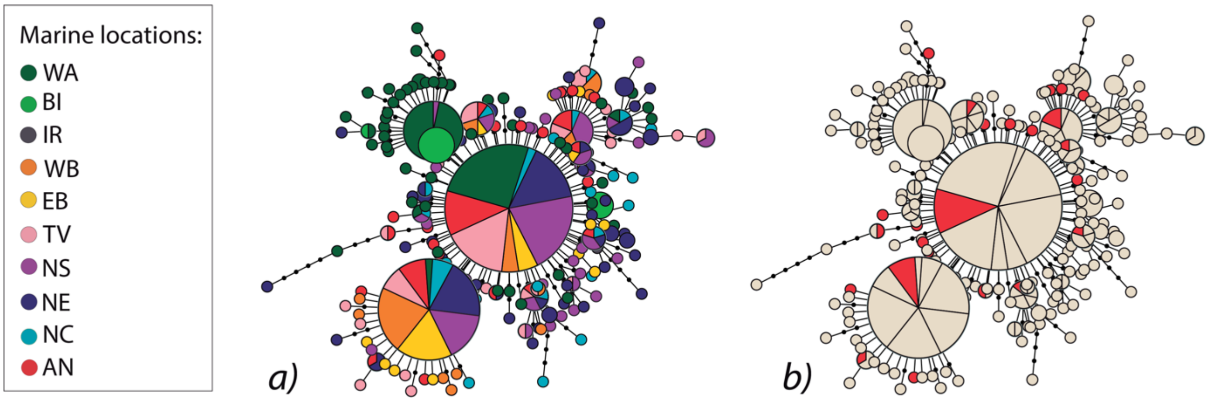


Figure 4. Star-like haplotype genealogy based on complete mitogenomes of Atlantic cod (see full details in **Paper I**). ***(a)*** Haplotype genealogy coloured by individual location. ***(b)*** Haplotype

genealogy highlighting ancient specimens as a single group (in red). Circle size is proportional to haplotype frequency.

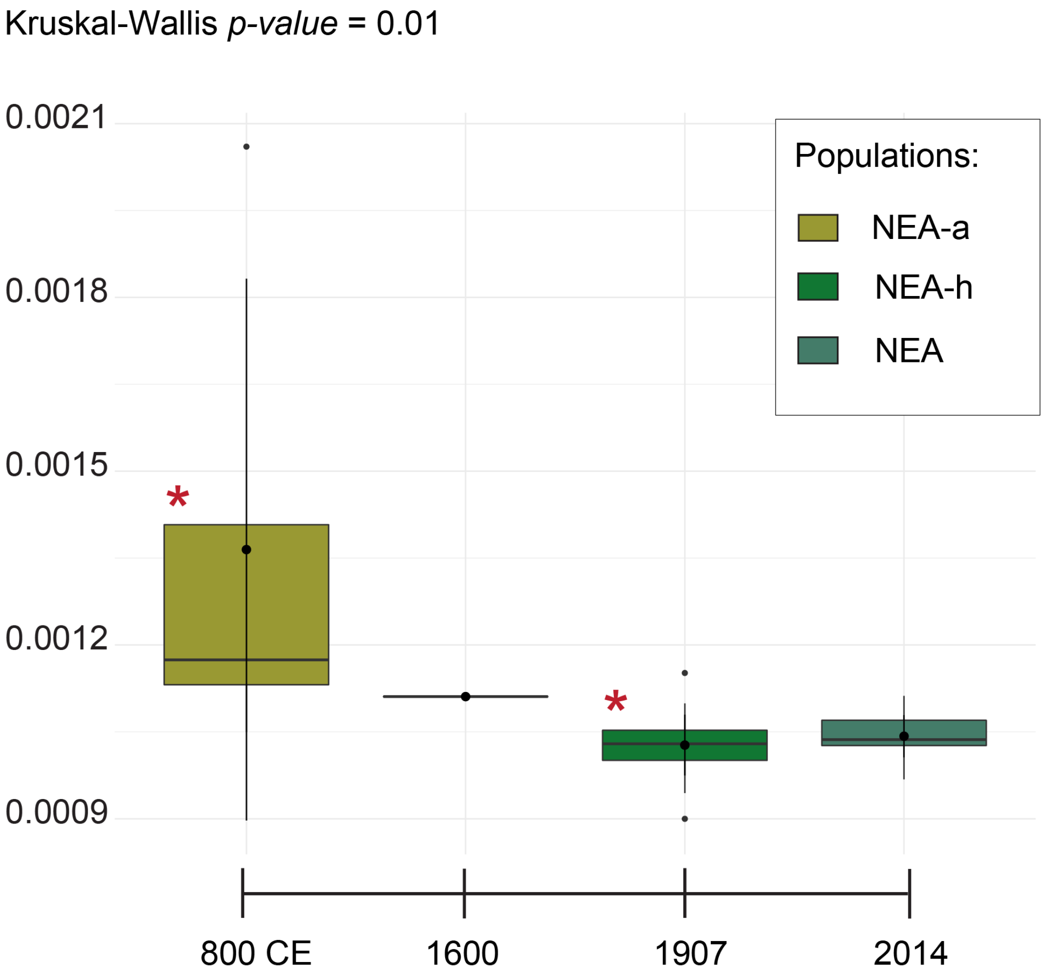


Figure 5. Heterozygosity differences across the Northeast Arctic (NEA) populations (see details in **Paper V**). Significant differences are highlighted with identical red shapes (see Table S5 in Paper V for details). The larger black dots represent the mean *He* in each period of time, while the black bar represents the interquartile range or median of the data. NEA-a: ancient population. NEA-h: historical population. NEA: modern population.

The climate in the past

Even though fishing activities over the last 1000 years may have negatively impacted populations like the Northeast Arctic, the role of climatic conditions in the population dynamics of a species has larger implications (i.e., shifts in distribution). As previously mentioned, in

Paper I and **Paper V**, results describe a large *Ne* in Atlantic cod, which coincides with climatic transitions from warm (cold) to cold (warm) temperatures. Specifically, in **Paper I**, three different population expansions of Atlantic cod around 150, 50, and 10 kya (Figure 6) may have overlap with interglacial periods experienced during glacial events (i.e., Last Glacial Maximum, the Wisconsinan and Illinoian glaciations) (Lait et al., 2018). Interestingly, the earliest population expansion showed a close agreement to the transformation of the Baltic Sea from a freshwater lake to the brackish water body (Wenne et al., 2020). Similar evidence was obtained in **Paper V**; however, (modern) western Baltic Sea and Northeast Arctic population expansions may be more related to an increase of colder temperatures (i.e., during the Little Ice Age-LIA (1300-1850 CE)), whereas (modern) population declines (last 250-200 years) appeared to be associated with warmer temperatures (Holocene Thermal Maximum-HTM (10,000-6000 years BP) or the Medieval Warming Period-MWP (900-1300 CE); Figure 7) (Crowley and Lowery, 2000; Mann, 2002; Sjögren, 2021). The only population with different demographic patterns is the North Sea, with a population increase during the last *ca.* 500 years. The reasons behind this population expansion remain unclear. Future studies should also examine the demographic patterns of ancient and historical specimens to observe if there are similarities between temporal populations. To this moment, one can only assume that the population growth of the North Sea may not be reflecting a proper expansion, but the actual decrease of the Northeast Arctic and the western Baltic populations resulting in the availability of novel habitats for such population. These observations reflect the complexity of the system and raise an intriguing inquiry, what is driving the different demographic reactions of Atlantic cod to certain climatic conditions in different periods of time?

A glance into the answer to this question may be found in **Paper II**, where I described the distribution of the Northeast Arctic cod at a site further south, *ca.* 4322-2092 years ago, than today. This result is remarkable considering the warmer temperatures (as seen from today) previously described, and reconstructed, for the late Holocene (*ca.* 5900 years BP to present) (Wanner et al., 2008). Considering the northward shift of marine species as a result to the current increase in sea temperatures (Figure 1) and the actual damage warmer temperatures can arise in Atlantic cod (i.e., low recruitment) (Righton et al., 2010; Freitas et al., 2015), one would expect a northwards distribution *ca.* 4000 years ago. Boreal and generalist species, like Atlantic cod can be benefited by warmer temperatures (i.e., 11°C to 16°C for growth), nevertheless, temperatures can indeed be too warm (or too cold) and have a direct negative effect on Atlantic cod (Righton et al., 2010). According to **Paper II**, the historical distribution (and possibly *Ne* sizes from **Paper I** and **Paper V**) of Atlantic cod does not depend on temperature alone. A larger extension of sea-ice coverage in the Barents Sea and reduce primary productivity in this area could have shifted southwards the feeding and spawning grounds of the Northeast Arctic cod. These results bring forward the importance of the ecological conditions and interactions that Atlantic cod was, and is, exposed to, to understand the response of marine species to present-day and future climate changes.

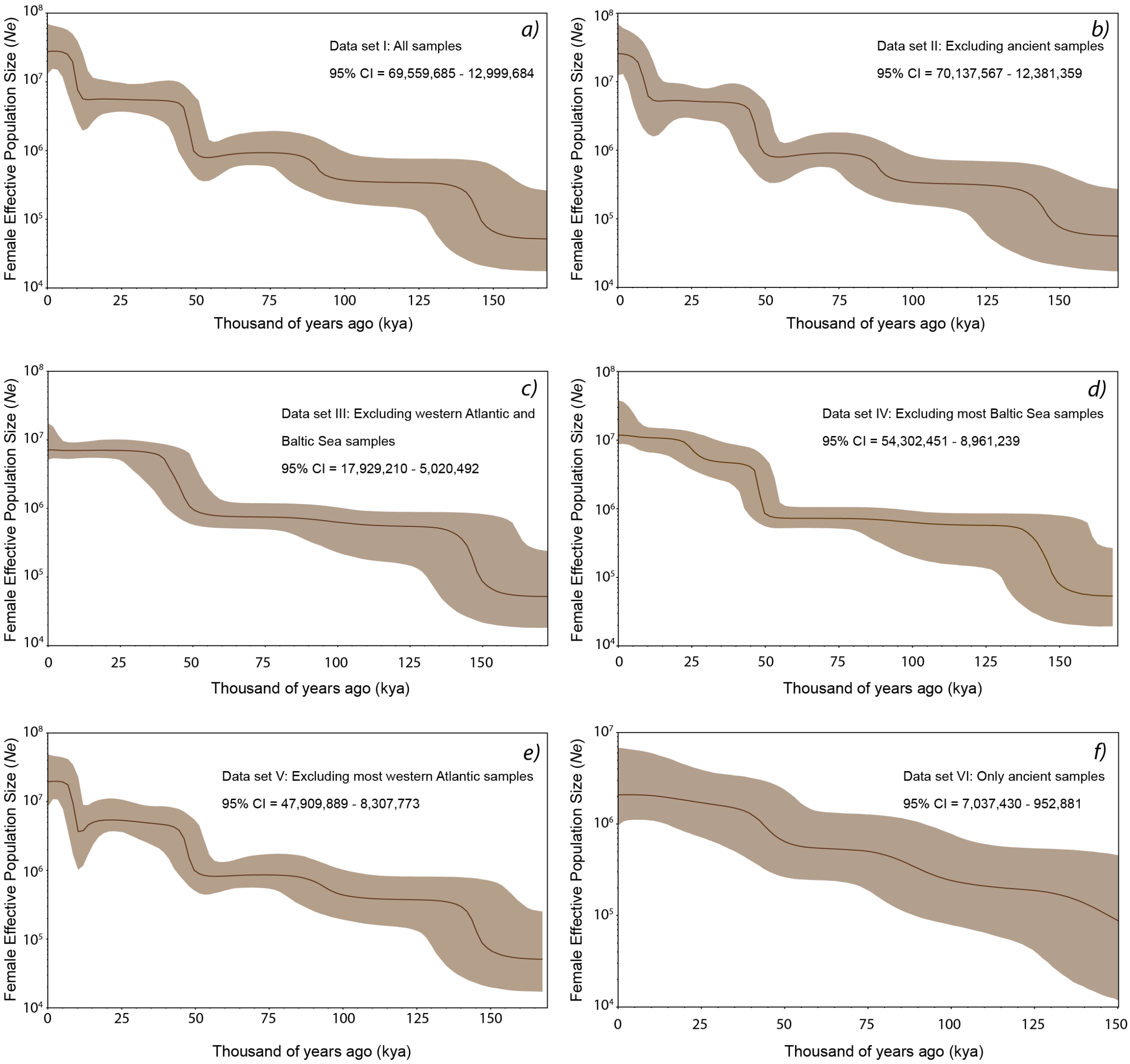


Figure 6. Atlantic cod demographic history representing the fluctuations of effective female population size (*Ne*; in dark brown line; see details in **Paper I**) based on ***(a)*** 524 sequences, ***(b)***

476 modern sequences (excluding 48 ancient samples), ***(c)*** 273 sequences (excluding clades associated with most western Atlantic and Baltic Sea samples), ***(d)*** 368 sequences (excluding the clade associated with most Baltic Sea samples), ***(e)*** 429 sequences (excluding clades associated with most western Atlantic samples) and ***(f)*** 48 ancient sequences (excluding all modern samples). The 95% CI corresponds to the light brown area. The most recent 95% CI at time 0 is shown in each respective panel.

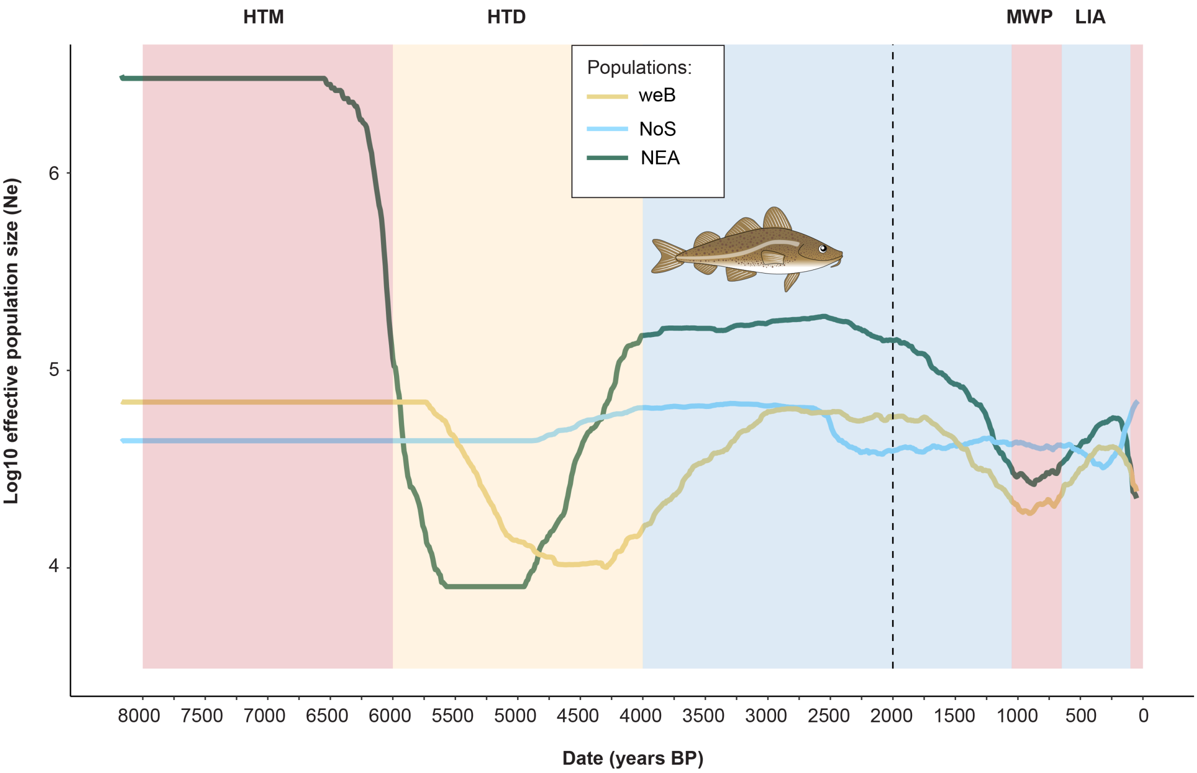


Figure 7. Temporal reconstruction of effective population sizes (*Ne*) in three Atlantic cod modern populations: the western Baltic Sea (in yellow), the North Sea (in blue) and the Northeast Arctic (in green; see details in **Paper V**). Coloured background shades indicate historical events: a red background highlights the approximate duration of the Holocene Thermal Maximum (HTM, 10,000-6000 years BP) and the Medieval Warming Period (MWP, 900-1300 CE). A yellow background highlights the transition from the MWP to the Holocene Thermal Decline (HTD, 6000-4000 years BP). A blue background highlights colder temperatures during the late Holocene (in comparison to those from the HTM) and the Little Ice Age (LIA, 1300-1850 CE).

Concluding remarks

The past leading to the future

"If ever there was a fish made to endure, it is the Atlantic cod –the common fish. But it has among its predators man, an openmouthed species greedier than cod –Mark Kurlansky"

It has been a long time since Atlantic cod has been fished for. It has lived through complex scenarios, including rather drastic changes in the environment and continuous fishing activities that together have disrupted its habitat. Current sustainable fisheries of Atlantic cod, or any other marine species, can be benefited from the available genetic information referring to the past population dynamics of the species. Failing to recognize the if and how a population has change can have catastrophic consequences for the species itself and for the marine ecosystem (e.g., actual loss of a population). The findings in this thesis are of valuable importance for understanding the evolutionary, demographic and distributional responses of an economically important fish species. These observations raise new questions about the past and specially about the future population dynamics of Atlantic cod, which can be of great use for long-term sustainable fishing strategies. They can further have potential implications in the demographic recovery of this marine fish species and they could serve as a baseline to raise awareness about other economically important marine species.

Together, **Paper I** and **Paper V** suggest that Atlantic cod populations have been impacted by climatic pressures, while **Paper V** also provides additional information about the putative impact of anthropogenic pressures in the Northeast Arctic cod stock (earliest exploited population over a 1000 years ago). **Paper II** stresses the complexity behind the population dynamics in Atlantic cod, and highlights the changes in the historical distribution of the Northeast Arctic cod. **Paper III** and **Paper IV** describe a larger extension of long-distance trading since the eleventh century and indirectly highlight that the Northeast Arctic and Iceland populations have been targeted by fishing activities before the onset of long-distance cod-fisheries.

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Paper I

Historical demographic processes dominate genetic variation in ancient Atlantic cod mitogenomes

Deep roots are not reached by the frost.
J.R.R. Tolkien

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Historical Demographic Processes Dominate Genetic Variation in Ancient Atlantic Cod Mitogenomes

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Ancient DNA (aDNA) approaches have been successfully used to infer the long-term impacts of climate change, domestication, and human exploitation in a range of terrestrial species. Nonetheless, studies investigating such impacts using aDNA in marine species are rare. Atlantic cod (*Gadus morhua*), is an economically important species that has experienced dramatic census population declines during the last century. Here, we investigated 48 ancient mitogenomes from historical specimens obtained from a range of archeological excavations in northern Europe dated up to 6,500 BCE. We compare these mitogenomes to those of 496 modern conspecifics sampled across the North Atlantic Ocean and adjacent seas. Our results confirm earlier observations of high levels of mitogenomic variation and a lack of mutation-drift equilibrium—suggestive of population expansion. Furthermore, our temporal comparison yields no evidence of measurable mitogenomic changes through time. Instead, our results indicate that mitogenomic variation in Atlantic cod reflects past demographic processes driven by major historical events (such as oscillations in sea level) and subsequent gene flow rather than contemporary fluctuations in stock abundance. Our results indicate that historical and contemporaneous anthropogenic pressures such as commercial fisheries have had little impact on mitogenomic diversity in a wide-spread marine species with high gene flow such as Atlantic cod. These observations do not contradict evidence that overfishing has had negative consequences for the abundance of Atlantic cod and the importance of genetic variation in implementing conservation strategies. Instead, these observations imply that any measures toward the demographic recovery of Atlantic cod in the eastern Atlantic, will not be constrained by recent loss of historical mitogenomic variation.

Keywords: population structure, fisheries, human exploitation, phylogenomics, population expansion, demographic history

INTRODUCTION

Continuous human activities and a changing climate have influenced terrestrial and marine ecosystems for millennia (Venter et al., 2016; Rodrigues et al., 2019; Mitchell and Rawlence, 2021), impacting the evolutionary potential and population demography of a range of species (Seersholm et al., 2018). Ancient mitochondrial DNA (mtDNA) has been widely used to understand long-term genomic consequences of such impacts (Shapiro et al., 2004; Nyström et al., 2006; Stiller et al., 2010; Paijmans et al., 2013; Fortes and Paijmans, 2015; Casas-Marce et al., 2017). Nonetheless, most ancient mtDNA studies have focused on terrestrial species, and studies that investigate the impacts of long-term human activities and/or climatic variation on fish, using whole genome sequencing approaches, are relatively rare. Long-term commercial fisheries—covering many centuries—have contributed to the decline of economically and ecologically important marine species (Exadactylos et al., 2007; Pinnegar and Engelhard, 2008; Barrett, 2019). The consequences of intensive fishing in recent times may be difficult to assess as this requires an understanding of historical population dynamics (Selim et al., 2016). The analysis of long-term biological and demographic fluctuations can therefore help to improve guidelines for sustainable fisheries management and optimal conservation measures (Barrett, 2019). In order to provide a long-term perspective on fishing exploitation impacts, the use of archeological evidence, such as fish bone remains, is essential for those periods for which little or no historical data are available. Recent developments in whole genome aDNA methods now allow the inference of demographic histories and the estimation of genetic fluctuations over time from fishbone samples (Oosting et al., 2019; Ferrari et al., 2021). Such combined molecular analyses of historical and modern samples can potentially provide an understanding of the association between human-environmental impact and population declines (Hofman et al., 2015).

Several studies have shown the utility of temporal mtDNA analyses in the marine environment. For instance, ancient mitogenomes have investigated impacts of climate and hunting on the Atlantic walrus (Star et al., 2018; Keighley et al., 2019; Barrett et al., 2020), narwhals (Louis et al., 2020), and the extinct great auk (Thomas et al., 2019). In fish, such studies remain limited to partial mitogenome data. For example, a shift in sturgeon species distributions was detected during the Holocene in the North East Atlantic based on CytB amplicon data (Nikulina and Schmölcke, 2016). Moreover, impacts of habitat destruction and human activities during the 1800s were associated with a reduction of the mtDNA diversity of Chinook salmon from the Columbia River in the 12S and control region by comparing ancient and modern samples (Johnson et al., 2018). Similarly, impacts of human exploitation and climate oscillations were associated with losses of haplotypic CytB variation in Atlantic cod during the 15th to 16th centuries in Iceland (Olafsdottir et al., 2014). In contrast, comparable levels of ancient mtDNA genetic diversity were found between ancient and modern samples of herring specimens, despite continuous human exploitation (Speller et al., 2012). Notwithstanding these examples, human-environmental impacts and population declines remain unclear for a wide range of marine species and populations.

Atlantic cod (*Gadus morhua* L. 1758) is a benthopelagic predatory fish with high reproductive rates and with a fundamental ecological role in marine ecosystems (Barth et al., 2017; Edvardsson et al., 2019). It has been one of the most exploited fish species in the North Atlantic Ocean (Carr et al., 1995; Árnason et al., 2000; Nicholls et al., 2021). The distribution of this species extends through the cold waters of North America, across the continental shelves of Greenland and Iceland, and northern Europe (Lait et al., 2018). Relatively large population sizes have been characteristic throughout its entire distribution even during the expansion of long-distance fish trading during the 12th to 13th centuries in the eastern Atlantic and at the beginning of the 16th century in the western Atlantic (Barrett et al., 2004, 2011; Orton et al., 2014; Castañeda et al., 2020). However, intensive fishing activities during the 20th century (Mieszkowska et al., 2009; Jonsson et al., 2016; Brattey et al., 2018) resulted in the severe depletion of several stocks, for instance the North Sea stock, which was decimated from annual landings of 354,000 to 50,000 tons during this period (Bannister, 2004). In addition to past human exploitation, climatic events like the Little Ice Age—a cooling period that varied regionally in timing and duration but occurred between ca. 1300–1850 CE—may have caused large declines between the sixteenth and 17th centuries (Edvardsson et al., 2019).

The genomic consequences of such population dynamics and declines in Atlantic cod remain unclear. Based on partial and whole mtDNA data, Atlantic cod populations between the western and eastern Atlantic Ocean show significant structure (Árnason, 2004; Jørgensen et al., 2018; Lait et al., 2018), whereas low to no mtDNA differentiation has been found across a wide range of eastern Atlantic locations (Carr et al., 1995; Árnason and Palsson, 1996; Árnason et al., 1998, 2000; Sigurgíslason and Árnason, 2003). Here, we compared modern and ancient Atlantic cod mitogenomes—dated up to 6500 BCE—from different fishing locations in northern Europe. We evaluated whether Atlantic cod in the eastern Atlantic have experienced any loss

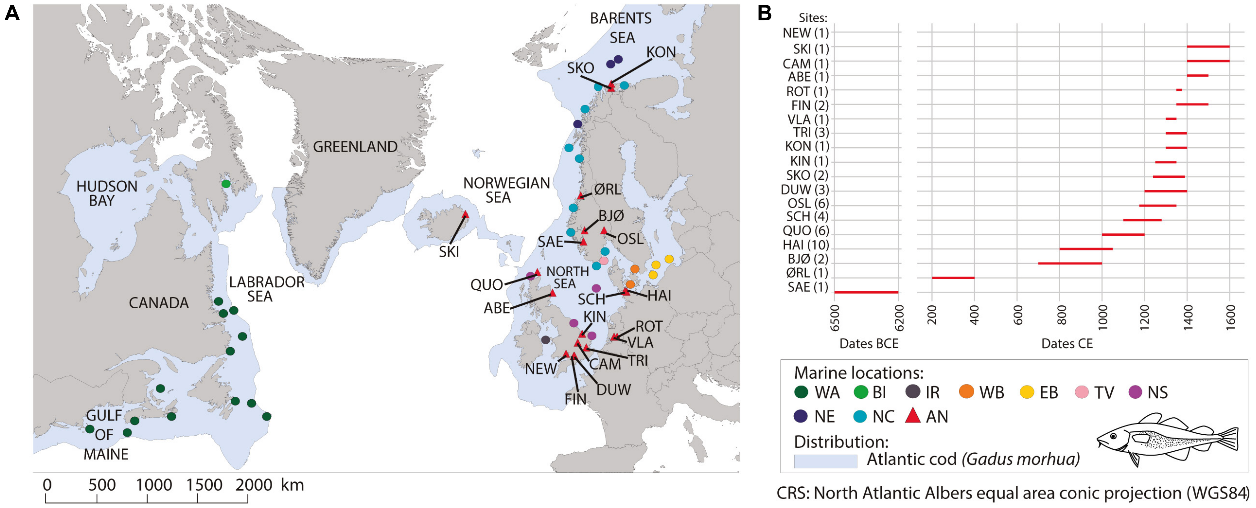

FIGURE 1 | (A) Ancient and modern Atlantic cod sampling locations. The abbreviated name of archeological sites is shown in the map (see also **Supplementary Table 1**). Modern data were obtained from Jørgensen et al. (2018); Lait et al. (2018) and Barth et al. (2019). Sample sites are grouped according to larger oceanographic location, ecotype and modern or ancient specimens: western Atlantic (WA) = dark green, Baffin Island (BI) = light green, Irish Sea (IS) = gray, North Sea (NS) = purple, western Baltic (WB) = orange, eastern Baltic (EB) = yellow, Tvedestrand fjord (TV) = pink, North East Arctic (NE) = dark blue, Norwegian coast (NC) = blue, and Ancient specimens (AN) = red (i.e., Saevarhelleren = SAE, Ørland Kampflybase = ØRL, Bjørkum = BJØ, Haithabu = HAI, Quoygrew = QUO, Schleswig Schild = SCH, Oslo Mindets Tomt = OSL, Bristol Dundas Wharf = DUW, Skonsvika = SKO, King's Lynn Raynham House = KIN, Kongshavn = KON, London Trig Lane = TRI, Vlaardingen Gat in de Markt = VLA, Bristol Finzel's Reach = FIN, Rotterdam, Hoogstraat 13–26 = ROT, Aberdeen = ABE, Cambridge Grand Arcade = CAM, Skriðuklaustur = SKI, Newport Ship = NEW). Modern distribution of Atlantic cod is shown in light blue. Fish illustration drawn by Geir Holm. **(B)** Cleveland plot showing the age range of ancient samples. Sample age is estimated based on archeological context. The number of individuals per site is represented in brackets after the site name (**Supplementary Table 1**).

of genetic variation, analyzed long term patterns of effective population size, and related any observed decline to the impact of commercial fisheries or climate change.

MATERIALS AND METHODS

Sample Collection

Ancient samples of Atlantic cod (n = 48) were obtained from 11 excavation sites (**Figure 1** and **Supplementary Table 1**) and were stored dry and unfrozen. The specimens were all supplied by the relevant archeological organizations, or sampled with permission on their premises. The shipment of Atlantic cod bones does not require CITES or other wildlife regulation permits for transport or analysis. Where practicable, only a subsample of bone was employed for the aDNA research, leaving material for other studies. Dating of the samples (**Supplementary Table 1**) was based on archeological context. Ancient samples were morphologically and genetically identified as Atlantic cod. A total of 472 available modern mitogenomes were obtained from Jørgensen et al. (2018), Lait et al. (2018), and Barth et al. (2019). Novel mtDNA sequence data from modern specimens sampled in 2016 in Orkney, United Kingdom (n = 24) were also included (**Figure 1** and **Supplementary Table 2**). The collection of the Orkney specimens complied with the Nagoya Protocol and Convention on Biological Diversity, which the United Kingdom signed up to in 2016. All specimens were deceased when the fin clip was collected.

DNA Extraction, Amplification and Sequencing

DNA extraction and library preparation from ancient samples were performed in the aDNA laboratory at the University of Oslo under rigorous measures (Cooper and Poinar, 2000; Gilbert et al., 2005). All ancient samples were processed with the same DNA extraction and library protocols according to Ferrari et al. (2021). In short, bones were UV-treated for 10 min per side and pulverized using a stainless-steel mortar (Gondek et al., 2018). Per specimen, two aliquots containing between 150 and 200 mg of bone powder were used as starting material for DNA extraction. Double-indexed blunt-end sequencing libraries were built from 15 to 16 μl of DNA extract using the Meyer-Kircher protocol (Meyer and Kircher, 2010; Kircher et al., 2012) with the modifications listed in Schroeder et al. (2015) and the single-tube (BEST) protocol (Carøe et al., 2018) with the modifications described in Mak et al. (2017). Sequencing reads were processed using PALEOMIX v1.2.13 (Schubert et al., 2014). Trimming of residual adapter contamination, filtering and collapse of reads was done using AdapterRemoval v.2.1.7 (Lindgreen, 2012). Sequencing reads shorter than 25 bp were discarded. Mapping of remaining reads was performed against the Atlantic cod GadMor3.0 nuclear genome (RefSeq assembly accession GCF_902167405.1; Star et al., 2011; Tørresen et al., 2017) and mitochondrial genome (Johansen and Bakke, 1996) using BWA v.0.7.12 (Li and Durbin, 2009) with the aln algorithm, disabled seeding and minimum quality score of 25. The resulting BAM files were indexed with *samtools* v.1.9 (Li et al., 2009) and

DNA postmortem damage assessed using MapDamage v.2.0.9 (Jónsson et al., 2013). DNA from modern Orkney samples were extracted using a DNeasy Blood & Tissue kit (Qiagen). Libraries were assembled with a TrueSeq DNA PCR-Free Preparation Kit and sequenced on an Illumina HiSeq 2,500. Modern alignment—including Orkney and Barth et al. (2019) samples, and the outgroup Alaska pollock (*Gadus chalcogrammus*; Malmstrøm et al., 2016) – was carried out using BWA v.0.7.12 with the mem algorithm, and a minimum quality score of 25.

Mitogenomic Analysis

Variant calling was performed using GATK v.4.1.4. (McKenna et al., 2010) simultaneously in all ancient, modern Orkney and Barth et al. (2019) samples, including the outgroup. gVCF files were created for each sample using GATK HaplotypeCaller (with ploidy set to 1). Individual genotypes were combined in one file using GATK CombineGVCFs and GenotypeGVCFs. Filtering was performed using *bcftools* v.1.9. (Li et al., 2009) and *vcftools* v.0.1.16. (Danecek et al., 2011) with the following thresholds: FS < 60.0, SOR < 4, MQ > 30.0, QD > 2.0, SnpGap = 10, minGQ = 15, minDP = 3, remove indels = yes, biallelic loci = yes, meanDP < 30 and read depth > 3. Consensus sequences were built using *bcftools* consensus and aligned using MAFFT v.7.429 (Katoh and Standley, 2013). Available modern mitogenomes obtained from Jørgensen et al. (2018) and Lait et al. (2018) were manually inspected using MEGA v.7 (Kumar et al., 2016) to set as missing the consistent nucleotide differences (between 50 and 100%; **Supplementary Table 3**) between their Illumina, Sanger and/or Roche 454 technologies with the Illumina sequenced mitogenomes in this study. Control region and half of the tRNA-Pro sequences from all the mitogenomes were excluded from further analyses as these two regions were not fully complete (i.e., 15,696–15,815 positions) after aligning sequences obtained from Jørgensen et al. (2018) and Lait et al. (2018) with the sequences presented in this study and Barth et al. (2019) samples. Thus, all sequences analyzed had 15,695 bp in length. Validated SNPs were annotated as transversion and/or transition using *SNP-sites* (Page et al., 2016). Checked and modified modern sequences (Jørgensen et al., 2018; Lait et al., 2018) were added and aligned to our multi-fasta alignment using MAFFT v.7.429. Unique sequences were identified with IQTREE v.1.6.12 (Nguyen et al., 2015). File formats required for different software and/or packages were obtained with seqinr and ape (i.e., nexus format; Paradis and Schliep, 2019; Charif et al., 2020), and phylotools (i.e., phylip format; Zhang et al., 2017) packages implemented in R.

Different sample combinations were used to compare the genetic diversity of the ancient samples to those of the modern conspecifics. Given the low spatial structure in the eastern Atlantic region (Árnason and Palsson, 1996; Árnason et al., 1998; Sigurgíslason and Árnason, 2003) and lack of consistent spatial structure amongst specimens (**Supplementary Figures 3, 5, 6**), all 48 ancient samples were compared as a single group to modern samples grouped into larger marine locations (according to their geographical proximity or ecotype; **Figure 1**). In addition, a comparison of subsets of multiple specimens from two archeological locations (Quoygrew and Haithabu) for which a more specific temporal pair from the same geographical region could be identified, was performed (**Supplementary Table 1**). Quoygrew specimens were locally sourced (Harland and Barrett, 2012; Star et al., 2017). Therefore, modern specimens sampled in the same area (i.e., modern Orkney) provide a logical, spatially consistent temporal comparison. However, specimens from Haithabu, were sourced from northern Norway (Star et al., 2017), and belonged to the North East Arctic ecotype. For these traded specimens, the North East Arctic ecotypes provide a spatially relevant temporal comparison, rather than North Sea or western Baltic specimens.

Haplotype (h) and nucleotide diversities (π), number of haplotypes (Nh) and number of polymorphic sites (S) were calculated using DnaSP v.6 (Rozas et al., 2017). To allow direct comparison with earlier CytB results (Árnason, 2004; Olafsdottir et al., 2014; Jørgensen et al., 2018), specific CytB haplotypes based on 250 bp gene fragment as previously reported by Árnason (2004) were identified using MEGA v.7. Demographic histories were determined by Tajima's D (TD) and Fu's F (F) neutrality in DnaSP v.6. A different number of specimens were obtained for ancient and modern locations. We corrected for such differences in sample size by randomly downsampling the modern specimens for each of the temporal comparisons (North East Arctic and Orkney) using 1,000 bootstrap replicates. A 95% confidence interval of the genetic parameters; genetic variation (π) and patterns of population demography (TD and F) was calculated from these 1,000 bootstrap replicates that were sampled using a without replacement approach with the *sample* function implemented in R (R Core Team, 2020) and the *fasta.sample* function in the FastaUtils package also in R (Salazar, 2020). For the bootstrapping test, π, TD and F from temporally spaced modern locations were re-calculated with the *pegas* (Paradis, 2010) and *PopGenome* (Pfeifer et al., 2020) packages implemented in R. Relationships among ancient and modern samples were visualized for whole mitogenome and CytB sequence data, by constructing a mitochondrial haplotype-genealogy graph using Fitchi (Matschiner, 2016) with the ML-based phylogenetic tree obtained with IQTREE v.1.6.12 as input.

Population Dynamics and Demographic Reconstruction

An analysis of molecular variance (AMOVA, 1,000,000 permutations) and population pairwise genetic distances (Φ_{ST}) were obtained in Arlequin v.3.5 (Excoffier and Lischer, 2010), to determine the distribution of variation between marine locations and temporally spaced locations. Divergence and coalescent analyses were based on unique sequences only ($n = 525$ sequences including the outgroup). Substitution model selection for unique sequences was performed using PHYML v.3.1 (Guindon et al., 2010) as implemented in JMODELTEST v.2.1.10 (Guindon and Gascuel, 2003; Darriba et al., 2012). Model selection was determined on the following partitions: 1st, 2nd, and 3rd codons from protein coding regions, rRNAs and tRNAs. Best-fitting models were selected according to the Aikaike Information Criterion (AIC; **Supplementary Table 4**). Based on these results, phylogenetic estimates were obtained using BEAST v.2.6.3 (Bouckaert et al., 2019).

Bayesian settings for all phylogenetic analyses included two sets of partitions: coding region and non-coding region. Three independent runs to test for chain convergence were run under the Coalescent Constant Population Tree Prior. Tip ages (ancient and modern dates) were included for each set of runs (**Supplementary Tables 1, 2**). Sample dates for ancient specimens were rounded to a midpoint date—from a given range—where necessary. To achieve high effective sample sizes (ESS = $\geq$ 200), chain lengths were run 800,000,000 under a substitution rate of 1.14×10^{-8} substitution/site/year as per Lait (2016) assuming a GTR + I (for coding regions) and TIM1 + I (for non-coding regions) models of evolution and a strict clock. Tracer v.1.71 (Rambaut et al., 2018) was used to check for convergence of MCMC and to ensure sufficient sampling. Consensus trees were obtained using TreeAnnotator v.2.6.2—implemented in BEAST v.2.6.3—after 10% burn-in. Final phylogenetic trees were viewed and edited in FigTree v.1.4.4.

Finally, a Coalescent Bayesian Skyline (CBS) analysis was completed to reconstruct the demographic history—including female effective population size (*Ne*) —of Atlantic cod through time. To assess any confounding effect of past or contemporary population structure (Heller et al., 2013), we analyzed demographic history using 6 different data sets (excluding the outgroup): (I) all 524 sequences, (II) 476 modern sequences (excluding 48 ancient samples), (III) 273 sequences (excluding clades associated with most western Atlantic and Baltic Sea samples), (IV) 368 sequences (excluding the clade associated with most Baltic Sea samples), (V) 429 sequences (excluding clades associated with most western Atlantic samples) and (VI) 48 ancient sequences (excluding all modern samples). The specific clades that were excluded in III, IV and V can be found in **Supplementary Figure 4**. We used the same MCMC sampling procedure described before with 3 independent runs reaching convergence at high effective sample sizes (ESS = $\geq$ 200). Chain lengths were run 800,000,000 for data sets I, II and V with a number of bPopSize and bGroupSize of 10; while chain length for data sets III and IV were run 500,000,000 and 50,000 for data set VI with a number of bPopSize and bGroupSize of 5.

RESULTS

Mitogenomic Variation

Sequencing reads from all ancient specimens showed the expected patterns of DNA fragmentation and deamination rates that were consistent with those of authentic aDNA (**Supplementary Figure 1**). We obtained 48 mitogenomes with at least 3-fold average coverage. We also obtained mitogenomes for 24 modern Orkney specimens (**Supplementary Table 2**). A total of 2135 SNPs ($\sim$13% of mitogenome positions) were identified among all 545 samples – including the outgroup species Alaska pollock –: 1219 SNPs corresponded to informative sites and 916 SNPs were singletons (**Supplementary Table 5**).

Nucleotide diversity (π) between modern locations ranged between 0.002 and 0.003 (**Table 1**) and π of ancient samples did not vary from the values obtained in modern locations. The temporal comparison of specific sites (Quoygrew-Orkney and Haithabu-North East Arctic), showed limited significant differences between genetic statistics of temporally spaced ancient and modern locations (**Supplementary Table 6** and **Supplementary Figure 2**), where Haithabu has significantly lower π and higher *F* compared to the North East Arctic (**Supplementary Figure 2**).

Neutrality tests showed significant negative values for all Tajima's D (*TD*) and *F* statistics in most locations, except for the western location Baffin Island, and the eastern locations Tvedestrand fjord and western Baltic (**Table 1**). Overall, there were 486 haplotypes —- including the outgroup—across all 545 samples, of which only 26 were shared between individuals (**Figure 2** and **Supplementary Table 7**). Ancient CytB variation consisted of 7 different haplotypes, including four main haplotypes (A, C, D, and E) previously identified in modern mtDNA studies (Árnason, 2004; Jørgensen et al., 2018). Two novel variations of existing CytB haplotypes were found in western Baltic (haplotype ED) and North Sea (haplotype LI), while another 2 novel variations of existing CytB haplotypes were found among ancient samples (haplotypes LJ and TI). The most prevalent ancient haplotypes were A and E ($\sim$ 40 and 38%, respectively, **Supplementary Tables 1, 7**), which were also commonly found in modern samples (**Supplementary Table 8**). The haplotype genealogy for whole mitogenome and CytB sequence data showed an extensive distribution of ancient samples across marine locations (**Figure 2** and **Supplementary Figure 4**). Limited geographic mitogenome structure was observed, except for elevated divergence between western Atlantic and eastern Atlantic locations, and between locations in the western and eastern Baltic Sea and other eastern Atlantic locations (**Figures 2B,C** and **Supplementary Figures 4B,C**). A star-like topology is observed for the whole mitogenome and CytB genealogies (**Figure 2** and **Supplementary Figure 4**).

Demographic Patterns and Population Structure

The AMOVA assigned 7.58% of the variation between marine locations (including ancient samples as a single group) while 91.47% of the variation was represented between individuals ($\Phi CT = 0.076$, $p \leq 0.001$; $\Phi ST = 0.085$, $p \leq 0.000$). Pairwise Φ_{ST} values (**Figure 3** and **Supplementary Tables 9, 10**) showed significant differentiation levels between all ancient samples and western Atlantic, Baffin Island, western Baltic, eastern Baltic and Tvedestrand fjord. Ancient samples showed higher differentiation when compared to western Atlantic ($\Phi_{ST} = 0.117$), and Baffin Island ($\Phi_{ST} = 0.192$) in comparison to other eastern Atlantic locations. Among modern samples, western Atlantic, Baffin Island, western Baltic and eastern Baltic showed significant Φ_{ST} values when compared to all other locations (**Supplementary Tables 9, 10**). Φ_{ST} values were not significant between North Sea, North East Arctic, Norwegian coast and Ancient samples. Pairwise Φ_{ST} values between temporal spaced locations also showed no significant differentiation (Quoygrew and modern Orkney: $\Phi_{ST} = 0.000$; $p = 0.807$; and Haithabu and North East Arctic: $\Phi_{ST} = 0.000$; $p = 0.456$).

TABLE 1 | Estimates of genetic diversity statistics for Atlantic cod at whole mitogenomes from different marine locations or ecotypes in the North Atlantic (see **Supplementary Tables 1, 2, 5**).

	Location	Code	*N*	*h*	*Nh*	*S*	π	*TD*	*F*
Modern	western Atlantic	WA	124	1.000	124	759	0.002	−2.652*	−5.668*
	Baffin Island	BI	18	0.791	7	68	0.002	1.570	1.688
	western Baltic	WB	43	0.996	40	221	0.002	−1.719	−2.992*
	eastern Baltic	EB	36	1.000	36	249	0.002	−1.888*	−3.305*
	Tvedestrand (fjord)	TV	37	0.982	31	256	0.002	−1.570	−2.757*
	North Sea	NS	99	0.999	96	678	0.003	−2.350*	−4.421*
	North East Arctic	NE	97	0.999	92	716	0.003	−2.408*	−4.695*
	Norwegian coast	NC	41	1.000	41	377	0.002	−2.187*	−3.694*
Ancient	Ancient	AN	48	0.998	46	364	0.002	−2.283*	−3.906*

*Significant values are indicated with * ($p \leq 0.01$). N, sample size; h, haplotype diversity; Nh, number of haplotypes; S, number of polymorphic sites; π, nucleotide diversity; TD, Tajima's D; F, Fu's F. Locations with 1 sample are excluded for genetic analysis (i.e., Irish Sea = IS).*

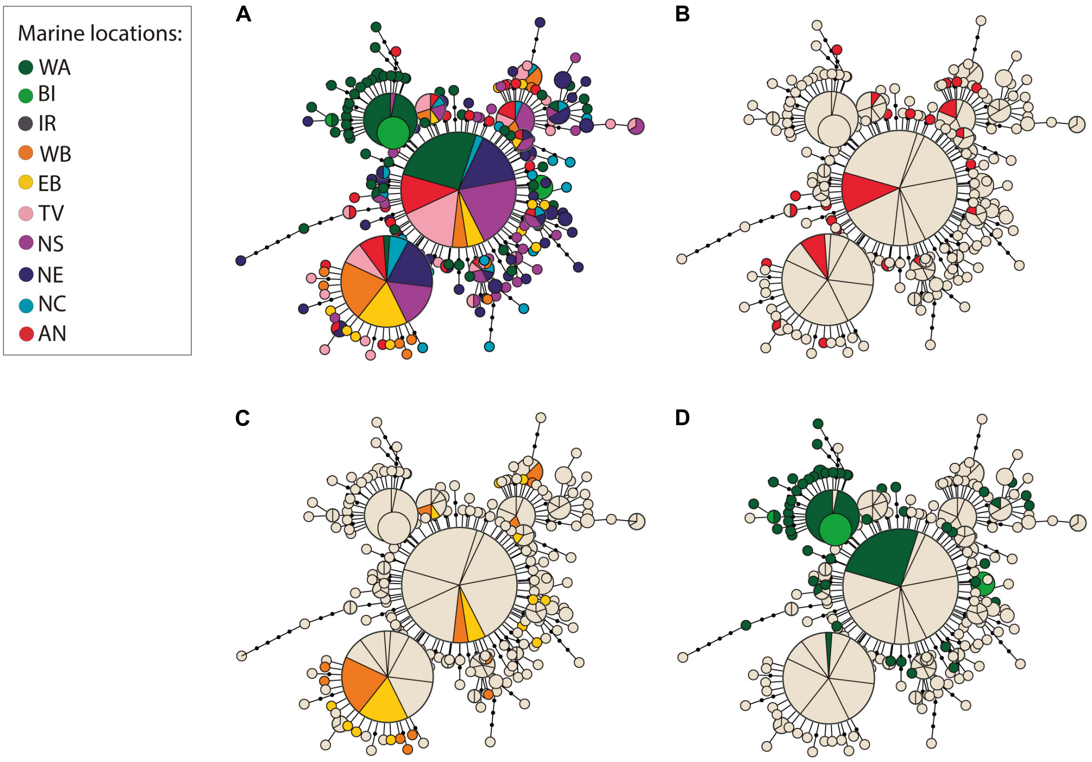

FIGURE 2 | Star-like haplotype genealogy based on complete mitogenomes of 545 samples of Atlantic cod. Only transversions (336) are depicted in this genealogy. **(A)** Haplotype genealogy colored by location. **(B)** Haplotype genealogy highlighting ancient specimens as a single group (in red; see **Figure 1** and **Supplementary Table 1**; for specific distribution of sampling sites of ancient samples see **Supplementary Figure 3**). **(C)** Haplotype genealogy highlighting samples from the western (orange) and eastern (yellow) Baltic. **(D)** Haplotype genealogy highlighting western Atlantic (green) and Baffin Island (light green) specimens. Circle size is proportional to haplotype frequency. Black dots indicate intermediate haplotypes not found among the specimens and black lines connecting black circles correspond to mutation steps between haplotypes.

The time-calibrated Bayesian phylogeny for ancient and modern Atlantic cod samples resulted in 2 main clades with an estimated divergence from the most recent common ancestor at 220 kya (95% highest posterior density (HPD) = 194,780—249,980 kya; **Figure 4**). The first clade, which is not further divided, includes mitogenomes from 6 different widely scattered localities. The second clade was composed by 16 subclades with posterior probability > 0.8, with divergence times of ca. 100 kya. Clades and subclades in the phylogeny were not geographically structured, with the exception of most samples from western Atlantic, and most samples from western and eastern Baltic, which clustered together (**Figures 2**, **4**).

The Bayesian skyline analysis using different subsets of the data revealed a consistent pattern of step-wise population expansions followed by periods of constant population size. Expansions around 150, 50, and 10 kya are present in most

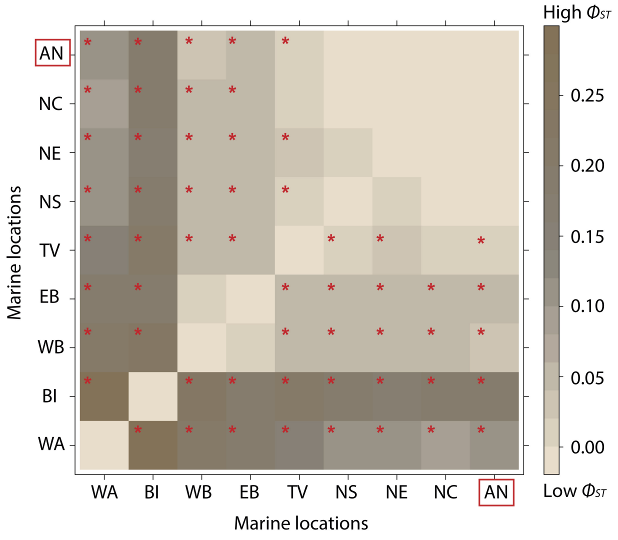

FIGURE 3 | Heatmap of differentiation pairwise (Φ_{ST}) between 10 marine locations of Atlantic cod: western Atlantic (WA), Baffin Island (BI), western Baltic (WB), eastern Baltic (EB), Tvedestrand fjord (TV), North Sea (NS), North East Arctic (NE), Norwegian coast (NC), and Ancient specimens (AN). Dark brown colors indicate higher levels of differentiation (Φ_{ST} = 0.15–0.30). Light brown corresponds to lower levels of differentiation (Φ_{ST} = ≥ 0.10). Significant *p*-values ($p \leq 0.05$) are indicated with a red **p*-values are listed in **Supplementary Tables 9, 10**.

subsets (**Figure 5**). A population expansion of Atlantic cod was identified ca. 50 kya in all subsets. The most recent expansion identified (around 10 kya), is only present in data sets that include clades with most Baltic Sea specimens (**Figures 5A,B,E**). Despite such differences, all analyses agree with a high and increasing female effective population size (*Ne*) of Atlantic cod (*Ne* = ca. 1,000,000—10,000,000) during the last ca. 100 kya, with highest estimates of *Ne* during the last few millennia (**Figure 5**).

DISCUSSION

Here, we compared modern and ancient mtDNA diversity in Atlantic cod to investigate whether observed historical and contemporaneous census population declines (Hutchinson et al., 2003; Hylen et al., 2008; Limburg et al., 2008; Bartolino et al., 2012; Jonsson et al., 2016; Brattey et al., 2018) have had mitogenomic consequences. The temporal comparison of 48 ancient specimens to 496 modern conspecifics did not reveal consistent significant mitogenomic changes or measurable effective genetic population declines through time. Below, we discuss reasons why such genomic impacts may not be observed.

First, mitogenomic variation is high in modern Atlantic cod and is characterized by limited genetic differentiation between populations and incomplete lineage sorting over large spatial scales across its range in the North Atlantic (Jørgensen et al., 2018; Lait et al., 2018). Low observed genetic differentiation (Φ_{ST}) between Tvedestrand fjord and other Norwegian coastal locations, as well as between the North Sea, the North East Arctic and the Norwegian coast confirm this lack of geographic structuring over large parts of the eastern Atlantic (**Figure 3**). Indeed, the non-significant differentiation of all ancient samples with modern North Sea, North East Arctic and Norwegian coast is fully consistent with their presumed geographical origin and highlights the long-term lack of mtDNA structure in this region. Non-significant ΦST values between the Norwegian coastal locations and Tvedestrand fjord indicate possible recent migration of fish between such coastal communities and more restricted fjord populations (Knutsen et al., 2011). Compared to many terrestrial ecosystems, where populations can often be isolated by physical barriers—which restrain interbreeding and dispersal—(Hauser and Carvalho, 2008; Exadactylos et al., 2019), in marine ecosystems the absence of physical barriers promotes larger panmictic populations and Atlantic cod is no exception (Berg et al., 2016, 2017; Sodeland et al., 2016; Barth et al., 2017). Thus, a combination of low spatial resolution of mtDNA data as a result of continuous gene flow and connectivity may mask any local temporal erosion of mitogenomic diversity (Welch et al., 2012) in Atlantic cod.

Second, we determined high long-term estimates of effective population size (*Ne* = ca. 1,000,000–10,000,000; **Figure 5**), which is in agreement with earlier observations in Atlantic cod (Hardie et al., 2006; Therkildsen et al., 2010; Pinsky et al., 2021). Estimates of *Ne* can remain high in economically important fish species, even if their populations have experienced a large biomass decline (Hauser and Carvalho, 2008) since it takes hundreds of generations (i.e., depending on the generation time of the species; Amos and Balmford, 2001; Frankham et al., 2002) for the actual population numbers and breeding populations to be reflected in *Ne* (Hauser and Carvalho, 2008). In fact, simulations have shown that a population with theoretical *Ne* of 100 (which is several orders of magnitude lower than observed in Atlantic cod) would retain 75% of heterozygosity after 57 generations (Frankham et al., 2002; Welch et al., 2012). Given that such population declines take a very long time to lead to measurable genomic consequences, mtDNA—as a single locus—will have limited power to record such changes in populations of high *Ne* (Allentoft et al., 2014; Johnson et al., 2018; Thomas et al., 2019; Spencer, 2020). The absence of significant genetic changes in this study is consistent with the absence of such changes in genome-wide data using historical samples of Atlantic cod from the western and eastern Atlantic (Pinsky et al., 2021) and with the absence of such changes in mitogenomic data from other taxa that have similarly high estimates of *Ne* as Atlantic cod, such as the Pacific herring (Speller et al., 2012; Moss et al., 2016), the Hawaiian petrel (Welch et al., 2012) and even extinct species such as the New Zealand moa (Allentoft et al., 2014), the passenger pigeon (Murray et al., 2017) and the great auk (Thomas et al., 2019).

In contrast, temporal losses of mitogenomic diversity and/or declines in *Ne* have been reported in species that have suffered population fragmentation (e.g., resulting in small effective population sizes) or that have experienced limited connectivity, such as the steppe bison (Shapiro et al., 2004), the Scandinavian arctic fox (Nyström et al., 2006), cave bears (Stiller et al., 2010), the Iberian lynx (Casas-Marce et al., 2017), the Iberian salmon

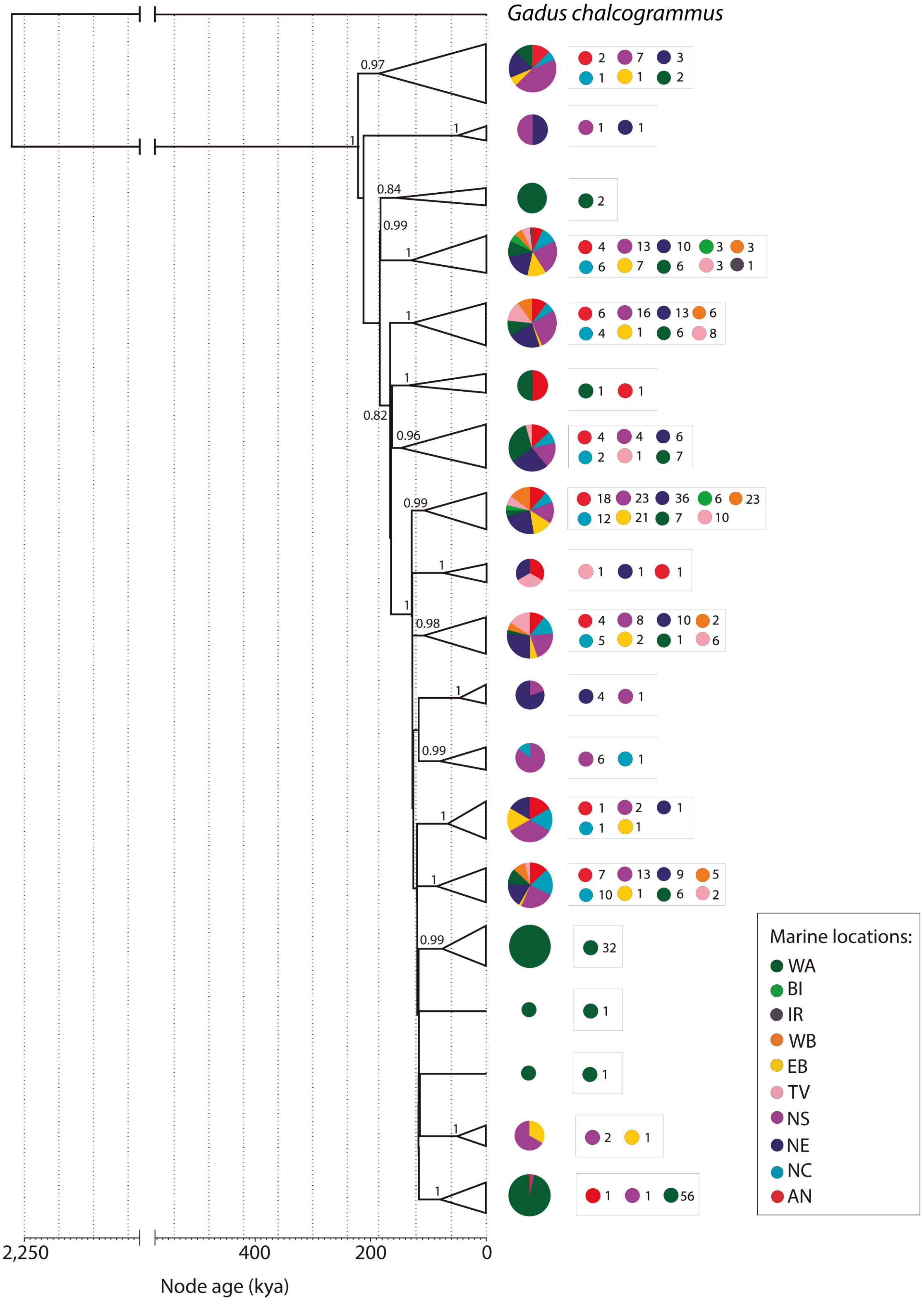

FIGURE 4 | Time calibrated collapsed Bayesian phylogeny of full mitogenomes from 525 Atlantic cod specimens using Alaska pollock (*Gadus chalcogrammus*) as an outgroup. Pie charts represent the marine locations distributed in each clade. Numbers beside pie charts indicate the number of individuals from each marine location distributed in each clade. Only branches with posterior probability > 0.8 are indicated next to the corresponding clade/subclade. For specific distribution of sampling sites of ancient samples see **Supplementary Figure 6**.

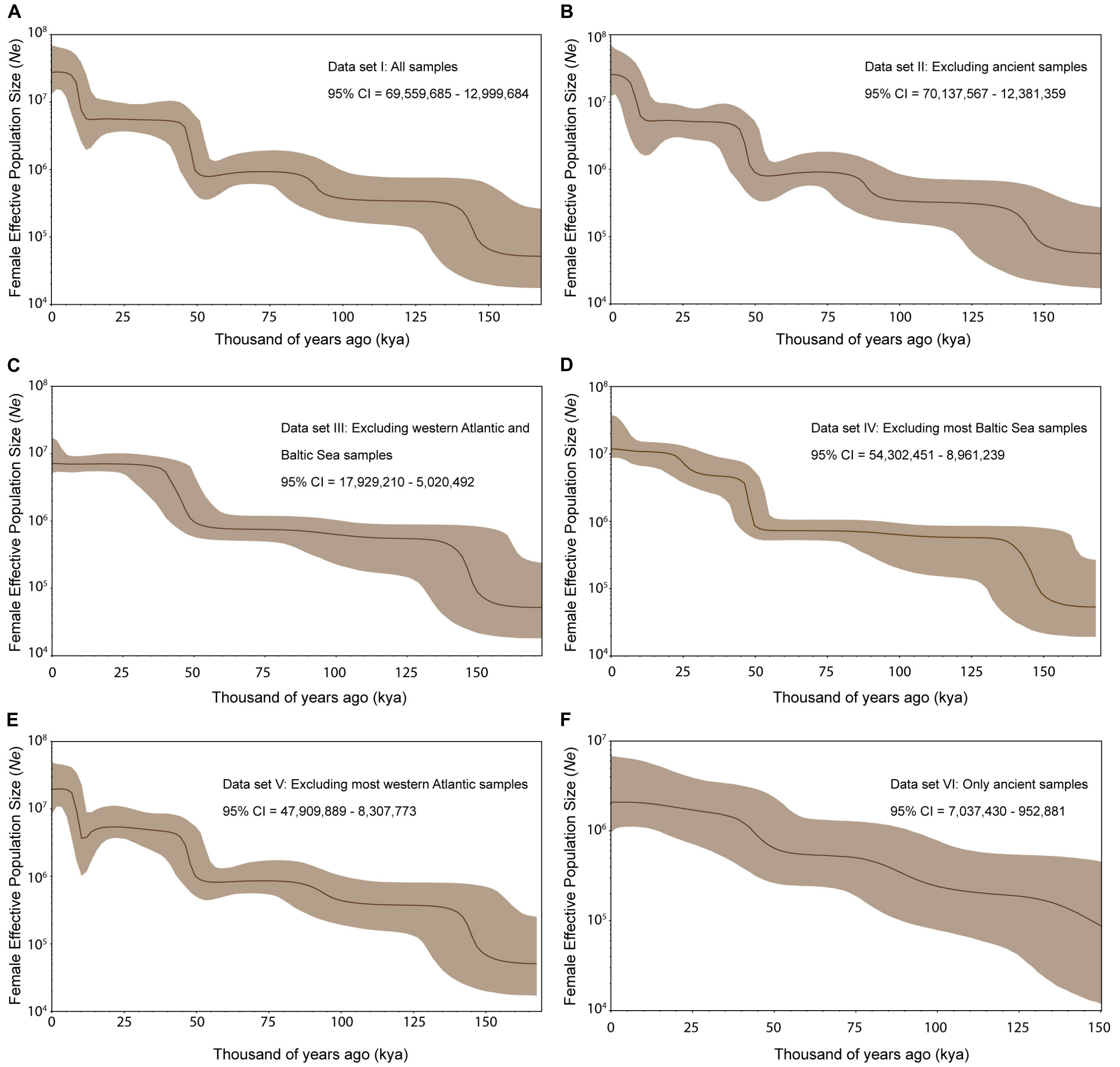

FIGURE 5 | Atlantic cod demographic history representing the fluctuations of effective female population size (*Ne;* in dark brown line) based on **(A)** data set I: all 524 sequences, **(B)** data set II: 476 modern sequences (excluding 48 ancient samples), **(C)** data set III: 273 sequences (excluding clades associated with most western Atlantic and Baltic Sea samples), **(D)** data set IV: 368 sequences (excluding the clade associated with most Baltic Sea samples), **(E)** data set V: 429 sequences (excluding clades associated with most western Atlantic samples) and **(F)** data set VI: 48 ancient sequences (excluding all modern samples). The 95% CI corresponds to the light brown area. The most recent 95% CI at time 0 is shown in each respective panel. Description of the clades excluded in data sets III, IV, and V can be found in **Supplementary Figure 7**.

(Consuegra et al., 2002) and the common bream (Ciesielski and Makowiecki, 2005). Interestingly, a loss of haplotypic variation has been identified—using CytB sequence data—for a single period (i.e., 15th to 16th centuries, out of 6 temporal periods investigated) in an Icelandic population of Atlantic cod (Olafsdottir et al., 2014). There are two potential explanations for this discrepancy. First, nearly all substitutions that comprise the CytB haplotypes can be affected by post-mortem deamination (i.e., they consist of C > T and G > A substitutions). Most of the ancient sequences (90%) investigated in Olafsdottir et al. (2014) were obtained in a single round of PCR without evaluation of such post-mortem deamination. Therefore, such bias due to post-mortem damage cannot be excluded. Second, our sampling does not include many specimens from Iceland (**Figure 1** and **Supplementary Table 1**), and it remains possible that—with 156 samples—a local effect has been observed in Olafsdottir et al. (2014), which we do not detect in our data.

Third, we do not observe major novel mtDNA lineages in the ancient data, nor observe a significant loss of such lineages over time. Instead, the majority of Atlantic cod mtDNA lineages observed in ancient and modern samples today have originated ca. 100–150 kya (**Figure 4**), during a period of population expansion (**Figure 5**). Therefore, the gain of such lineages—and associated population expansions—in Atlantic cod is more likely caused by changes in abundance driven by major historical climatic events such as eustatic oscillations in sea level, and the interglacial and warming periods experienced during the last glacial maximum ca. 23,000 kya (Bigg et al., 2008) and the Wisconsinan (ca. 110–120 kya) and Illinoian (ca. 200–130 kya) glaciations (Gibbard and Van Kolfschoten, 2005) as described by Lait et al. (2018). For instance, we only observe the most recent population expansion ca. 10 kya (**Figures 5A,B,E**) when including those mtDNA clades which are strongly associated with the Baltic Sea. The timing of this expansion is in agreement with the development of the Baltic Sea (ca. 7,000–8,000 years; Ojaveer et al., 2010; Wenne et al., 2020) which has led to genetically distinct Atlantic cod populations that have adapted to local environmental conditions (i.e., salinity and temperature; Johannesson and Andre, 2006; Berg et al., 2015; Wenne et al., 2020). Therefore, the observed changes in *Ne* reflect past population demography rather than recent and contemporary demographic changes (Lombal et al., 2020).

It is clear from zooarcheological evidence that Atlantic cod has periodically experienced intense exploitation in the distant past, particularly around the North Sea and the Baltic Sea (Barrett et al., 1999; Enghoff, 1999; Olson and Walther, 2007; Orton et al., 2011). This fishing pressure became even greater in the 19th and 20th centuries (e.g., Thurstan et al., 2010). Landings of Atlantic cod exceeded 4,000,000 tons during 1960–1990s in the North Atlantic Ocean (Shelton and Morgan, 2014). In particular, landings surpassed 600,000 tons in Iceland by ca. 1930s (Drinkwater, 2006), 354,000 tons in the North Sea during ca. 1970s (Bannister, 2004), 200–400,000 tons in the eastern Baltic during 1960–1990s (MacKenzie et al., 2002), 650,000 tons in North East Arctic between 1937 and 1938 up to 800–1,200,000 tons in ca. 1950s (Sætersdal and Hylen, 1964; Hylen, 2002). Such high levels of exploitation led to major reductions in present abundances of most Atlantic cod populations (i.e., Food and Agriculture Organization [Fao], 2020-2021a,b). Nonetheless, for the reasons discussed above, our results indicate that such population declines of Atlantic cod did not lead to a detectable impact on the mtDNA genome on the time scale we investigated here.

Taken together, our results highlight that historical and contemporaneous anthropogenic pressures such as commercial fisheries have had little impact on the ancient mitogenomic diversity of a wide-spread marine species with high gene flow such as Atlantic cod. Future ancient DNA studies should consider the inclusion of nuclear genomic data and extensive sampling on a local scale—considering a temporal comparison of specimens from the same geographical region—to assess the effects of climate and human exploitation with greater statistical power. Finally, our observations do not contradict evidence that overfishing has had negative consequences for the abundance of Atlantic cod and they do not oppose information about the important implications of genetic variation in evolutionary biology, ecology and conservation biology. Instead, our observations suggest that conservation management measures aimed toward the demographic recovery of Atlantic cod in the eastern Atlantic, if achievable by conservation management measures, will not be constrained by recent loss of historical mitogenomic variation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/ena, PRJEB42959.

AUTHOR CONTRIBUTIONS

BS and JB: conceptualization, project design, and supervision. GF and LM-G: laboratory work and data curation. LM-G with input from GF, TO, LA, BS, and JB: formal analysis. HB and MS: analytical advice. JB, IY, IJ, JH, RN, DO, SH-D, US, AH, and RB: ancient Atlantic cod specimens. FB: modern Orkney specimens. JB, IY, IJ, JH, RN, DO, SH-D, US, AH, and RB: archeological context information. LM-G and BS: data visualization. JB, BS, KJ, SJ, and DO: funding acquisition. LM-G and BS with input from JB: writing—original draft. All authors writing—review and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.671281/full#supplementary-material

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Paper II

Ancient DNA reveals a southern presence of the Northeast Arctic cod during the Holocene

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J.R.R. Tolkien

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Population ecology

Ancient DNA reveals a southern presence of the Northeast Arctic cod during the Holocene

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Climate change has been implicated in an increased number of distributional shifts of marine species during the last century. Nonetheless, it is unclear whether earlier climatic fluctuations had similar impacts. We use ancient DNA to investigate the long-term spawning distribution of the Northeast Arctic cod (*skrei*) which performs yearly migrations from the Barents Sea towards spawning grounds along the Norwegian coast. The distribution of these spawning grounds has shifted northwards during the last century, which is thought to be associated with food availability and warming temperatures. We genetically identify *skrei* specimens from Ruskeneset in west Norway, an archaeological site located south of their current spawning range. Remarkably, ^{14}C analyses date these specimens to the late Holocene, when temperatures were warmer than present-day conditions. Our results either suggest that temperature is not the only driver influencing the spawning distribution of Atlantic cod, or could be indicative of uncertainty in palaeoclimate reconstructions in this region. Regardless, our findings highlight the utility of aDNA to reconstruct the historical distribution of economically important fish populations and reveal the complexity of long-term ecological interactions in the marine environment.

1. Introduction

Significant poleward shifts in the distribution of marine species have been observed during the last century and have been associated with global warming [1]. The description of species distributions under a changing climate may yield fundamental insights into ecosystem dynamics and responses to future climate change. Nonetheless, we still have a poor understanding of the historical distribution of marine species during the late Holocene.

Atlantic cod, an economically important and highly exploited fish species in the North Atlantic Ocean, comprises various stocks with different life-history characteristics. Along the Norwegian coast, two distinct ecological ecotypes of Atlantic cod have been identified. The 'stationary' ecotype (Norwegian coastal cod, NCC) spawns along the Norwegian coast and has limited migration between spawning and feeding areas [2,3]. By contrast, the 'migratory' ecotype (Northeast Arctic cod, NEAC), also known as '*skrei*' (from the old Norse 'the wanderer'), migrates every year during winter–spring (March to beginning of

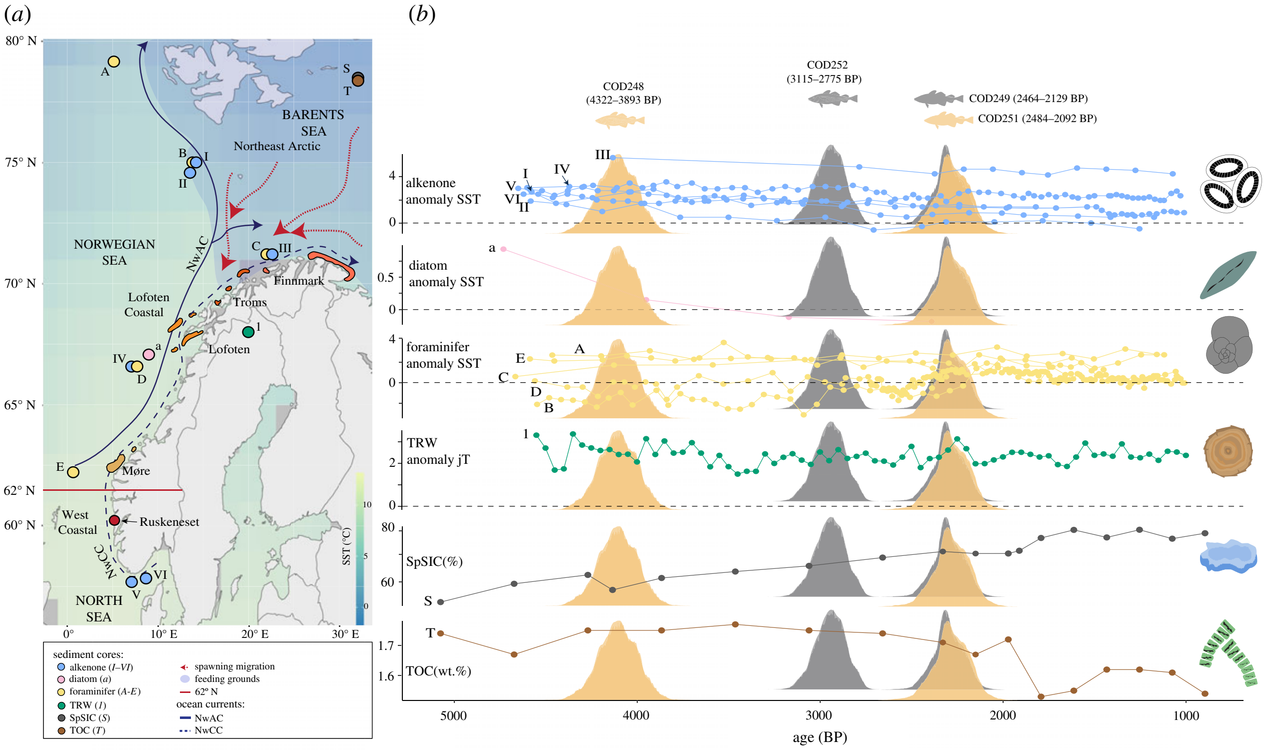

Figure 1. (*a*) Distribution of spawning sites for the migratory *skrei* ecotype from top to bottom: Finnmark (orange), Troms (dark orange), Lofoten (light orange) and Møre (light brown). Spawning map and details are adapted from Sundby and Nakken [4]. Blue arrows indicate the pathway of the NwAC and the NwCC. Red arrows indicate the spawning migration of *skrei* from feeding grounds. No *skrei* is currently observed below 62° N [3]. The background colour indicates average Norwegian and Barents Sea sea-surface temperature (SST) during January to December 2021. The distribution of the sediment core locations used in the study are highlighted per colour according to each proxy: alkenone (in blue: I, II, III, IV, V and VI), diatom (in pink: a), foraminifer (in yellow: A,B,C,D and E), tree-ring (TRW, in green: 1), spring sea-ice composition (SpSIC, in grey: S) and total organic carbon (TOC, in brown: T). (*b*) Historical climate reconstructions presented as an individual line for each sediment core for each proxy (electronic supplementary material, figure S2 for individual locations) with a dotted reference at 0° C in all temperature graphs. SST and July temperatures (jT) anomalies were calculated with respect to the long-term 1981–2010 average for their specific location (see electronic supplementary material, methods for details on long-term means). ^{14}C dating range (orange for *skrei* and grey for stationary ecotype) are shown for each ancient Atlantic cod (see electronic supplementary material, figure S1 and table S1 for details). Specimen COD253 was not dated due to insufficient bone material. Fish illustrations were drawn by Geir Holm. Tree-ring, diatom, foraminifer, alkenone, sea ice and TOC illustrations were drawn by Lourdes Martínez-García.

May) from colder feeding grounds (down to −1.5° C) in the Barents Sea towards warmer (up to 8° C) spawning areas along the Norwegian coast like Finnmark, Troms, Lofoten and Møre (figure 1*a*) [4,5]. In particular, the Lofoten archipelago has been the major spawning ground of *skrei* since at least medieval times, when relevant historical records first appeared [6]. Right after spawning, *skrei* eggs, larvae and juveniles will drift *ca* 600–1200 km towards the northeast of the Barents Sea, following the Norwegian Coastal Current (NwCC) and the Norwegian Atlantic Current (NwAC; figure 1*a*) [3,7].

Recent observations have shown a pronounced northward re-distribution of *skrei* [3,5]. The causes for this shift have been debated [2,4,8], although a northward movement of prey in the Barents Sea, directly influenced by an increase in sea temperatures, has been implicated [4]. Displacement of *skrei* feeding grounds lengthens migration distances to southern spawning locations (i.e. Møre) and could potentially influence the spawning latitude of *skrei* [5]. Nonetheless, it is unclear whether historical climate fluctuations along with ecological interactions (i.e. prey–predator interaction) have similarly influenced such distributions over longer temporal scales. Archaeological bone assemblages of *skrei* and the stationary ecotype are morphologically similar and difficult to distinguish with certainty [6]. Yet, the two ecotypes are genetically different, with significant population differentiation in several chromosomal inversions [6,9]. Genome-wide scans of such chromosomal regions allow the identification of individual *skrei* ecotypes with high confidence [6], even when using low-coverage sequence data from poorly preserved archaeological specimens [10].

We used ancient DNA (aDNA) to study the long-term spawning distribution of the stationary and migratory Atlantic cod ecotypes. We obtained genome-wide data of five archaeological specimens (*ca* 4322–2092 cal. BP) from Ruskeneset, west Norway (figure 1*a* and electronic supplementary material, table S1). Given the low latitudes of Ruskeneset, overall warmer climatic conditions and low sea-ice conditions in the Barents Sea during the late Holocene (within the past *ca* 5000 years, figure 1*b* and electronic supplementary material, figure S2) [11], and the contemporary temperature-related shifts in distribution, we expected that our ancient specimens would comprise stationary ecotypes.

2. Material and methods

(a) Sample collection and age calibration

Ancient samples ($n = 6$) retrieved in 1914–1916 at the archaeological site Ruskeneset in the municipality of Bergen, west Norway (60.23° N – 5.15° E) [12] were used to extract DNA. The zooarchaeological assemblage (bones) from Ruskeneset are in the osteological collections at the Natural History Department, the University Museum, University of Bergen.

Ruskeneset is a rock-shelter area in the western coast of Norway which preserves evidence of human activities (e.g. bones, shells and archaeological elements) dating back to the late Neolithic and Bronze Age [13]. During the Bronze Age, the shelter would have been nearly inaccessible from land due to steep cliffs on both east and west, with easier access from the seaside by boat (N. Anfinset pers. comm.). Moreover, the fishing and hunting gear findings (e.g. harpoons, hooks, arrowheads and daggers) indicate that this was a hunting and fishing station rather than a permanent coastal settlement [13]. The site is located close to tidal current channels and is at a lower latitude than the current spawning grounds of *skrei* [3,4]. Four specimens were dated using ^{14}C content (figure 1*b*; electronic supplementary material, figure S1 and table S1). Age calibration of the samples was calculated in OxCal v. 4.4.4 [14] using the Marine20 calibration curve [15]. We used slightly different ΔR values for the stationary (−164 ± 29) and *skrei* (−144 ± 46) ecotypes to account for differences in the marine reservoir effect given that these ecotypes feed either around the coast of Norway or in the Barents Sea (figure 1*b*; electronic supplementary material, figure S1) [15,16].

(b) DNA extraction and library amplification

All ancient samples were processed in the aDNA laboratory at the University of Oslo under rigorous conditions [17,18]. DNA extraction and library preparation were according to Ferrari *et al*. [19]. Ancient read data for five specimens were processed using PALEOMIX 2.13 [20]. Sequencing reads were trimmed, filtered and collapsed using AdapterRemoval v. 2.1.7 [21], and aligned to the Atlantic cod gadMor2 nuclear genome [22,23] using BWA *backtrack* v. 0.7.12 [24] with a minimum quality score of 25. DNA postmortem damage was assessed using MapDamage v. 2.0.9 [25] and the resulting BAM files were indexed with samtools v. 1.9 [26]. Additional details of the laboratory protocols are provided in the electronic supplementary material.

(c) Genomic statistical analyses

Four different chromosomal inversions associated with migratory behaviour and temperature clines were investigated (LG1, LG2, LG7 and LG12) to determine the probability of the ancient Atlantic cod specimens to be *skrei* [9,27–30]. These chromosomal inversions differ in their affinity towards a particular geographic area as previously described in Star *et al*. [6]. The BAMscorer pipeline [10] was used to assign inversion haplotypes. First, the Atlantic cod reference SNP database from Ferrari *et al*. [10] was used to associate divergent SNPs to different haplotypes. This reference SNP database includes 276 Atlantic cod individuals from three geographical locations (western Atlantic, eastern Atlantic and Baltic Sea) [31,32] across the species' range. Second, five ancient Atlantic cod specimens were compared to the reference dataset with *score_bams*. Ancient specimens were identified as *skrei* or stationary Lofoten Coastal or stationary (Norwegian) West Coastal individuals using the population specific chromosomal inversion frequencies obtained from Star *et al*. [6] and Johansen *et al*. [33].

(d) Reference palaeoclimate datasets

To describe the climate as reflected during the late Holocene, particularly during the period of the Atlantic cod ancient samples (*ca* 4322–2092 cal. BP), a range of previously published marine and terrestrial palaeoreconstructions were compiled using temperature, spring sea-ice conditions (SpSIC) and total organic carbon (TOC) reconstructions along the Norwegian coast, Scandinavia and northern Barents Sea (electronic supplementary material, table S2). The localities of these datasets overlap with the spatial distribution of spawning and feeding areas of *skrei* (figure 1*a*).

Marine palaeoreconstructions are established from reference sea-surface temperature (SST) datasets based on three different proxies: alkenone ($U^{K'}37$) [34–36], planktic foraminifer [34,37,38] and diatom assemblages [39]. Reference SpSIC dataset is based on the seasonal sea-ice biomarker IP_{25} [40], while TOC is based on the open water phytoplankton biomarkers brassicasterol and HBI III [40]. For further comparisons, SpSIC previously reported in Pieńkowski *et al*. [41] was included. This dataset includes recent observations of persisting levels of seasonal sea-ice during the Holocene Thermal Maximum (6000–10 000 cal. BP; electronic supplementary material, figure S2). The terrestrial palaeoreconstruction is established from a reference July temperature (jT) dataset based on tree-ring width (TRW) data [42]. TRW was selected because tree growth is a reliable and sensitive proxy for climatic conditions (e.g. temperatures, precipitation and drought) [43]. All temperatures are presented as an individual line for each sediment core for each proxy (figure 1*b*) and as individual graphs (electronic supplementary material, figure S2) to avoid introducing uncertainty between proxies. Full details of climate datasets are provided in the electronic supplementary material and electronic supplementary material, table S2.

3. Results

We successfully extracted aDNA from five out of six Atlantic cod specimens and radiocarbon dated four specimens (electronic supplementary material, table S1). Sequencing reads showed the patterns of DNA fragmentation and deamination rates that are associated with authentic aDNA (electronic supplementary material, figure S3). Our sequencing results yield approximately 59 million paired reads, with between 1% and 7% endogenous DNA and approximately 74 000 to approximately 1 million aligned reads for five specimens (electronic supplementary material, table S1). This is sufficient coverage to unequivocally determine the genotype of the four major chromosomal inversions of Atlantic cod (LG1, LG2, LG7 and LG12; electronic supplementary material, table S3). Two out of five specimens (40%) were identified as *skrei* with a near 100% probability (figure 2; electronic supplementary material, tables S3 and S4). The specimens were dated to three different periods approximately 4300, approximately 3100 and approximately 2400 cal. BP which is consistent with previous dates obtained for Ruskeneset [13,44]. These specimens represent the oldest genetically identified southern *skrei* to date. Although our sample size remains limited, our findings suggest a presence of *skrei* at Ruskeneset between *ca*. 4322 and 2092 cal. BP at overall warmer temperatures than present-day conditions (figure 1*b*; electronic supplementary material, figure S2).

4. Discussion

Several reasons may explain the historical *skrei* presence at lower and warmer latitudes than today. First, *skrei* could have been obtained from northern latitudes and transported to Ruskeneset. Such transport would indicate the mobility of human settlements during the late Neolithic and/or Bronze Age from northern to southern Norway. Nonetheless, bone material and artefact evidence indicate that Ruskeneset was a hunting site associated with local marine exploitation [13]. Transport of *skrei* from northern Norway to this location therefore seems improbable.

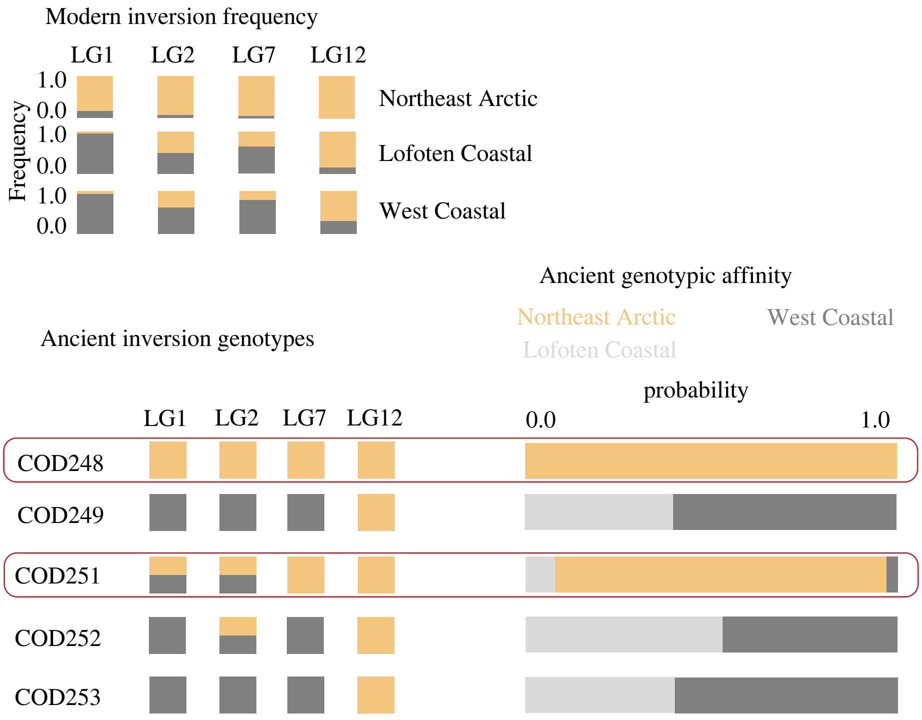

Figure 2. Modern inversion frequencies for LG1, LG2, LG7 and LG12 in Northeast Arctic, Lofoten Coastal and (Norwegian) West Coastal populations, and individual ancient inversion status. Binomial probability calculations identify two Atlantic cod specimens as *skrei* following Star *et al*. [6] (see electronic supplementary material, tables S3 and S4 for complete assignment probabilities).

Second, it is possible that the association of these inversion haplotypes with the *skrei* ecotype is of a recent evolutionary origin, and that the adaptive association we use to distinguish each ecotype has not remained stable across time. Nevertheless, the evolutionary origin of these inversions is dated to 0.4 and 1.66 million years ago, and they have been selectively maintained within the Atlantic cod populations ever since [9,30]. It would seem unlikely that the association of such evolutionary ancient genetic variants with this distinct behaviour evolved as recently as the last millennia.

Third, although no *skrei* is currently observed below 62° N (figure 1*a*), they were sporadically observed below 62° N during the start of the twentieth century [8]. The impact of fishing has been hypothesized for the current absence of such southern spawners—through the removal of larger individuals with greater capacity for migration—however, larger fish are not associated with increased migration distance [2]. The reasons for these observations, therefore, remain unknown. The archaeological southern latitude of *skrei* could reflect such sporadic spawning events, possibly during short cold spells—representing annual inter-variability—experienced within the date range of each individual and the temperature variability resolution of the palaeoclimate reconstructions (figure 1*b*; electronic supplementary material, figure S2). Moreover, the complexity of marine reservoir correction on tissues from animals feeding at latitudes higher than 50° N adds uncertainty to the precision of radiocarbon dates. Regardless, the probability of observing such sporadic southern spawning events would appear low, given that only a few specimens were sampled over a *ca* 4000 years period of natural history. Our results, therefore, tentatively suggest more frequent southern spawning of *skrei* during the late Holocene.

Finally, there may be uncertainty around the climatic reconstructions in the Barents Sea. A recent observation has identified persisting levels of seasonal sea-ice during the entire Holocene Thermal Maximum (6000–10 000 cal. BP) in this region [41] (electronic supplementary material, figure S2). Consequently, as the climate in the Barents Sea further cooled during the late Holocene (*ca* 5900 cal. BP to present) [11], this region may have had reduced primary productivity and more significant ice cover than currently estimated [40]. Such a scenario could have resulted in more southern located feeding grounds and decreasing migration distance towards lower latitude spawning areas. Our observations would agree with such more extensive presence of sea-ice than currently assumed during the late Holocene in the Barents Sea.

Taken together, we here identify the oldest known migratory ecotype in an archaeological Atlantic cod fishbone assemblage. Although the reasons for their southern distribution during the late Holocene remain unclear, our results highlight the utility of aDNA to reconstruct the historical distribution of economically important fish populations. Our findings indicate that the response of marine species to present-day and future climate change may be more complex than currently anticipated.

Ethics. No modern specimens were sampled for this study. Sampling of the zooarchaeological assemblage (bones) from Ruskeneset was approved by the University Museum, Department of Culture, Bergen with reference no. 2018/13857.

Data accessibility. Modern reference raw sequence data have been released earlier under ENA accession nos. PRJEB29231 and PRJEB41431. The raw reads for the ancient specimens for this study are released under ENA accession no. PRJEB49220.

The data are provided in the electronic supplementary material [45].

Authors’ contributions. L.M.-G.: conceptualization, data curation, formal analysis, visualization, writing—original draft and writing—review and editing; G.F.: data curation, methodology and writing—review and editing; A.K.H.: resources and writing—review and editing; K.S.J.: funding acquisition and writing—review and editing; S.J.: funding acquisition and writing—review and editing; J.H.B.: conceptualization, formal analysis, funding acquisition, resources, supervision, visualization and writing—review and editing; B.S.: conceptualization, formal analysis, funding acquisition, project

administration, supervision, visualization, writing—original draft and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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Paper III

Ancient DNA evidence for the ecological globalization of cod fishing in medieval and post-medieval Europe

To the sea, to the sea! The white gulls are crying.
The wind is blowing, and the white foam is flying.
West, west away, the round sun is falling.
Grey ship, grey ship, do you hear them calling,
the voices of my people that have gone before me?
J.R.R. Tolkien

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Ancient DNA evidence for the ecological globalization of cod fishing in medieval and post-medieval Europe

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Understanding the historical emergence and growth of long-range fisheries can provide fundamental insights into the timing of ecological impacts and the development of coastal communities during the last millennium. Whole-genome sequencing approaches can improve such understanding by determining the origin of archaeological fish specimens that may have been obtained from historic trade or distant water. Here, we used genome-wide data to individually infer the biological source of 37 ancient Atlantic cod specimens (*ca* 1050–1950 CE) from England and Spain. Our findings provide novel genetic evidence that eleventh- to twelfth-century specimens from London were predominantly obtained from nearby populations, while thirteenth- to fourteenth-century specimens were derived from distant sources. Our results further suggest that Icelandic cod was indeed exported to London earlier than previously reported. Our observations confirm the chronology and geography of the trans-Atlantic cod trade from Newfoundland to Spain starting by the early sixteenth century. Our findings demonstrate the utility of whole-genome sequencing and ancient DNA approaches to describe the globalization of marine fisheries and increase our understanding regarding the extent of the North Atlantic fish trade and long-range fisheries in medieval and early modern times.

1. Introduction

The expansion of long-range fish trade, not least of Atlantic cod (*Gadus morhua*), has partly driven the development of urbanized market economies across European societies during the last millennium [1–3]. The importance of this trade is well documented by historical sources from the fourteenth century and can be glimpsed in anecdotal historical records and archaeological evidence from the late eleventh, twelfth and thirteenth centuries [4,5].

Ancient DNA (aDNA) and stable isotopes have previously shown the early transport of air-dried Arctic Norwegian cod (*stockfish*) to Haithabu in Germany by *ca* 1066 CE [6,7]. This exchange developed into a major and wide-ranging Atlantic cod trade across medieval northern Europe, linking towns in Scandinavia, Germany, England and the Low Countries (e.g. Bergen, Lübeck, King's Lynn, London and Deventer) [6–8]. In the Iberian Peninsula, the northern ports were developed as strategic trading posts for receiving and distributing luxury and foreign products from both the Mediterranean and northern Europe [9,10]. As a consequence, distant-water fisheries and fish trade along the Atlantic coast, from Sevilla to western Ireland and Flanders, started to receive more interest within the Iberian market [11,12]. Subsequently, post-medieval European expansion to the western Atlantic, especially to Newfoundland, linked the above-mentioned northern and Iberian networks into competing and sometimes complementary long-range fisheries that were sources of both food and wealth [1,13]. For example, seventeenth-century English catches from Newfoundland were often traded to southern Europe, in an economically significant triangular trade that also entailed salt and wine [14].

Tracing the origin of Atlantic cod specimens harvested for these medieval and post-medieval trade networks contributes to our understanding of economic history and historical ecology. Historical and archaeological sources have revealed the extension of distant-water fisheries and trading networks through time and space [8,13]. However, the geographical and biological resolution of text-based and archaeological sources is often limited, and the level of detail in such sources often decreases with time depth [15]. Determining the biological origin of archaeological bone assemblages of species such as Atlantic cod can therefore provide important information about the populations targeted through distant-water fishing and/or trade. Since archaeological cod bones can represent local or long-distance (even intercontinental) fishing, it is important to distinguish between source populations. Thus, there has been an increased interest in the use of aDNA and stable isotope methods to identify the origin of archaeological remains to trace the development of the globalization of marine fisheries [6,16–20]. Here, we use novel whole-genome aDNA approaches to greatly improve the spatial specificity and resolution regarding the inference of source populations of archaeological Atlantic cod bones [21].

We assess the biological origin of 37 Atlantic cod specimens from medieval England (London) and post-medieval Spain (Barcelona, Álava (Castillo de Labastida), Madrid and Sevilla) using low-coverage genome-wide data. We genetically assign such specimens according to patterns of spatial genome-wide differentiation among modern populations of Atlantic cod [22–25]. We specifically investigated significant differentiation in polymorphic chromosomal inversions (i.e. LG1, LG2, LG7 and LG12) [6,26,27] that are associated with migratory behaviour and temperature clines [22,27–30]. Their genetic differentiation can therefore indicate the assignment of specimens towards a particular geographical area [6,31]. Through these methods, we aim to distinguish source populations with improved discriminating power in relation to previous stable isotope and aDNA approaches [6,15,16].

2. Materials and methods

(a) Sample collection

English samples ($n = 32$) were obtained from eight archaeological locations in London (figure 1*b*; electronic supplementary material, table S1). Based on archaeological evidence and stable isotope analysis, three of the locations (Finsbury Pavement, Seal House and Trig Lane) have previously been inferred to have imported preserved cod [16]. Specimens from the additional five locations (Billingsgate 1982, Cheapside 120, New Fresh Wharf, Nonsuch Palace and Swan Lane) were included to provide a continuous fishing time series from the eleventh to sixteenth–seventeenth centuries CE (figure 1*c*). Seven of the archaeological sites in London were urban when occupied. The eighth location (Nonsuch Palace) was a royal residence originally outside London, which was later surrounded by the modern metropolis. Spanish samples ($n = 5$) were obtained from four different archaeological locations: a monastic-upper class context from La Cartuja (Sevilla, late fifteenth–early sixteenth centuries), an urban context from Plaza de Oriente (Madrid, seventeenth–eighteenth centuries), a context from the fishermen's quarter from Barraques de pescadors (Barcelona; *ca* seventeenth century) and a rural castle (Álava) that acted as a military centre during the nineteenth century (JA Quirós 2022, personal communication; figure 1*a*; electronic supplementary material, table S1). Atlantic cod bones are very rare in Iberian archaeological sites [12]; therefore, the present five Spanish specimens represent those available for this study.

Cranial (articular, premaxilla, frontal, dentary and parasphenoid) and postcranial (vertebra and cleithrum) bones were included (electronic supplementary material, table S1 and figure S2). Cranial bones are more likely to represent local fishing because many preserved fish products were decapitated [32], although, complete fish (and/or preserved fish heads) were sometimes traded over long distances [6,33,34]. Cleithra (which support the pectoral fin just behind the cranium) can be found together with cranial remains, or (if fish were decapitated anterior to this element) with postcranial bones [15]. Here, we considered cleithra belonging among the postcranial bones.

After field collection, all samples were stored dry and unfrozen. Dating of the samples was based on archaeological context. Qualitative date ranges were converted into calendar years as per Orton *et al*. [15] considering an 'early' century the first half of that century (e.g. '00 to '50), 'mid' century as '25 to '75 and 'late' century as the second half of that century (e.g. '50 to '00). The archaeological Atlantic cod samples were morphologically and genetically identified to species.

(b) aDNA extraction and library preparation

We processed 18 English (London) fish-bone samples in the aDNA laboratory at the University of Oslo [35,36] (electronic supplementary material, table S1). Treatment of samples prior to DNA extraction was according to Ferrari *et al*. [37] and Martínez-García *et al*. [38]. In short, fish bones were UV treated for 10 min per side and milled using a stainless-steel mortar [39]. Milled fish-bone powder was divided in two aliquots per specimen (150–200 mg per aliquot) as starting material for DNA extraction. Genomic DNA was extracted from the fish-bone samples using the mild Bleach treatment and Double-Digestion step (BleDD) protocol [40]. In addition, we added to our initial

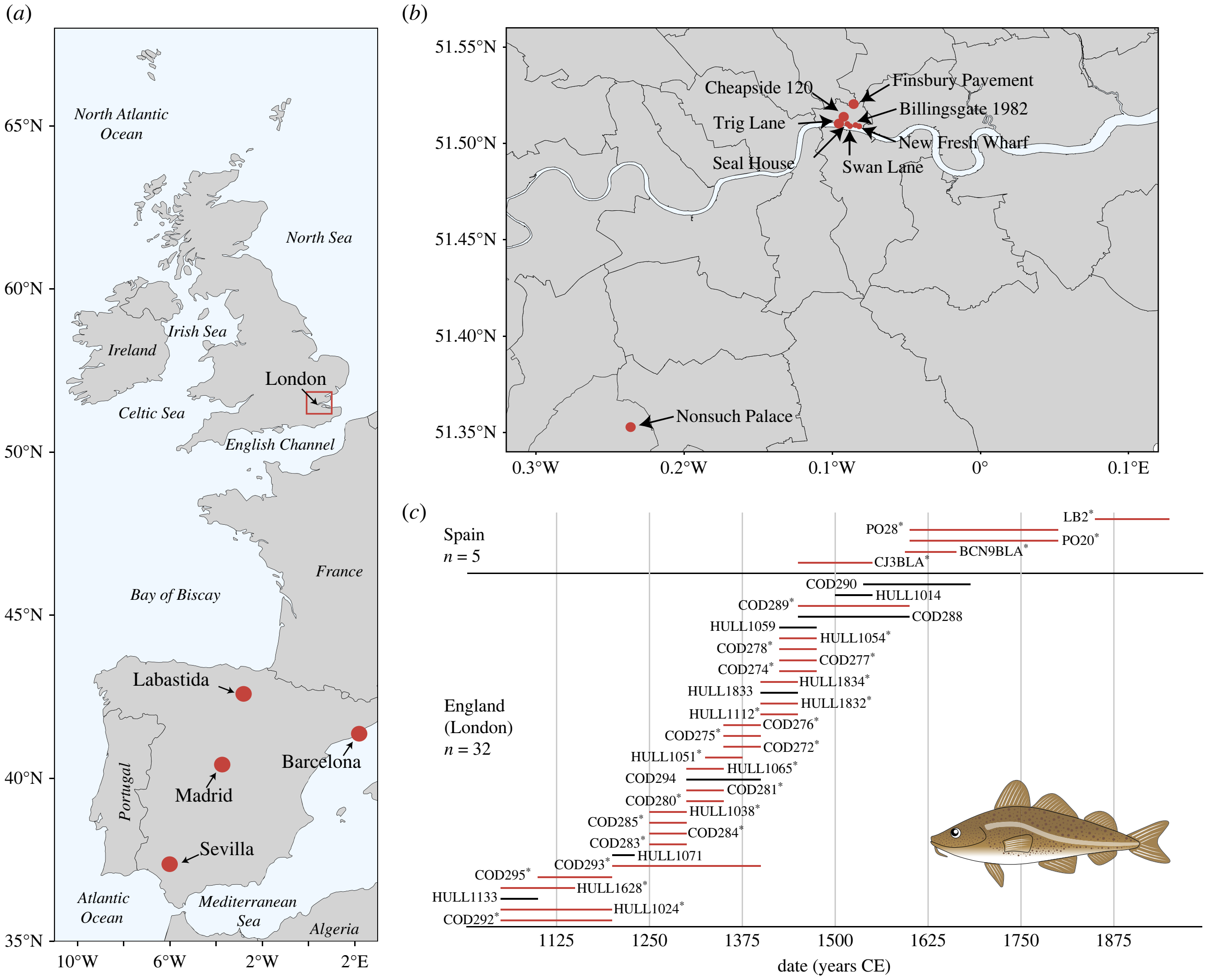


Figure 1. (*a*) Distribution of archaeological Atlantic cod specimens in England (London) and Spain. Spanish locations are highlighted (in red) on the map. (*b*) Detailed distribution (in red) of English archaeological locations in London from which Atlantic cod bones were obtained. (*c*) Date range of the 37 archaeological specimens as estimated based on archaeological context. Samples in red and with 'asterisk' (*) yielded sufficient data to allow more detailed genomic assignments. Other samples could only be assigned to major geographical regions (see Results section for explanation). For details regarding the sample codes, see electronic supplementary material, table S1. Fish illustration was drawn by Lourdes Martínez-García. (Online version in colour.)

London assemblage aDNA from 14 English fish-bone samples previously processed at the University of Hull following the protocols in Hutchinson *et al*. [19] (electronic supplementary material, table S1). Three out of the 14 samples were previously inferred to have a southern and central North Sea biological origin (electronic supplementary material, table S1) [19]. Furthermore, we analysed aDNA from five Spanish bone samples previously analysed and processed using a modified protocol of Yang *et al*. [41] at BioArch, University of York. In short, samples were decontaminated with 6% sodium hypochlorite (bleach) for 5 min and then rinsed three times in distilled water. Samples were further UV treated for 10 to 20 min per side. Samples were powdered prior to the addition of a lysis buffer (EDTA) and Proteinase K. Samples were incubated overnight at 50°C while kept in rotation. After incubation, samples were centrifuged to separate bone powder from buffer solution. The supernatant was transferred to an Amicon Ultra-4, Centrifugal Filter Device, 10 000 NMWL tube to concentrate the solution and the Qiagen QiaQuick MinElute™ kit was used for DNA purification. Contamination controls were taken during every step of the extraction and amplification procedure.

Double-indexed blunt-end sequencing libraries were built from 16 or 20 µl of DNA extract from all samples using the double-stranded Meyer-Kircher protocol [42,43] with the modifications listed in Schroeder *et al*. [44] or the single-stranded Santa Cruz Reaction (SCR) protocol using tier four adapter dilutions [45] (see electronic supplementary material, table S1 for specifications). Multiple extraction and negative controls during all library sessions were used to detect possible contamination. All samples were assessed for library quality and concentration using a high-sensitivity DNA Assay on the Bioanalyzer 2100 (Agilent) or with a high-sensitivity NGS Fragment Analysis Kit on the Fragment AnalyzerTM (Advanced Analytical). Successful libraries were sequenced using the Illumina HiSeq 4000 with 150 bp paired reads, or on a Novaseq 6000 with 150 bp paired reads at the Norwegian Sequencing Centre. Sequencing reads were demultiplexed allowing zero mismatches in the index tag and they were processed using PALEOMIX v1.2.13 [46]. Trimming of residual adapter contamination, filtering and collapsing of reads was done using AdapterRemoval v.2.1.7 [47]. Mapping of remaining reads was performed against the gadMor2 reference genome [48,49] using BWA v.0.7.12 [50] with the *backtrack* algorithm, disabled seeding and minimum quality score of 25. aDNA deamination patterns were determined using MapDamage v.2.0.9 [51] and BAM files were indexed with samtools v.1.9 [52].

(c) Genomic and statistical analysis

To infer the biological origin (source population) of the archaeological samples of Atlantic cod, we followed a hierarchical procedure (figure 2). First, we used BAMscorer, a software that can assign low-coverage sequences to biological populations following the methodology described in Ferrari *et al*. [21]. This approach creates databases of spatially divergent genetic data, analysing high-coverage whole-genome data of modern individuals of known provenance. These modern specimens were obtained from Barth *et al*. [53] and Pinsky *et al*. [24] and comprise 276 Atlantic cod individuals that represent three broad geographical regions of the species' range: the western Atlantic Ocean, the eastern Atlantic Ocean and the Baltic Sea. All of these regions are genetically differentiated and represent potential sources of distant-water fishing and fish trade over the chronology of our study [24,53]. Three major genetic clusters (here named western Atlantic, eastern Atlantic and Baltic Sea) can be identified and genetically assigned using genome-wide data (excluding the four large chromosomal inversions in Atlantic cod: LG1, LG2, LG7 and LG12, figure 2*a*). Based on such genome-wide differentiation, the first assignment was used to determine an overall eastern or western Atlantic Ocean origin. Subsequently, using a similar approach, all specimens first assigned to a putative eastern Atlantic region were analysed to determine a possible Baltic Sea origin [21].

Then, again using the BAMscorer pipeline [21], we identified specific individual genotypes of the four large chromosomal inversions in our low-coverage ancient samples. These inversions have highly divergent genetic haplotypes [22,27–30] whose spatial distributions show elevated differentiation between different Atlantic cod ecotypes and spawning regions within the western and eastern Atlantic cluster [22–25]. Therefore, these genotypes can be used to further assign each ancient specimen to a more specific source population within these three genome-wide clusters (electronic supplementary material, table S2). For this, we used the binomial sampling method as per Star *et al*. [6], to infer the overall probability of obtaining a specific composite inversion genotype based on the inversion frequencies of the specific source populations [22–25]. In short, this approach investigates if ancient individuals statistically differ in their affinity towards a particular modern population based on their inversion genotypes. The probability of obtaining an individual inversion genotype follows a binomial distribution given the underlying allele frequencies of a modern population. Since the inversions are located on different chromosomes, we can assume independence between inversion loci. We then calculated the overall probability of obtaining a composite ancient inversion genotype—based on the modern populations' respective allele frequencies—as a measure of an individual's affinity toward a specific population (electronic supplementary material, table S2).

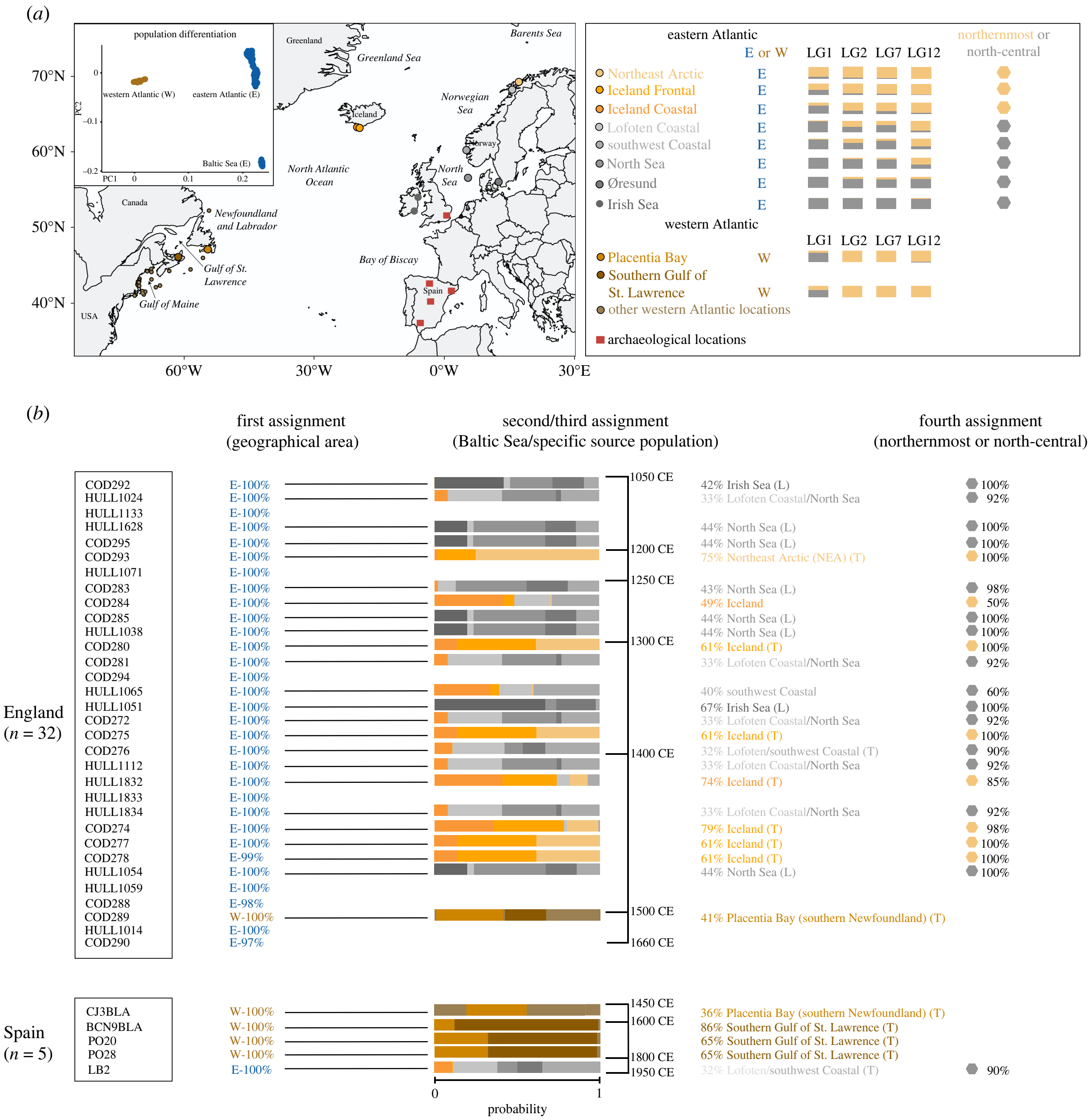
Given the low-coverage data, we used the negative controls from the library sessions as a baseline to include or exclude specimens for specific genomic assignments. We included those samples with greater than 2000 aligned reads and at least one magnitude more of nuclear coverage and endogenous DNA than our negative controls. Subsequently, we only infer the genotype of samples with at least three chromosomal inversions with greater than 75% probability (see details in electronic supplementary material, tables S1 and S2). Comparative modern inversion frequency data were compiled for a range of different populations [22–25]. From the eastern Atlantic, we included: the Northeast Arctic, the Norwegian Coast (Lofoten and the southwest), the North Sea, the Irish Sea, Øresund and Iceland (both coastal and frontal ecotypes, which differ in their migratory behaviour). Western Atlantic populations included a number of populations south and north of Newfoundland (figure 2*a*; electronic supplementary material, tables S3 and S4). The highest specific assignment probabilities are reported as percentages (%) in tables and figures, representing the confidence with which one individual is assigned to a specific population (i.e. 75%). In the event of similar assignment probabilities for more than one population (e.g. 50% and 50%), both populations are reported as the putative origin of the individual (i.e. Norwegian Coast or North Sea, see electronic supplementary material, tables S3 and S4 for specific details of assignment percentages). Finally, we recognized that most eastern Atlantic individuals could be further classified with high confidence towards two spatially distinct groups; an overall assignment to a northernmost (Northeast Arctic and Iceland) or north-central (Norwegian Coast, North Sea, Irish Sea and Øresund) distribution (by adding the scaled probabilities of source populations; see details in the electronic supplementary material, tables S3 and S4).

We performed a Fisher's exact test to assess for the existence of an association between bone element and specimen origin (i.e. sourced through trade versus local landings from the North Sea or Irish Sea). The test was implemented in the *stats* and *ggstatsplot* libraries in R [54,55] using 22 samples that were assigned to a specific source population (figure 2*b*; electronic supplementary material, S3). We excluded samples with a northernmost or north-central assignment with less than 75% probability and samples with an indistinguishable origin (see Results section for more details).

3. Results

We sequenced 37 specimens and obtained a total of approximately 342 million paired reads, approximately 9 million aligned reads and endogenous DNA content between less than 0.01% and 34% (electronic supplementary material, table S2). Sequencing reads showed the patterns of DNA fragmentation and deamination rates that are consistent with those of authentic aDNA (electronic supplementary material, figure S1). We successfully assigned these 37 sequenced specimens to one of the two broad geographical areas (eastern or western Atlantic Ocean), finding a total of five samples (England = 1, Spain = 4) from the western Atlantic and 32 specimens (England = 31, Spain = 1) from the eastern Atlantic (figure 2*b*; electronic supplementary material, tables S1 and S2). Within the eastern Atlantic specimens, we found two that could be assigned to the Baltic Sea at 54% and 98% probability (electronic supplementary material, table S2), however, because of low numbers of obtained sequence reads such assignments were not considered for our final chronology. Subsequently, we assigned a total of 29 out of the 37 samples (England = 24, Spain = 5) to a more specific source population based on the assignment probabilities of their composite inversion genotypes (electronic supplementary material, table S2; figure 2*b*). For samples assigned to a specific source population, we could identify 102 out of 116 inversion genotypes with more than 95% probability (electronic supplementary material, table S2). Specific assignments are dependent on the source populations provided for comparison, which can result in low probability assignments as several populations can share similar inversion genotype frequencies (e.g. less than 50% probability; figure 2*b*). In agreement with postcranial bones being commonly assigned to non-local sources, we found a statistically significant association between the bone element (cranial or postcranial) and the origin (local or traded, respectively) of the specimen ($p = 0.01$; electronic supplementary material, table S1 and figures S2 and S3).

For London, of the 31 specimens assigned to the eastern Atlantic Ocean, 24 passed the initial quality baselines required for specific assignment analysis and had sufficient data for estimating genotypes of inversion loci. We assigned 15 specimens to a north-central haplotype group (with greater than 60%



COD292	E-100%	42% Irish Sea (L)	100%
HULL1024	E-100%	33% Lofoten Coastal/North Sea	92%
HULL1133	E-100%		
HULL1628	E-100%	44% North Sea (L)	100%
COD295	E-100%	44% North Sea (L)	100%
COD293	E-100%	75% Northeast Arctic (NEA) (T)	100%
HULL1071	E-100%		
COD283	E-100%	43% North Sea (L)	98%
COD284	E-100%	49% Iceland	50%
COD285	E-100%	44% North Sea (L)	100%
HULL1038	E-100%	44% North Sea (L)	100%
COD280	E-100%	61% Iceland (T)	100%
COD281	E-100%	33% Lofoten Coastal/North Sea	92%
COD294	E-100%		
HULL1065	E-100%	40% southwest Coastal	60%
HULL1051	E-100%	67% Irish Sea (L)	100%
COD272	E-100%	33% Lofoten Coastal/North Sea	92%
COD275	E-100%	61% Iceland (T)	100%
COD276	E-100%	32% Lofoten/southwest Coastal (T)	90%
HULL1112	E-100%	33% Lofoten Coastal/North Sea	92%
HULL1832	E-100%	74% Iceland (T)	85%
HULL1833	E-100%		
HULL1834	E-100%	33% Lofoten Coastal/North Sea	92%
COD274	E-100%	79% Iceland (T)	98%
COD277	E-100%	61% Iceland (T)	100%
COD278	E-99%	61% Iceland (T)	100%
HULL1054	E-100%	44% North Sea (L)	100%
HULL1059	E-100%		
COD288	E-98%		
COD289	W-100%	41% Placentia Bay (southern Newfoundland) (T)	
HULL1014	E-100%		
COD290	E-97%		
CJ3BLA	W-100%	36% Placentia Bay (southern Newfoundland) (T)	
BCN9BLA	W-100%	86% Southern Gulf of St. Lawrence (T)	
PO20	W-100%	65% Southern Gulf of St. Lawrence (T)	
PO28	W-100%	65% Southern Gulf of St. Lawrence (T)	
LB2	E-100%	32% Lofoten/southwest Coastal (T)	90%

Figure 2. (*a*) Geographical distribution of inversion frequencies of chromosomal inversions in Atlantic cod (LG1, LG2, LG7 and LG12) across the North Atlantic Ocean. The assignment of specific haplotypes to a geographical area is either eastern (E, in blue) or western (W, in brown) Atlantic. The population PCA plot was modified from Ferrari *et al*. [21] and shows the differentiation between the eastern and western Atlantic Oceans, and the Baltic Sea (in blue as it is located within the eastern Atlantic Ocean). Specific alleles associated with a northernmost composite genotype distribution are assigned in orange. Alleles associated with a more temperate north-central genotype distribution are assigned in grey. The archaeological locations are indicated with red squares. The modern populations used as possible source populations of our ancient specimens are located in the map. (*b*) First genomic assignment: overall percentages (%) represent the minimum probability obtained to be from either the eastern or the western Atlantic Ocean. Second/third genomic assignment: source population percentages (%) represent the highest probability to be assigned to a specific modern location (electronic supplementary material, tables S2–S4). Iceland assignment is obtained by adding the probabilities of both frontal and coastal Icelandic ecotypes. Only two locations from the western Atlantic region have divergent inversion genotype frequencies; thus, they have a specific assignment colour (Placentia Bay and Southern Gulf of St. Lawrence). Other western Atlantic locations are represented in light brown colour (electronic supplementary material, tables S3 and S4). The approximate age (CE) of the specimens is indicated on the right side of the bar plot. Specific time periods are found in figure 1, the electronic supplementary material, table S1 and figure S2. A local (L) or traded (T) assignment follows putative source population. Specimens are considered to be of local origin with considerable North Sea or Irish Sea assignments, specimens are considered to have been obtained through trade with significant Northeast Arctic, Norwegian coast, Iceland or western Atlantic assignments. Individuals with ambiguous origin (i.e. HULL1024, COD281, COD272, HULL1112 and HULL1834) or with a northernmost or north-central origin below 75% probability (i.e. COD284 and HULL1065) are not identified as local or traded in this chronology. Individuals COD276 and LB2 are identified as traded specimens as their likely origin is a remote population: Norwegian coast (Lofoten or southwest). Fourth assignment: percentages (%) representing either the northernmost or north-central genotype distribution after adding the scaled probabilities of selected source populations. For details of the sample codes, see electronic supplementary material, table S1. (Online version in colour.)

probability). This includes eight specimens with a possible source population like the North Sea or the Irish Sea (42–67% probability), one specimen with a southwest Norwegian Coast origin (40% probability) and six specimens with indistinguishable associations to the Norwegian Coast (both Lofoten and southwest) and the North Sea (32–33% probability). Nonetheless, these within-group assignments are not strongly supported as the inversion frequency differences of the reference populations are limited (figure 2*a*). Similarly, we assigned seven specimens to a northernmost composite genotype group (with greater than 85% probability), where we found one specimen possibly coming from northern Norway (Northeast Arctic, 75% probability) and six specimens likely coming from Iceland (61–79% probability). We calculated an overall Icelandic origin by adding the probabilities of being Icelandic frontal or coastal ecotypes. The genomic distinction between Icelandic cod and Northeast Arctic cod is predominantly driven by the higher frequency of north-central genotypes (in grey figure 2*a*) for the chromosomal inversion LG01 in Iceland [22]. However, similar inversion frequencies (for LG01) between deep water Icelandic cod and Northeast Arctic cod have been reported [56]. Considering such similarities, the assignments to Iceland or the Northeast Arctic should be taken with caution. Moreover, we found an unreliable assignment for one specimen to a northernmost or north-central composite genotype group (with 50% probability), resulting in an Icelandic assignment with low confidence (49%; figure 2*b*; electronic supplementary material, table S3). Our specific genomic assignments further agree with a presumed local origin of two specimens included in our final chronology previously used as 'local' (control) samples for aDNA analysis in Hutchinson *et al*. [19] (electronic supplementary material, table S1). These samples (HULL1038 and HULL1065) have a north-central haplotype group distribution with 60% and 100% probability, respectively. In combination with the significant association between the bone element (cranial or postcranial) and the origin (local or traded) of a specimen, these bones are therefore likely of a local origin. Finally, as noted above, we assigned one London specimen (dated between the late fifteenth and sixteenth centuries) to a western Atlantic origin (with 100% probability) including a possible low confidence assignment to Placentia Bay (41% probability; figure 2*b*; electronic supplementary material, table S3).

For Spain, we found four specimens assigned to the western Atlantic and one specimen assigned to the eastern Atlantic (figure 2*b*). The assignments to the western Atlantic (with 100% probability) tentatively included source populations along southwestern Newfoundland (Placentia Bay) or the Gulf of St. Lawrence (with 36–86% probability). We assigned the eastern Atlantic specimen (with 90% probability) to a north-central composite genotype group which includes the Norwegian Coast as a putative origin (indistinguishable association to the Lofoten and southwest coast with 32–33% probability; figure 2*b*; electronic supplementary material, table S4).

4. Discussion

We have used a novel genomic assignment approach to identify the biological source of individual archaeological Atlantic cod specimens from England and Spain. With high confidence, we assigned fish remains to a large-scale geographical origin (up to 100% assignment probability) and genotype groups within regions (greater than 85% assignment probability). With moderate to low confidence (less than 86% assignment probability), we tentatively identified several of the specimens to more specific spatially constrained populations. Below we describe the resulting spatio-temporal patterns observed in England and Spain, and consider the impact these findings have on our understanding of the globalization of marine fisheries over the last millennium.

(a) London: an increasingly North Atlantic trade

Previous zooarchaeological evidence and stable isotope data [15,16] implied that Atlantic cod trade stemmed predominantly from local fisheries during the eleventh to twelfth centuries, after which longer distance imports appeared during the thirteenth to fourteenth centuries. Our individual assignments provide confident aDNA evidence that supports this chronology, with fish assigned to northern Atlantic regions appearing in increasing frequency over time. Assignments to specific source populations are often associated with lower individual probabilities (approx. 32% probability); therefore, these should be considered as indicative only. Interestingly, our assignment analysis indicates that imported Atlantic cod dated between the thirteenth and the fourteenth centuries derived not only from northern Norway but possibly from Iceland (figure 2*b*). According to historical evidence, Iceland first became a major supplier of dried cod to England during the fifteenth century, when fishermen and merchants from England and Germany first defied the Norwegian royal monopoly on trade with Iceland [57,58]. However, exports of *stockfish* from Iceland via Norway commenced *ca* 1300 CE or earlier [59,60]. Our results are consistent with this chronology. Icelandic cod in medieval London would probably have reached England via Bergen, on Norwegian, English, and/or Hanseatic ships that are known to have traded between Norway and ports of the English east coast [61,62].

Furthermore, England's participation in the western Atlantic cod fisheries expanded in south-eastern Newfoundland during the late sixteenth century (figure 2*b*; electronic supplementary material, table S4) [63]. Therefore, the observation of a specimen from the western Atlantic dated to the late fifteenth to the sixteenth centuries currently represents one of the earliest known genetic examples of this trans-Atlantic expansion. This chronology is consistent with existing knowledge regarding the emergence of trans-Atlantic cod trade, although, as discussed further below, English catches in North America were often destined for southern Europe rather than home markets like London [14,64].

(b) Spain: an increasingly trans-Atlantic trade

Similar to the single late fifteenth- to sixteenth-century London cod bone, our findings in Spain are consistent with known western Atlantic fishing expansion of the early modern period [65]. In a historical context, the Basques and Galicians provided Atlantic cod for Spain throughout the sixteenth century [64–66]. The fifteenth- to sixteenth-century sample from Spain assigned to waters of the western Atlantic is consistent with historical sources, which indicate that Basque fishermen (from Spain and France) and Galicians acquired fish from southern to western Newfoundland (i.e. Placentia Bay, the Gulf of St. Lawrence, St. Pierre and

Miquelon) to fulfil the demand for Atlantic cod in Spain [65,67–70]. The three later specimens (seventeenth century and seventeenth to eighteenth centuries) could have derived from Spanish fishermen operating in Newfoundland [66,70] or as trade items with English or French fishermen that had been operating in these grounds since the sixteenth century. In fact, the English engaged in unofficial trade even when political hostilities disrupted relations with Spain [64]. These specimens may thus relate to the triangular trade involving an exchange of Atlantic cod from the western North Atlantic for wine and salt from southern Europe [14]. By the late nineteenth to early twentieth century, dried Atlantic cod from across the Norwegian waters could have been used to provision the military centre in Álava (JA Quirós 2022, personal communication). Our most recent Spanish sample originating from the Norwegian Coast (figure 2*b*; electronic supplementary material, table S4) is therefore consistent with the supply of northern European air-dried Atlantic cod since the eighteenth century [71,72].

5. Conclusion

Altogether, our results provide genetic evidence for an expanding trade and increasing demand for marine fish leading to the exploitation of a great diversity of distant-water sources already in the Middle Ages. Our evidence also tracks the culmination of the marine fisheries extension with European exploitation of the western Atlantic fishing grounds around Newfoundland, starting in the sixteenth century. Our findings emphasize the utility of whole-genome sequencing and aDNA methods to describe the increasing demand for Atlantic cod for European societies during medieval and post-medieval periods. We expect that the inclusion of more archaeological sites and larger sample sizes through space and time will reveal additional patterns about those populations targeted for long-distance trade in the past and possibly provide greater insight about which populations have experienced long-term impacts of fisheries. Overall, our results corroborate and significantly increase existing knowledge about the globalization of marine fisheries and fish trade in medieval and early modern times.

Data accessibility. The raw reads for the ancient specimens are released under the ENA accession number PRJEB52865. This manuscript is available as a pre-print in BioRxiv at https://www.biorxiv.org/content/10.1101/2022.06.03.494519v1 [73].

The data are provided in the electronic supplementary material [74].

Authors' contributions. L.M.-G.: conceptualization, data curation, formal analysis, methodology, visualization, writing—original draft and writing—review and editing; G.F.: data curation, methodology and writing—review and editing; A.C.: methodology and writing—review and editing; L.M.A.: methodology and writing—review and editing; B.L.-A.: methodology and writing—review and editing; M.C.: methodology and writing—review and editing; L.L.-R.: methodology, resources and writing—review and editing; A.M.-M.: resources and writing—review and editing; E.R.-I.: resources and writing—review and editing; J.A.Q.: resources and writing—review and editing; R.M.-M.: resources and writing—review and editing; B.H.: resources and writing—review and editing; W.H.: funding acquisition, resources and writing—review and editing; K.S.J.: funding acquisition and writing—review and editing; S.J.: funding acquisition and writing—review and editing; D.O.: funding acquisition, resources and writing—review and editing; B.S.: conceptualization, funding acquisition, project administration, supervision, visualization and writing—review and editing; J.H.B.: conceptualization, funding acquisition, project administration, resources, supervision and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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