Tracing the Early Origins of the Atlantic Herring Trade Using Ancient DNA

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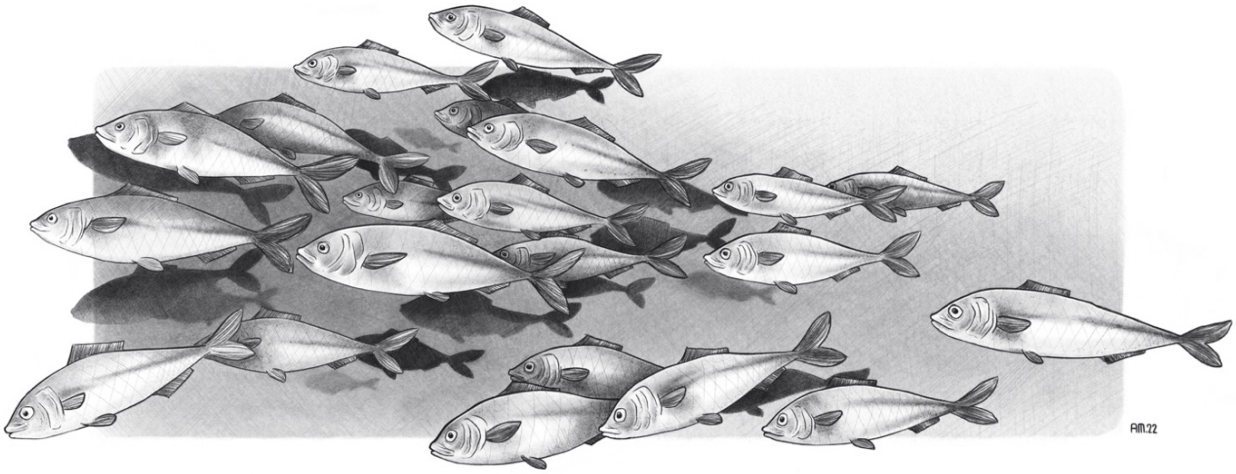

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Acknowledgements

Marine historical ecology and the wider field of ancient DNA are often referred to as "inherently interdisciplinary," transecting the fields of archaeology, history, statistics, ecology, evolutionary biology, bioinformatics, and genetics. To some extent this is true; depending on where I am presenting, I describe myself variably as a "biomolecular archaeologist," a "population geneticist," an "evolutionary biologist," or an "ecologist." (I have also been known to state emphatically that I am "NOT an archaeologist" on more than one occasion.) This presents challenges and benefits to any research project, namely in the challenge and reward of attempting to integrate various modes of knowing to get closer to some form of the truth. However, it is also true that the practical needs of research mean that specialization is required. My practical research sits firmly in the realm of molecular ecology, including the associated considerations of evolutionary biology and ecology as well as the practical tools involved, namely bioinformatics and population genetics theory. I work in a wet lab, write code, employ statistical methods, and deal with giant databases of genome sequences. I read widely, but I do not directly engage in interdisciplinary modes of knowledge production. The interdisciplinary aspect of the research is, thus, inherently collaborative. In designing sampling techniques and research questions, I consult with archaeologists and historians, just as I do to contextualize results. I read archaeology and history, but I am not an archaeologist or a historian, much as I read fisheries policy and science but I am not a fisheries scientist. This doctoral work is therefore the result of many willing to collaborate (however skeptically or enthusiastically) with a PhD researcher who wanted to use miniscule herring bones for molecular research. I am further indebted to those who have spent their careers exhaustively combing through historical records to provide us with such a detailed picture of Europe and its fisheries in the Middle Ages. Thank you to all my collaborators, including Daniel Makowiecki, Lembi Lõugas, Liz Quinlan, Katrien Dierickx, Magie Aiken, Fabricio Furni, Simone Häberle, José Granado, Rachel Blevis, and Anne-Karin Hufthammer. Thank you to Alberto Marcías for the gorgeous herring drawings throughout the thesis.

Thank you to Inge van der Jagt for hosting me in Amersfoort and introducing me to *maatjesharing.* My enormous gratitude goes to Carl André for ensuring not only that my analysis of herring population structure in Chapter 4 was of the highest standard, but providing the modern tissue samples that made all of it possible. Giada, I would never have made it through lab work without you, much less the long COVID lockdowns. I'm also grateful for the large team surrounding me, both in the office day-to-day and in the Seachanges network. We may not all have been working on the same projects, but the shared knowledge and sense of camaraderie was so important in successfully completing a PhD in a new country during a global pandemic. Thanks to Lulú for griping with me and being a friend throughout; Oliver for gossip and chats about bioinformatics; Emma for showing me the fun side of Oslo. Of course, many thanks go out to the rest of the UiO Archaeogenomics team and to my supervisors. James, you believed in the Baltic story long before I did and you were very right to suggest exploring it. Bastiaan, it's been a pleasure. I feel lucky to have had a supervisor I can learn from as well as disagree with. I look forward to collaborating in the future.

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Summary

This is a thesis about the Atlantic herring – a small, unassuming silver fish that holds a special place in the hearts and history of many northern Europeans. Herring have been fished in Europe for thousands of years, but it was not until the medieval era that they began to be targeted in massive quantities. Herring were one of the first fish targeted for commercial production, thus providing wealth and impetus for technological advancement to nation states such as England, Denmark, and the former Dutch Republic. It is said that cities like Amsterdam and Copenhagen were built on herring, so integral was the fishery to their foundational wealth. Today, the Norwegian herring fishery is one of the largest in the world in terms of profits, and the spring-spawning herring stock around Norway may be the largest commercial fish stock on the planet. Herring have further been credited with providing enough mysteries about their life history and ecology as to have founded the field of fisheries science. Thus, much has been said about the impact herring have had on humans. We owe a lot to this small fish that flocks to our shores and has fed people around the world for centuries. But what has *our* impact been on the herring?

In order to assess the impact humans have had on the Atlantic herring, I collected archaeological herring specimens from around Europe and used these bones for ancient DNA analysis. These analyses were then used to reconstruct herring demography from the last 1200 years and compare demographic trends with existing historical, archaeological, and palaeoclimate data. Yet, in order to conduct such work several key issues had to first be solved: 1) A proper theoretical framework for integrating ancient biomolecules into marine historical ecology; 2) How to use miniscule bone samples from herring in ancient DNA; 3) How to maximize the usability of poor-quality genome sequence data retrieved from said small bones.

Chapter 1 provides a full theoretical framework for integrating ancient DNA into marine historical ecology. A system of thresholds is proposed that encompasses both shifting baselines syndrome as well as previously-proposed theories of thresholds in ecology and evolution. Instead of relying merely on ecological and evolutionary thresholds, an additional classification of threshold in human culture is proposed. By including human cultural evolution, ancient biomolecular research can be better contextualized and designed, as ecology and evolution of non-human species does not exist in a vacuum without anthropogenic influence. This is particularly important when discussing marine historical ecology focused on exploited species. **Chapter 2** illustrates the use of small herring bones for ancient DNA. While the typical ancient DNA laboratory pipeline calls for 50-200mg of bone powder as starting material, the use of individual herring bones weighing as little as 1mg is here evaluated. Bone weight is not found to drive variation in DNA sequence quality, which is instead largely explained by differences in site of origin. This indicates the field of ancient DNA should interrogate assumptions inherent in laboratory work and strive to further minimize waste of irreplaceable archaeological material by using smaller quantities of bone powder. **Chapter 3** describes a novel software program that allows biological assignment of DNA sequences with as few as 5,000 reads (~0.0001X coverage). Many ancient DNA sequences are extracted from archaeological material only to be discarded due to insufficient quality. This chapter provides a novel pathway for gleaning important biological information from extremely low-quality sequence data that not only greatly expands the proportion of usable sequences in a typical aDNA workflow, but also has applications to other types of sequence data.

These chapters provided the framework for being able to assess the impact of fishery development on the Atlantic herring over the last 1200 years. In **Chapter 4**, the evolutionary and ecological impact of a key cultural threshold – the advent of commercial fishing – on the Atlantic herring is assessed using both ancient and modern whole-genome sequences. In this chapter we report the earliest-known commercial herring trade in Europe ~800-850 CE in the Baltic region. The demographic trajectories of key herring stocks are further assessed, revealing demographic independence, differential response to climate change, and patterns of serial exploitation that are consistent with classic resource depletion. Research such as this can provide crucial information for sustainable management as we as a society deal with the issues of anthropogenic pollution and climate change and ongoing overexploitation in the marine environment.

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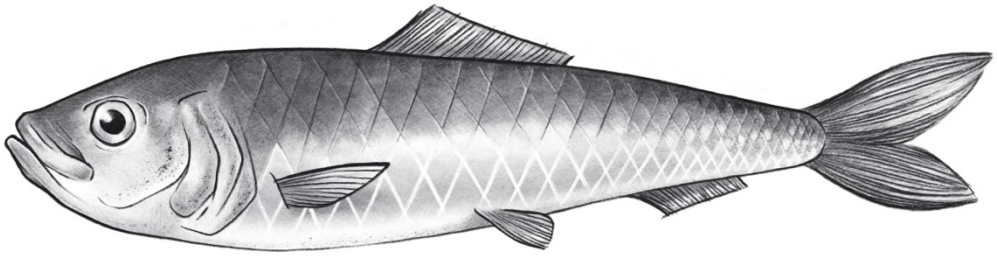
Let all the fish that swim in the sea,
Salmon and turbot and cod and ling,
Bow down the head and bend the knee,
To herring their king – to herring their king.

Perceval Graves, Bishop of Limerick, 1846

Introduction



Atlantic herring are often called the most important fish in history; nations and peoples have risen and fallen on the Atlantic herring trade (Barrett, 2016; Hoffmann, 2001; Holm, 2016; Lõugas, 2016; Makowiecki et al., 2016). Wealthy nations in northern Europe founded much of their wealth on the growing herring trade during the Middle Ages. Indeed, it's said that Amsterdam and Copenhagen were built on herring (Hunt, 2017; Roberts, 2007). Herring fisheries in the past landed fish in quantities comparable to – and sometimes more than – modern fisheries. Today, the Norwegian Spring Spawning herring industry alone is one of the most valuable stocks in the world, with claims laid to it by Norway, Russia, Iceland, and the UK (Marine Stewardship Council, 2022; The Fishing Daily, 2022). Herring are critical components of the agricultural industry (European Commission 2021), the aquaculture industry (Pihlajamäki et al., 2018), and are increasingly highlighted as the next great sustainable food choice (Koehn et al., 2022).

"In value and renown the herring takes an unassailable position as the lord of the fishes"

- Walter Wood, author of "North Sea Fishers and Fighters" (1911)

Atlantic herring have a long, intertwined history with humans. They have been exploited for thousands of years, with the first "industrial" fishery appearing 800 years ago in northern Europe (Holm, 2016). Yet, while herring's impact on human history has been a popular avenue of research, less attention has been given to how these developments changed the Atlantic herring. The impact of this long exploitation history is poorly characterized, largely due to challenges in studying small, pelagic fish. When did commercial herring trade begin and where? What has the impact of this fishery been on the Atlantic herring as a species? As individual stocks? How well do current management policies address herring conservation? Were historical fisheries intensive enough to impact herring stocks? With the rise of the genomic era and the knowledge that ancient DNA (aDNA) can be recovered from archaeological fish bone, some of these questions can finally be answered. This thesis is the world's first foray into the historical ecology of the Atlantic herring from a biomolecular perspective. This chapter provides background for understanding the Atlantic herring in an ecological, evolutionary, and historical context. I then provide an overview of methodology and research undertaken for this thesis.

The Atlantic Herring

Atlantic herring (*Clupea harengus*) are a small pelagic forage fish found across the northern Atlantic, from the northeast of the US and Canada to northern Norway and the Baltic Sea. Across this geographical span, they inhabit a diverse range of local environments mainly differentiated by the existence of specific ocean currents, exposure to sunlight, temperature, and level of salinity. In this thesis, I focus on those herring stocks found in the eastern Atlantic, which are associated with the earliest herring fisheries in Europe.

Recent research into herring population structure and genomics has revealed strong signals for local adaptation despite the incredibly low genome-wide diversity across the species (Han et al., 2020). One of the key differences in Atlantic herring stocks is in adaptation to salinity (Han et al., 2020; Pettersson et al., 2019). Those stocks that inhabit the Baltic Sea are exposed to much lower salinity concentrations than their Atlantic counterparts. This has resulted in a suite of adaptations including chromosomal inversions and loci associated with vision in brackish

water (Han et al., 2020) and different egg characteristics to deal with differences in osmosis and water density (Martinez-Barrio et al., 2016).

The second major axis of variation in Atlantic herring is spawning season. Atlantic herring are separated into two main metapopulations: autumn spawners and spring spawners. The difference in spawning season as well as demonstrated strong spawning site fidelity in herring has resulted in a degree of reproductive isolation between herring stocks (Limborg et al., 2012; Teacher et al., 2013). It has been postulated that the evolution of two spawning seasons in herring may be an example of adaptive synchrony to maximize species-wide survival and local adaptations (Schindler et al., 2010). There may be plasticity in spawning season in rare instances (Han et al., 2020), yet spawning season appears to be a strongly selected trait, with only 2 alleles responsible for the difference between autumn and spring (Chen et al., 2021). Signatures of selection for spawning season have been demonstrated across the entirety of the Atlantic (Lamichhaney et al., 2017).

Herring spawn benthically, releasing eggs that attach to the substrate in thick mats before hatching into the pelagic zone. Each herring female can release as many as 200,000 eggs, which are then fertilized by free-floating milt from the males in the spawning aggregation. Prior to spawning, herring are fat and full of nutrients, whereas afterwards they are "spent" and thin. Thus, a spawning aggregation – which can comprise millions of fish – is a major ecological event, drawing predators in the form of other fish, birds, marine mammals, and humans. Throughout their life cycle, herring are an important prey species for many other marine and coastal species. They are thus a foundational species across the north Atlantic, including for human consumption.

"The silver of the sea": A brief history of herring exploitation in Europe

Herring must have seemed inexhaustible in past centuries. The word for herring is thought to come from the Germanic words *heer* (German) or *haer* (Danish/Norwegian), which mean "army" (Roberts 2007; Hunt 2017). The schools arrived on coasts in such numbers that they appeared a massing army in the shallows. Shoal size was "*...such as to alter the very appearance of the ocean ... [the school was] divided into distinct columns, of five or six miles in length, and three or four abroad; while the water before them curls up, as if forced out of its bed*" (Goldsmith, 1776). Descriptions such as these stagger the imagination, mainly because no such aggregation of herring has been seen in living memory. While still one of the most abundant fish in the world, it appears our oceans are nothing like what they were in the past. This begs the question, what happened to the Atlantic herring?

Herring in the Middle Ages

Atlantic herring is known to have been exploited for thousands of years in Europe. The first written reference to herring fishing is from the Roman historian Solinus in 247 CE, writing of the people in the Hebrides who subsisted off "fish and milk" (Hunt, 2017). In the Middle Ages, as Christianity spread and religious requirements increased fish consumption in accordance with fasting, herring consumption also increased (Hoffmann, 2005). Yet, early exploitation levels had two major limitations: 1) herring are pelagic fish that spend most of their lives in the open ocean, therefore requiring seafaring technology for harvesting fish at sea; 2) herring spoil quickly and require materials for curing, such as salt and brine. Thus, the earliest exploitation efforts were mainly opportunistic harvesting of coastal spawning aggregations (Holm, 2016; Kowaleski, 2016). As Europe entered the Medieval era, trade networks and burgeoning urbanism resulted in increased capacity for salt production and trade (Lehmann et al., 2021).

As one of the most abundant species in northern Europe, herring was one of the key target species for the new industries (Roberts 2007; Barrett et al., 2004b). It also served as a veritable superfood for the Middle Ages, packing up to 24.6g of protein, 12.4g of fat, and 217 calories per serving as well as high quantities of crucial vitamins and nutrients, including omega-3 fatty acids, vitamin D, and vitamin B12 per 85g (USDA 2022). Herring spawning aggregations were so numerous the fish occasionally glutted the market, resulting in herring being used as rent tax and tithes across Europe, and giving it an enduring place in many European cultures (Kowaleski, 2016). Historical records document subsequent growth in herring industries in Europe over the last 1000 years in areas that mirror the regions in which major herring populations exist today, most notably Sweden, Denmark, Poland, the Netherlands, and England (Barrett et al., 2004a; Holm, 2016; Makowiecki et al., 2016; Poulsen, 2008; Skre, 2007). The earliest herring fisheries in Europe were coastal enterprises designed to take advantage of spawning aggregations. Fishing later developed into high seas operations as technologies developed (e.g., tanks for storing live fish on board ships, and advanced preservation techniques) (Hoffman 2000). I here overview the major historical fisheries according to chronology and geography.

The Baltic

The earliest fisheries in the Baltic were coastal fisheries targeting spawning aggregations (Holm, 2016). The Baltic herring fishery, taking off in the 13th century, has been called the "first industrial fishery" in Europe due to the sheer quantity of herring caught in the area. The Øresund fishery – as it is now called – was controlled by the Hanseatic League, which took much of its wealth from the Scanian Herring Market (Holm, 2016; Sahrhage and Lundbeck, 1992). The commercial success of the Øresund fishery lasted until the 16th century, at which point it collapsed. Whether this collapse was due to climate change, overfishing, or shifting herring markets has been debated for decades (Cushing, 1988; Holm, 2016; Lehmann et al., 2021). In Chapter 4, I weigh in on this debate in light of new genomic evidence.

England and Scotland

The coastal herring fishery in Scotland and England was an early important fishery beginning in the Middle Ages and continuing until the early 20th century (Roberts 2007). In England, herring was so abundant from coastal fisheries that it was used as money – called *herringlode* or *herringsilver* – for tithes, taxes, and rent as early as the Domesday Book in 1086 (Kowaleski 2016). Indeed, the sudden presence of abundant herring bones in English archaeological sites is in part responsible for Barrett et al. (2004a) to designate 1050 CE as the "Fish Event Horizon."

As fishing technology developed, the English government began to subsidize the industry. They reasoned that a large population of capable, well-paid fishers was the equivalent of a standing navy (Roberts, 2007). Barreled herring was also used to form some of the first army rations in England. One example of the use of herring as crucial military supplies is amusingly documented the *Journée des Harengs* (The Battle of the Herrings in English). On February 12th, 1429, 300 English wagons carrying weapons and barreled herring to troops at Orléans, France, were attacked by 4,000 French soldiers. The English commander defended his troops using the herring wagons as temporary fortification, but in April 1429 Joan of Arc arrived at Orléans and routed the English despite their supply of herring (Casavant, 2021).

*"**The herring is one of those products whose use decides the destiny of empires**. The coffee bean, the tea leaf, the spices of the torrid zones, the worm which spins silks, had less influence on the wealth of nations than the northern ocean."*

-18th century French naturalist Bernard-Germain-Étienne de La Ville sur-Ilan, Comte de Lacépède (from Hunt, 2017; emphasis added)

The Dutch Republic

Perhaps the most famous herring fishery of the past was the Dutch herring industry, which dominated the market from the 16th-18th centuries. Together with new technological advancements, including barreling their catch at sea, the Dutch Republic successfully inaugurated the first high seas herring fishery in Europe (Poulsen, 2008). The Dutch product took over the European market, with nearly 80% of total catch exported to countries around Europe (Poulsen, 2008). By the mid 17th century, nearly 1/5th of the Dutch population worked for the Colleges van der Grote Visserij, the regulatory body founded to handle the fishery (Coenen, 1577; Poulsen, 2008). The salted, barreled Dutch product was highly prized and a good source of protein during Lent and other Christian religious days when meat was not allowed (Hoffman 2001). The herring fishery was so profitable it's said that Amsterdam was built on herring.

Herring as an early commercial industry

Determining the timing of onset for the emergence of herring as a commercial product is important for several reasons. First, it illustrates a change in the relationship between humans and the world around them concurrent with increased urbanization and globalization. Hoffman (Hoffmann, 2001) proposed that this shift occurred around the 11th century with a shift in consumption patterns from subsistence to something "beyond the bounds of natural local ecosystems". Hoffman (2005) further argued that this shift included increased pelagic resource use. Archaeologists have – somewhat tongue-in-cheek – suggested the 11th century was a "Fish Event Horizon" (FEH) in which improving technologies for salt production and trade, continued urbanization, and demand for fish protein resulted in unprecedented marine fisheries expansion which has continued to this day (Barrett et al., 2004a). The proposed FEH, however, is based exclusively on archaeological material from England. Understanding when this shift occurred elsewhere provides insight into the varied cultural history of Europe, as well as providing insight into the level of exploitation in the past.

Herring commercialization was a key component in the rise of the modern market economy, as trade became dominated by staple commodities rather than luxury goods (Barrett et al., 2004b). Throughout the centuries, this product became more standardized and available across the continent in various forms (red herring, salted herring, fresh herring, etc) with corresponding values placed on each form (Kowaleski, 2016; Pitcher and Lam, 2015). Efforts to maximize efficiency in catch and market product were fine-tuned by the Dutch herring industry in the 16th-17th centuries by the Colleges van der Grote Visserij (Poulsen 2008). Herring were barreled into "lasts" consisting of fish that were all the same size, then shipped in large quantities across Europe.

The ecological impact of these early intensive fisheries has long been debated, with some historians arguing for dramatic overexploitation (Lehmann et al., 2021) and others concluding fishing was not extensive enough to impact marine ecology (Poulsen, 2008). Yet, contemporary evidence suggests that fishers were concerned about overexploitation throughout history. In 1357, fishers successfully petitioned Edward III to regulate the East Anglian herring fishery due to concerns over overfishing (Jones, 2018; Pitcher and Lam, 2015; Roberts, 2007). Fishers were also concerned about the invention of the trawl in the 1300s and its continued use and development particularly after the invention of steam power as it was often used to fish predators feeding on herring spawn, thereby dramatically reducing herring recruitment (Jones, 2018; Roberts, 2007). The true impact of historical fisheries can be difficult to estimate using historical sources, which inevitably rely on sources like catch records and taxes. These documents can be used to great effect (e.g., Holm et al., 2021). Yet, they also come with

caveats: who was writing the documents? For what purpose? How much stock should we place in the author? By analyzing other sources – including but not limited to ancient molecules – we can attempt to triangulate a better answer to the impact of fishing on ocean ecology. I further expand this point below and in Chapter 1.

Herring in the industrial era

Unsurprisingly, herring fishing continued into the late 19th and 20th centuries, as it remained one of the most profitable fisheries in Europe (Roberts 2007). The major new herring industry was the Norwegian spring spawning fishery (also sometimes called the Atlanto-Scandian fishery). In the late 19th century, the Norwegian herring industry grew from a small, coastal enterprise to a full-fledged industrial high seas fishery target the Norwegian spring-spawning herring, one of the largest fish stocks in the world. New inventions such as purse seines – a large wall of netting designed to entirely surround schools of fish (NOAA, 2019) – and steam-powered ships meant ever-increasing catches for fishers (Pitcher and Lam, 2015).

Yet, these large catches were masking a detrimental impact human activity was having on herring. To understand this point, it is crucial to consider fishing through the concept of "catch per unit effort" (Gulland, 1974). This term describes the amount of fish harvested given a certain amount of effort, e.g., sail-power vs steam-powered vessels. For example, if you control for changes in technology when measuring herring fishing, was the fishery actually more profitable than it had been in the past? The answer was a resounding "no" – earlier fishers caught the same amount or more fish with little effort (Roberts 2007). European herring fisheries were on a treadmill, running faster and faster to remain in the same place, much as in the "Red Queen Hypothesis" in evolutionary biology (Van Valen, 1973). Of course, what this also meant is that herring were declining across Europe by the early 20th century.

Thus, prior to WWI, herring stocks in the North Sea were already overexploited. The years during both WWI and WWII offered respite for the herring stocks, which exhibited rapid population growth in the absence of fishing pressure (Holm, 2012). It was a decade after WWII, however, that the most devastating herring crashes in recent memory occurred. Technological advancements in WWII – including faster and bigger ships, GPS, radar, and sonar – meant that fishing effort was higher than ever before (Holm, 2012; Pitcher and Lam, 2015). During the immediate post-war era there was little regulatory oversight on fishing across Europe (Claireaux et al., 2021). With stocks rebounding during the war years, the ocean seemed as productive as ever. Yet, the era of free-for-all herring fishing was short-lived; in 1955 the East Anglian herring fishery collapsed, marking the beginning of a decades-long sequences of herring fishery collapses across the Atlantic (Dickey-Collas et al., 2010; Hannesson, 2022).

In the early 1970s, the North Sea autumn-spawning herring stock suffered a total commercial collapse (Dickey-Collas et al., 2010) shortly followed by the Norwegian spring-spawning herring (Claireaux et al., 2021; Røttingen and Tjelmeland, 2012), the two most important stocks in Europe. In 1977, a herring moratorium was announced for the North Sea (Dickey-Collas, 2016). Moratoria in other regions followed as unexpected stock collapses forced a reckoning. These collapses helped pave the way for European-wide management policies that set limits such as maximum catch allowances and minimum fish size to keep the stock viable (Claireaux et al., 2021). Fishing grounds reopened in the 1980s and 1990s, with initial years of high catches in some regions, but the long-term sustainability of herring stocks are still in question (Dickey-Collas, 2016; Dickey-Collas et al., 2010; ICES, 2021; Marine Stewardship Council, 2022).

Herring in the 21st century

Herring fishing continues in the same regions as the ancient fisheries – Scotland, England, Denmark, Poland, Sweden, the North Sea, and the Norwegian Sea. Despite the long history of fishing and ample time to develop sustainable practices, up to 80% of fisheries in Europe are estimated to be overexploited (Guénette and Gascuel, 2012). This measure includes several herring stocks, most notably those in the Baltic. Nearly all Baltic herring stocks are currently assessed as overfished, with the International Council for the Exploration of the Sea (ICES) consistently recommending maximum sustainable yield (MSY) of zero each year (ICES, 2021, 2020, 2019). Despite these recommendations, the Total Allowable Catch (TAC) is consistently set high to satisfy the fishing industry and a growing demand for fishmeal (Baltic Eye 2021). Even stocks that have been deemed sustainable in recent years have started to decline. For example, the fishery in Norway lost sustainability accreditation from the Marine Stewardship Council in 2020 due to failures of management and overexploitation (Marine Stewardship Council, 2022).

Climate change is also starting to have dramatic effects on herring populations. Herring are a cold-loving species that likely have limited fitness in warm periods (Niiranen et al., 2013; Rönkkönen et al., 2004). Ongoing climate change is expected to be playing a role in dramatically lowered recruitment in several herring populations, including in the Baltic (Niiranen et al., 2013; Polte et al., 2021) and in the Norwegian Sea (Tiedemann et al., 2021). The Norwegian herring industry has been responding to weak year-classes and low recruitment in the last 10 years by reducing TAC. Yet, even with consistent revision of fishing effort, the stock has declined by 40% since 2009 (Tiedemann et al., 2021). Sustainable parameters for herring fishing are often set without consideration of climate change or that long-term fishing caused population declines before the start of "fisheries science." This means that current policies may not accurately reflect the true health of a herring population, necessitating a revision of what "MSY" might mean in light of a long exploitation history and a changing climate.

But no one eats herring anymore!

Until relatively recently, herring was a staple food across Europe. Herring are still culturally important in many countries, for example as as *surströmming* in Sweden and *maatjesharing* in the Netherlands (Hunt 2017). Yet, in many countries with a historical relationship to herring, consumption has decreased in recent decades (European Commission 2021; Pihlajamäki et al., 2018;). This decline in herring consumption tracks the recent history of herring fishing in Europe – closure of many European fisheries during WWII and the moratoria after stock collapses in the 60s, 70s, and 80s followed by increased fishing in recent decades. Herring is still the most-highly consumed pelagic fish in Europe, but per capita consumption is less than half that of fish like salmon and tuna (EU Fish Market 2021). Yet, the herring market is clearly still booming (The Fishing Daily, 2022), so where is that fish going?

Herring fished around the world today is often relegated to use other than human consumption (Casavant, 2021; Pihlajamäki et al., 2018). Atlantic herring are also frequently processed into fish oil, fish meal, and fertilizer, a use that began in the mid-20th century (Pitcher and Lam, 2015). In the Baltic, 66% of all herring caught is used to produce fish meal, which is then fed to salmon in aquaculture pens (Pihlajamäki et al., 2018). During the 20th century, as catches increased along with industrialization, herring increasingly became part of the agro-industrial complex, as the oil and meal was used as fertilizer (Pitcher and Lam, 2015).

Herring are additionally often used as bait for other fisheries (Driscoll and Chan, 2022; Grabowski et al., 2010; Masilan and Neethiselvan, 2018). The practice of using small fish as bait is also not new; for example, small herring from what was then the Zuiderzee in the Netherlands were often used as bait for cod in the 15th-16th centuries (Coenen, 1577). However, this practice has increased in recent years as our appetites have shifted to predatory species like salmon, tuna, and lobster. Recent studies have indicated that salmon and trout aquaculture, rather than decreasing wild fish harvests to more sustainable levels, have dramatically increased pressure on wild forage fish like herring as more of them are required for fishmeal production (Cottrell et al., 2021). Further research has shown a similar pattern in wild fisheries that use herring as bait. The Maine lobster fishery is a net consumer of nutrients due to the inefficiencies of using herring as bait for lobster traps (Driscoll and Chan, 2022). If using herring as fishmeal and bait is such an inefficient strategy, what should we do instead?

Atlantic herring appear to be the perfect food for a sustainable lifestyle. They have a low carbon footprint, a high edible biomass yield, and are rich in omega-3 fatty acids and protein (Marine Stewardship Council, 2022). They have long been important sources of nutrition in Europe. What would fishing pressure be if herring were consumed directly rather than used as bait and fishmeal? Would this be a sustainable fishery? Researchers have advocated for the EU to push for changing the public perception of herring to increase direct consumption in an effort to safeguard food security (Pihlajamäki et al., 2018). Much has been made of the ocean as salvation for climate change (European Environment Agency, 2016). Marine resources, we are told, will reduce our carbon footprints, make us healthier, and protect land from harmful agriculture (European Commission. Directorate General for Maritime Affairs and Fisheries., 2021; European Environment Agency., 2016; Koehn et al., 2022).

Herring, specifically, has been held up as a safe, sustainable fish with a very low carbon footprint (Koehn et al., 2022). Yet, simply ramping up production within the current paradigm is clearly not a sustainable strategy, considering many regions are considered currently overexploited (ICES, 2021; Marine Stewardship Council, 2022). Ecologists have already noted the disconnect between the reality of modern ocean environments and governments' proposed "sustainable" new marine industries (Thurstan and Roberts, 2014), although it is unclear whether this analysis holds true in all ecosystems. We cannot save the world by eating farmed salmon and lobster. It is time to revisit our use of small forage fish like herring.

Understanding what kind of impact varying consumption patterns might have on herring and other small forage fishes requires a long-term approach, both to understand what our true impact on this species has been, and to provide historical parallels. In the early Middle Ages, herring consumption increased dramatically across Europe. What was the impact of this significant subsistence shift at the time? How might this compare to what is being proposed today? Will increased consumption be sustainable? By looking backwards to historical exploitation, we may be able to provide a framework for such practices. If we can better understand the shared history between humans and Atlantic herring and the true impact we've had on this species, we may be able to facilitate the transition towards more sustainable marine resource consumption and provide benchmarks for safe fishing thresholds.

Mode of Inquiry: Marine Historical Ecology and Ancient Biomolecules

This thesis is grounded in the theory of historical ecology. Historical ecology expands the traditional realm of ecology to incorporate interdisciplinary methods that illuminate the past. Historical ecology is a field which attempts to reconstruct ecosystems of the past, "...unified by a core belief that understanding present biotic conditions requires viewing them through the

lens of past interactions with human societies" (McClenachan et al., 2015). In recent years, awareness of our long embeddedness in so-called "natural" environments has grown (Ellis et al., 2021; Rick and Erlandson, 2008; Rick and Sandweiss, 2020). This forces us to drastically revise the long-held belief in the western thought paradigm that we humans are separate from "nature," and to accept that human impacts on the environment were much more profound in the past than previously believed. When applied to marine systems, this approach has facilitated breakthroughs such as the realization of phenomena like "fishing down the food web," "shifting baselines syndrome," and the long, inter-connected history between human societies and the ocean (Jackson, 2001; Pauly, 1995). Marine historical ecology can therefore provide crucial information for conservation and management.

Science Europe noted MHE as one of "12 compelling cases for policy makers" in the past decade due to the highly relevant nature of this line of work in addressing the problems of ecosystem degradation and over-exploitation (Engelhard et al., 2016). By reconstructing past ecosystems through the MHE framework, we can better understand species responses to issues like overfishing, climate change, and differing management strategies. Further, ecological principles learned through MHE could provide policy makers and scientists with the knowledge necessary to not only recover damaged ecosystems, but to foster novel systems in the coming years (Alagona et al., 2012; Kittinger et al., 2015; Máñez et al., 2014).

With the increased accessibility of genomic sequencing techniques and ancient DNA laboratories, the field of palaeogenomics has truly come into its own in the last decade. This has opened up new applications for ancient DNA (Rawlence et al., 2021). Molecular approaches can bypass many of the limitations of traditional zooarchaeological analysis and provide key insight on issues such as species identification (Biard et al., 2017; Rodrigues et al., 2019), establishing origin .and demographic history (Nye et al., 2020). The use of aDNA and reconstructive genomics can provide a deeper time scale than historical catch records (Ferrari et al., 2021; Speidel et al., 2021), for instance allowing temporal assessments of overall population size or population of origin, something that is challenging to achieve using traditional archeological and historical methods (Star et al., 2017; van der Valk et al., 2021). By reconstructing past ecosystems using ancient DNA, it may be possible to see changing relationships between human society and the marine ecosystem, thereby providing a better understanding of what constitutes a sustainable ecosystem and key tools for future-oriented conservation efforts.

Since the discovery that ancient DNA can be extracted from fish bones, which commonly appear in archaeological sites and are otherwise difficult to extract information from (although certainly not impossible), a new window into the history of human maritime exploitation has opened (Hutchinson et al., 2008; Oosting et al., 2019). Yet, the place of ancient biomolecules is not fully characterized in marine historical ecology theory, often being used to define arbitrary "baselines" for species' population sizes. There are many theoretical issues surrounding this use of molecular methods such as aDNA. In Chapter 1, I provide a full consideration of the theoretical foundations of this thesis, as well as defining how ancient biomolecules should be integrated into marine historical ecology. In the rest of the thesis, I follow this theory and use ancient DNA in combination with modern DNA and historical and archaeological evidence to explore the relationship between developing Europe and the Atlantic herring. I place demographic reconstructions and analysis of biological traits in the context of historical trade, estimated exploitation pressures in the past, and modern management techniques.

Knowledge Gaps and Research Aims

We know that herring have a long history of exploitation, are culturally important, and have a complex ecology. Yet, the above literature review reveals areas in which knowledge gaps are extant. These include:

a) Resolution of fine-scale population structure
b) Onset and early origins of the herring trade in Europe (where, when, trade routes?)
c) The possible impact of exploitation on herring ecology and evolution
d) Long-term demographic responses of herring to climate change

The main limitation to addressing these knowledge gaps was that, at the beginning of this PhD, no comprehensive genomic database was publicly available for the Atlantic herring. Further, no ancient genomic sequences were available. Indeed, the viability of tiny archaeological herring bones for yielding ancient DNA was unclear. Thus, answering the above questions required extensive foundational work through genomic database generation and curation, laboratory work innovation and evaluation, and development of novel analytical tools. This foundational work is addressed in Chapters 2 and 3. The overarching goals for the research conducted during the course of the PhD project were designed to address these knowledge gaps:

a) Create a representative database of modern and ancient herring genomes for the east Atlantic and the Baltic
b) Characterize human impact and the development of intensive exploitation on the Atlantic herring populations through zooarchaeological analysis and whole-genome sequencing on a long time-series
c) Provide sustainable measures for informing fisheries policy in multiple countries relating to Atlantic herring
d) Gain understanding of herring responses to climate change in the past to inform management policies for the future

Sampling and Project Development

This project hinged on the capacity to build a database of herring specimens (and DNA sequences) from across time and space in Europe. It therefore required collecting archaeological specimens from researchers and depots in many different countries. Originally, field work was planned to conduct sampling. However, my doctoral work began shortly before the onset of the COVID-19 pandemic. With rolling university, museum, and border closures ongoing until mid-2021, all sampling field work trips were canceled after February 2020. Collecting specimens therefore went remote and slowed down drastically. Combined with delayed access to modern genomic data, this left me with almost no useable data for the first year of my PhD. In response, my focus at the beginning of my PhD shifted to theoretical work and methodological development. This resulted in two manuscripts, one outlining a theoretical framework for integrating molecular research into marine historical ecology (Chapter 1), and another presenting a newly-developed software program that allows us to use even ultra-low-quality DNA sequences (Chapter 3).

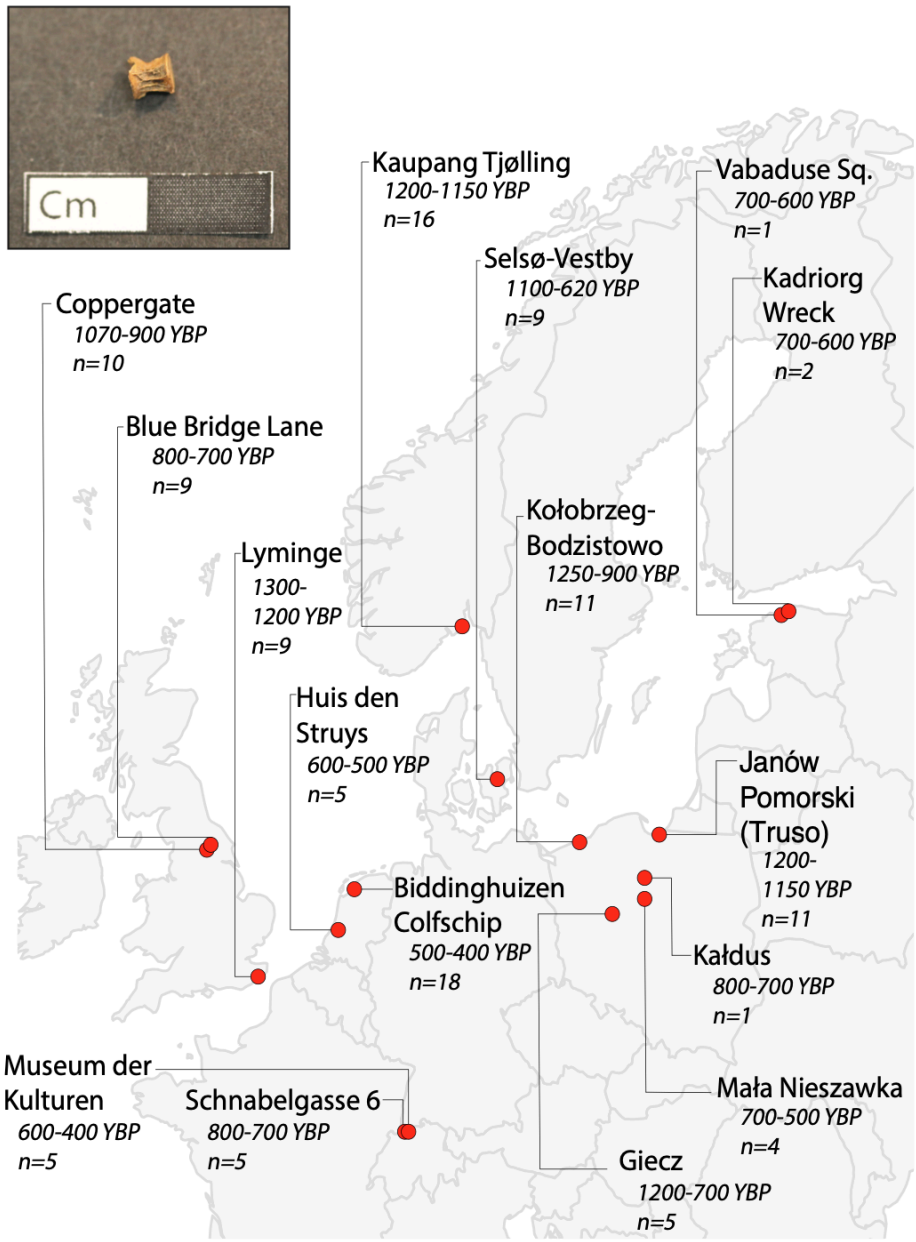


Fig 1 – Archaeological Sampling (from Chapter 2)

Persistence and easing restrictions finally paid off, resulting in a dataset of 121 herring skeletal elements from archaeological sites around Europe spanning the geographical and temporal range of known intensive exploitation of the species (see Fig 1). Of these 116 specimens, 74 yielded viable DNA sequences for nuclear analysis and 111 yielded viable DNA sequences for mitogenome analysis. As archaeological samples arrived at UiO, they were processed in the dedicated ancient DNA lab at Blindern. The aDNA laboratory workflow is long and involved (Orlando et al., 2021), requiring many hours in the lab and months in between the receipt of specimens and the production of sequences.

Ancient DNA work requires the modern genomic reference data for situating results and conducting comparative analysis. Prior to the commencement of the project, it appeared that there was a wealth of herring genomes available for download on platforms like NCBI and ENA. However, we soon realized that these genomes were not actually available or uploaded incorrectly. Luckily, Carl André, a collaborator of Bastiaan's, had many frozen tissue samples stored in his lab at Goteborg University in Sweden. After an unnecessarily lengthy hold-up at the border between Norway and Sweden – which was closed due to COVID – we received enough tissue to generate 53 sequences from modern herring spanning the Baltic, North Sea, and Norwegian Sea. At this point I was nearly 1.5 years into my PhD and had not been able to conduct any analysis on actual herring data. I processed these samples as fast as possible (with help from Bastiaan and Emma Eriksen, then our MSc student) and within a few weeks they were sent off for sequencing. By this time, other modern sequences had also been published and I was able to add 19 genome sequences obtained from published research (Han et al., 2020) and an additional 53 sequences were generated at UiO from herring tissue samples obtained at the University of Gothenburg. The resulting modern dataset covered all major herring populations in the eastern Atlantic as identified by Han et al. (2020) (see Fig 2).

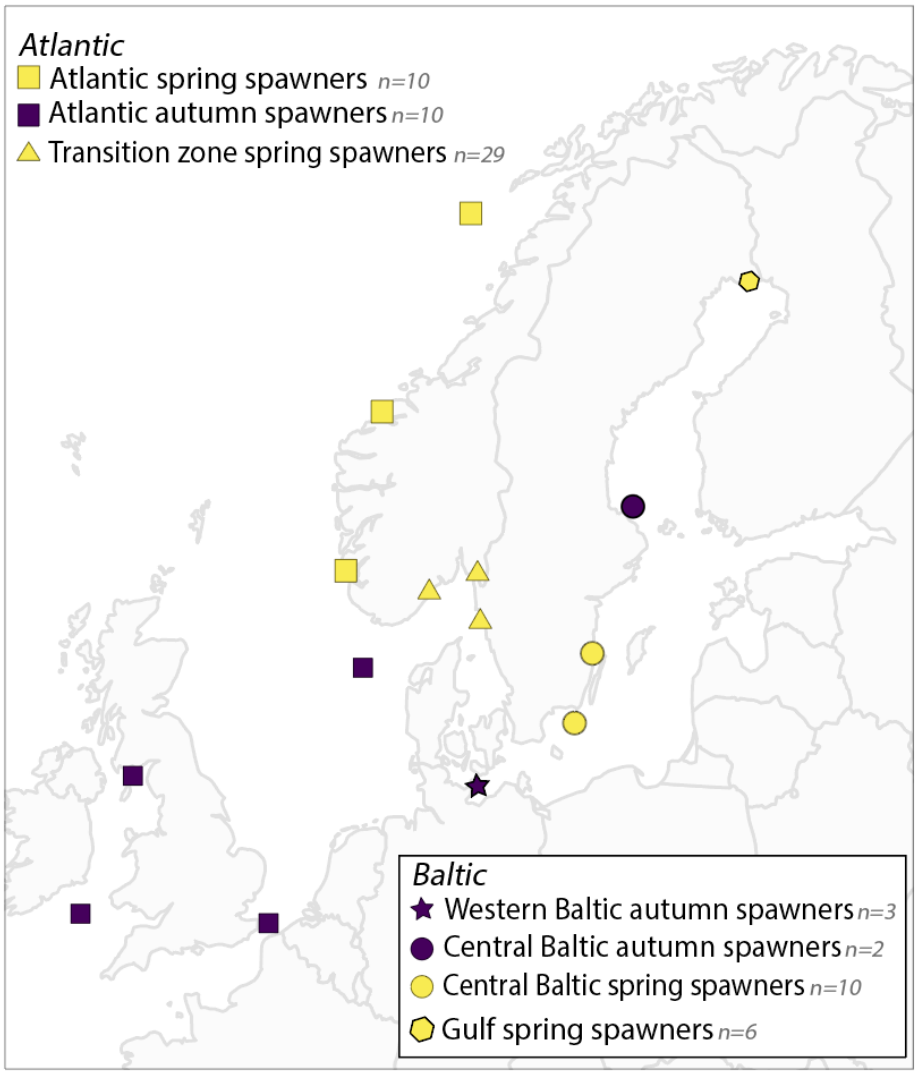


Fig 2 – Modern Herring Sampling (from Chapter 4)

By late 2021, all lab work was finally completed and the full genomic dataset was ready for analysis. I had worked to develop a bioinformatic pipeline for analysis during the prior two years of the PhD, thus all that remained was analysis, pipeline fine-tuning, and writing.

Research Projects

The chapters that follow are manuscripts resulting from my doctoral research. I here briefly overview the main components and subsequent chapters of the thesis.

Theoretical Underpinnings

Chapter 1: Shifting Baselines to Thresholds

The first chapter of this thesis, and the first project undertaken during my PhD, resulted in a manuscript on integrating molecular work into marine historical ecology. Developed with two other PhD fellows within the Seachanges ITN, the research framework is based on a system of *thresholds* and rethinking the concept of "baselines." It explicitly integrates human culture with ecological and evolutionary work, which we argue provides a more realistic basis for truly understanding marine ecosystems and the ways in which we can protect them in the future.

Technological Limitations of Ancient DNA

Ancient DNA research is subject to many challenges and limitations. One major problem is that of post-mortem degradation, in which DNA sequences are damaged after an organism dies, resulting in sequences that are of significantly lower quality than modern data (Orlando et al., 2021; Parks and Lambert, 2015). Because it is impossible to know exactly which bones will be suitable for destruction and DNA sequencing (Ferrari et al., 2021; Keighley et al., 2021; Tin et al., 2014), specimens are often processed and sequenced only to be discarded due to their low quality (e.g., Star et al., 2018; van der Valk et al., 2021). This results in a large amount of waste in time and resources for the researcher as well as exacerbating ethical issues that surround the destruction of irreplaceable archaeological and paleontological materials (Pálsdóttir et al., 2019; Wagner et al., 2020). In Chapters 2 and 3, I further discuss the limitations of aDNA research and introduce methodological advancements that address technological and ethical issues inherent in aDNA research.

Chapter 2: How to use small bones for ancient DNA

Prior to the start of my PhD, the viability of herring bones – which are often small enough to be blown away by an errant breath – for comprehensive aDNA analysis was unproven. A promising, yet unpublished, pilot study at UiO had successfully extracted DNA from a well-preserved site in Poland. Yet, it was unclear whether other sites would yield DNA or how the tiny size of herring bones impacted DNA sequence preservation and quality. Such small bones were further not accounted for in the DNA extraction protocols, which typically assumed 50-200mg of bone powder as starting material. Together with Giada Ferrari, a postdoc in the lab during the first year and a half of my PhD, I developed a work-around for grinding the tiny bones with micro-pestles. Luckily for me, this method worked and I was able to extract DNA. Even luckier for me, I was able to avoid the grueling hours of bone drilling and milling required for DNA extraction from some of the larger species.

During my sampling, I was frequently faced with skepticism that such small bone quantities could yield usable DNA. A review of the available literature further revealed that the smallest bone material used in a published aDNA protocol was 10mg and even then only when using petrous bone. Yet, here I was using bones as small as 1mg. We decided to evaluate the sequencing results – did the size of the bones affect the quality? Was the quantity of DNA

retrieved reliant on bone weight? These questions not only satisfied my curiosity, but could help address a continued issue in the field of ancient DNA research: ethical destruction.

Ethical destruction of archaeological material is a major ongoing issue in the field of aDNA. The field of aDNA typically uses between 50 to 200mg of minimum input weight of bone material for the extraction of DNA from archaeological remains. While laboratory and analysis techniques have focused on improved efficiency of extracting usable sequence data from older and poorer quality remains, bone material input requirements have rarely been critically evaluated. In this study, I evaluated my success in the aDNA laboratory in using extremely small Atlantic herring bones for DNA sequencing. I found that initial bone weight is not significantly associated with DNA sequence quality, rather that preservation is the key factor. This work expands the number of specimens considered suitable for aDNA analyses, and therefore facilitates efforts to minimize the destructive impact of aDNA research and mediate some of the ethical concerns surrounding destructive analysis.

Chapter 3: How to use terrible data
Despite my success in retrieving DNA from miniscule bone samples, the level of preservation was generally low. With traditional approaches and software packages, nearly all the sequences were unusable. This is a problem that plagues all ancient DNA research – months of work and many thousands of dollars are poured into extracting DNA from bones that ultimately gets thrown away. Unless the sequences are of a high enough quality *and* the researcher has enough money to devote to sequencing effort, there is often little that can be done with ancient DNA sequences. But what if there was a way to use these sequences rather than letting them languish?

To address this issue, we developed a software program that is capable of using extremely low-quality DNA sequences for biological assignment tests. I wrote the code for the program, which was co-developed between myself, my supervisor Bastiaan Star, and Giada Ferrari. This program is not specific to any type of genomic data or for use with ancient samples, thus is applicable to other types of low-quality sequence data as well, including those sequencing approaches which are popular in ecology such as reduced-representation sequencing. This new technology meant that from my own dataset I was able to use 64% of all destroyed specimens from 15 out of 16 archaeological sites, an unprecedented success rate in ancient DNA research.

<u>Fisheries' Impacts on the Atlantic Herring</u>
After laboratory work and foundational research projects were completed, I could then apply these theories and techniques to exploring the human impact on Atlantic herring. I designed several projects to address the research questions outlined above, spanning the Baltic to the Norwegian Sea. These included the development of the commercial herring fishery in Europe and impacts these fisheries have had on herring ecology and evolution.

Despite my ambitions, only one of the projects could be completed during my PhD. In May of 2022, with nearly 7 months remaining before submission, my right hand succumbed to a repetitive strain injury. At this point, I had just submitted the fourth chapter of this thesis for publication. Thinking I would be able to finish at least one of the other projects in time for submission, I took nearly two months of sick leave to allow my hand to heal. Unfortunately, the injury was too severe to heal on its own and is still with me at the time of writing five months later. While this has been, of course, a disappointment, I am bolstered by the fact that what now stands as the final chapter is an example of the type of work now made possible by the foundations laid in the first three chapters.

Chapter 4: 1200 years of herring fishing in the Baltic

Marine resource consumption has been a key component in European diet and culture since the Middle Ages, when fish consumption increased dramatically. Yet, the early origins of marine industries and the long-term ecological consequences of historical and contemporary fisheries remain debated. The Baltic Sea was home to the first "industrial" fishery ~800 years ago targeting the Baltic herring, a commercial species that is still economically and culturally important in the region today. In this study, I used ancient DNA to identify the oldest known long-distance commercial fish trade in northern Europe. Further, modeling past demography for four herring stocks in the Baltic revealed patterns of serial exploitation within the Baltic herring industry. Management strategies do not take into account long-term population dynamics of this species prior to the 19th century, which must be done in order to inform sustainable exploitation policies in the future. A second key finding was the differential response to climate change experienced by each of the stocks, demonstrating the importance of a fine-scaled understanding of population structure for determining the appropriate management strategies in the face of climate change.

Discussion

Finally, these chapters are followed by a discussion chapter which contextualizes my research results in the wider fields of ecology, fisheries science, and conservation as well as areas for future study. The discussion highlights key findings, such as an earlier start date to herring commercialization than previously understood. I further illustrate the combined impacts of climate change and exploitation on herring ecology as well as the utility of marine historical ecology for management.

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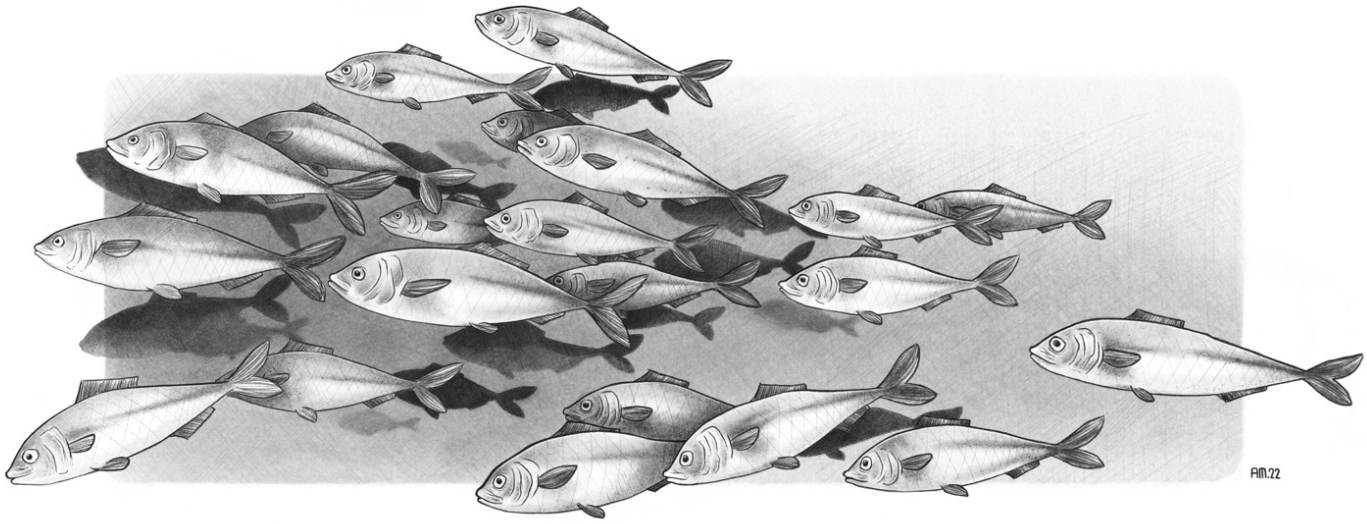
Chapter 1: Theoretical Underpinnings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Shifting Baselines to Thresholds: Reframing Exploitation in the Marine Environment

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Abstract

Current research on anthropogenic impacts on marine ecosystems often relies on the concept of a "baseline," which aims to describe ecosystems prior to human contact. Recent research is increasingly showing that humans have been involved in marine ecosystems for much longer than previously understood. We propose a theoretical framework oriented around a system of "thresholds" referring to system-wide changes in human culture, ecosystem dynamics, and molecular evolution. The concept of the threshold allows conceptual space to account for the fluid nature of ecosystems throughout time while providing a critical framework for understanding drivers of ecosystem change. We highlight practical research approaches for exploring thresholds in the past and provide key insights for future adaptation to a changing world. To ensure ecological and societal goals for the future are met, it is critical that research efforts are contextualized into a framework that incorporates human society as integral to ecology and evolution.

From Baselines to Thresholds

Humans have long been part of marine ecosystems worldwide, although the nature of this relationship has changed over time and space. Today many marine ecosystems are deeply threatened, including commercial fish stocks, coral reefs, and polar environments (Bindoff et al., 2019; Worm and Lotze, 2021). The ongoing crisis in the world's oceans threatens vulnerable species and ecosystems as well as economic stability and food security (FAO, 2018). Further, the world is shifting toward relying even more heavily on the ocean (Costello et al., 2020). From these trends, it is clear that research must address the maintenance of ocean ecosystems. To balance the various needs of all ecosystem actors, both human and non-human, it is crucial that we have language and theory that reflects the dynamic nature of marine ecosystems and our place in them.

Addressing the problem of ocean ecosystem degradation requires enormous interdisciplinary effort. This effort, however, is often stymied by the way in which knowledge creation occurs in marine science. In 1995, Daniel Pauly published a paper on the now-famous phenomenon known as "shifting baselines syndrome (SBS)," in which collective environmental knowledge and memory move forward in time with successive generations, resulting in gradual loss of knowledge regarding the previous state of an ecosystem (Pauly, 1995). Continuous environmental degradation can result in a misinterpretation of changes over time, frequently leading to under-valuation of environmental carrying capacities or species population sizes (Lotze and Worm, 2009; Rodrigues et al., 2019) making researchers ill-equipped to fully understand changing ecosystems. Yet, the environments being studied have been transformed by human actions, often for a much longer time period than is commonly assumed (Lotze and Worm, 2009; Rodrigues et al., 2019).

Although the prevalence of SBS has been clearly demonstrated, the concept itself relies on a problematic ecological assumption: that there is a "baseline" for any one species or ecosystem dynamic. This implies a singular, natural state for any particular ecosystem, which, in practice, becomes fraught with arbitrary decisions regarding how a "baseline" is determined (Lotze and Worm, 2009; Rodrigues et al., 2019). To illustrate this point, Collins et al. (2020) estimated historical population sizes of hunted North American mammals throughout history using two different historical time points as cut-offs for baseline calculation. They found that by pushing the timeline back 100 years, population size estimates increased by over 10%, suggesting the original estimates were likely misleading due to the arbitrary designation of a temporal baseline. Such studies are crucial in illustrating the arbitrary nature of baseline determination while acknowledging the equally important role baseline assumptions play in knowledge creation.

It is clear that humans have had a significant and deleterious effect on our surrounding ecosystems in our more recent history. Yet, it is also important to emphasize that humans have played a role in our environments for the entirety of our evolutionary history, including coastal and marine ecosystems (Rick and Erlandson(eds), 2008; Stringer et al., 2008). This role was neither inherently positive nor inherently negative; our long relationship with the marine environment and the increasingly appreciated dynamic nature of ecosystem stability illustrates the fact that the "baseline" concept itself is what should be shifting. Rather than focusing on a baseline, the concept of SBS should be incorporated into a wider theoretical framework of thresholds in marine environments which explicitly incorporates human culture as an integral component of marine ecosystems. As will be discussed below, the concept of the threshold allows conceptual space for the fluid nature of ecosystems throughout time. It also provides

markers for establishing baselines and a critical framework for understanding drivers of ecosystem change.

By incorporating SBS into a larger discussion of thresholds, the debate can be shifted back to understanding long-term ecosystem dynamics rather than elucidating concrete baselines. Further, the concept of thresholds expands the timescale further than that of SBS, allowing complete incorporation of ecological and evolutionary dynamics into the discussion of sustainable resource management. Finally, as we will show, SBS can be conceptualized as a key component within the wider threshold structure as a cultural characteristic that facilitates the crossing of a certain type of threshold—the tipping point.

We recognize that some of these concepts have been previously addressed in other studies and reviews (Groffman et al., 2006; Samhouri et al., 2010; Rodrigues et al., 2019). Here, we propose a framework that seeks not to disprove other attempts but to expand and enhance inquiry into past ecosystems. We propose a generalized research approach. We emphasize the way in which recent advances in molecular research can illuminate long-term ecosystem change when they are firmly grounded in theory that explicitly incorporates human society in our understanding of what constitutes an "ecosystem." We situate this framework within previous theoretical work, including SBS. We then highlight knowledge gaps in ecological and evolutionary thresholds that can be addressed through biomolecular methods that have become increasingly efficient and accessible in recent years, such as genomics and palaeo-/archaeo-genomics and stable isotopes analysis. Finally, we provide suggestions for future research and the impact such studies could have on providing crucial information for future environmental resource management

Defining Thresholds in Marine Ecosystems

The technical definition of a "threshold" in the *Oxford English Dictionary* is, "[t]he magnitude or intensity that must be exceeded for a certain reaction or phenomenon to occur" (OED, 2021). We use this definition of "threshold" to define various subtypes of threshold that are relevant for understanding marine ecosystems: the magnitude or intensity of some *driver* increases or decreases to the point that a *threshold* is crossed. Here we highlight three major threshold categories that pertain to change in marine ecosystems: *cultural*, *ecological*, and *evolutionary* (see Table 1) and discuss various research techniques to identify when a threshold has been crossed, e.g., a certain reaction or phenomenon has occurred. For each category, threshold identification is always dependent on the research question: it relies on the reaction or phenomenon of interest in the study.

When discussing marine resource exploitation, there are several major ways in which a threshold can be identified. The first is to pinpoint times in the past at which human societies experienced a major transition that coincided with changes in relationships to marine ecosystems: *thresholds in culture.* These thresholds are based on phenomena such as changes in perception of marine environments, technological loss/innovation, economic development, and changes in resource use fall into this category. Thresholds in culture signify a shift in human society that impacts societal-level relationships with the surrounding environment. There are several cultural thresholds that have been identified in human history and are widely known, including the Neolithic and Industrial "revolutions" and the rise of the Information Age. It should be noted, however, that shorter or seemingly less dramatic periods of flux can also be considered cultural thresholds though this is dependent on the scale of the study and the research question. Some examples of cultural thresholds include religious change,

technological advance, and political regime changes (including societal collapse). These changes often affect the relationship between humans and their environments, even if indirectly, through changing subsistence patterns, access to novel food sources through trade, and changing economic and political regulations. Cultural thresholds that impact human exploitation in the marine environment are varied and often relate to technological advance (e.g., seafaring technology or improved fishing techniques) (Unger, 1980; Couper, 2009; O'Connor et al., 2011; Montenegro et al., 2016), but can also be linked to cultural mores surrounding seafood consumption, as seen with the arrival of Christianity in northern Europe and the associated increase in fish consumption on religious holidays in lieu of meat (Hoffmann, 2001, 2005; Müldner and Richards, 2005). Although these cultural changes can (and often do) have ecological implications, impacting the ecology is not a requirement for a cultural threshold.

Table 1 – The three thresholds

TABLE 1 | The three thresholds.

Threshold	Definition	Examples of drivers	Examples of thresholds
Cultural	Major societal transitions that coincide with changes in relationships to marine ecosystems, often resulting in changed societal perception and use of nature and/or marine environments	The invention of trawling; the Industrial Revolution; Animal domestication events; Onset of the North American Fur Trade; The invention of motorized vessels	Dietary change; Economical shift; Urbanization of coastal areas
Ecological	A boundary in ecosystem dynamics that results in a balance shift within and/or between ecosystems. This can occur at the inter- and intra-species level or be related to abiotic factors	Glacial Periods; Climate Change; Extirpation events; Population density of particular species; Onset of pollution	Species richness; trophic interactions; populations connectivity; distribution of species; historical population size
Evolutionary	A heritable change in a species' genotype or phenotype that impacts a population larger than a single lineage	Growth/reductions in population size; Isolation; Changes in sexual selection; adaptation to environmental changes	The evolution of lactase persistence in humans; Creation of domesticated animal species/breeds; adaptive potential; accumulation of deleterious mutations; diversity gain or loss

The second category of thresholds characterizes ecosystem boundaries according to species biology or ecological traits. These are *thresholds in ecology*. Thresholds in ecology encompass relationships between species in an ecosystem (e.g., mutualism, predation, symbiotic relationships, trophic balance, etc.) and can refer to radical shifts in ecosystem dynamics as a whole. Again, the threshold is scalable depending on the research aims. Thresholds in ecology can be linked to anthropogenic impacts but may also occur naturally, such as in the process of ecosystem turnover. Thresholds in ecology can also be driven by abiotic factors, both extrinsic, such as the introduction of pollutants or chemicals, and intrinsic, such as biogeological processes like shifting currents and changes in upwelling. Such ecological thresholds are commonly used in exploitation policies today. For example, fisheries policies are often based on measures of spawning-stock biomass—the number of fish in a species that can reproduce in any given year—requiring that human extraction levels do not cause the population to dip below a certain threshold based on the minimum remaining stock size calculated for sustaining the population (De Lara et al., 2007). We here avoid the terms "ecosystem services" and "ecosystem function" as these have been used in the past to create hierarchies between human societies and their surrounding ecosystems and frame management goals purely in terms of how these ecosystems can benefit humans (de Groot, 1987; de Groot et al., 2002; Costanza et al., 2017). We recognize that the definition of the term "ecosystem function" has been under debate in ecological research for some time (Paterson et al., 2012). As it can be used as an underpinning for determining ecosystem services and occasionally synonymously with "ecosystem services" (e.g., Peterson et al., 2010; Oliver et al., 2015; Hillman et al., 2018), we choose to avoid this term altogether.

Finally, there are *thresholds in evolution*. Evolutionary thresholds are crossed when there is a heritable change in genotype or phenotype in a species. For simplicity's sake, we here discuss molecular evolution as the main example for crossing and evolutionary threshold. This includes demographic shifts such as bottlenecks and population expansions, selective processes and inbreeding, and other phylogenomic phenomena. Potential drivers of threshold crossing include: species migration, climate change, and abiotic factors such as ocean acidification and temperature changes. In recent history, many of these thresholds are likely reached due to human activity, but may have been non-anthropogenic in the past. In this paper, we focus on human exploitation as a critical example of how anthropogenic change can induce an evolutionary threshold. For example, the concept of fisheries-induced evolution postulates that intensive fishing pressures cause an evolutionary change in fish species, mostly by changing life-history traits for faster maturation (Heino et al., 2015; Pinsky et al., 2021). Fisheries-induced evolution occurs when fishing has reached a scale in which evolution is impacted, which may be temporally decoupled from the ecological or cultural thresholds.

Recent discourse in ecosystem management, on anthropogenic impacts on the environment, and on climate change has revolved around the notion of maintaining human activity and ecosystem dynamics within a set of sustainable boundaries often referred to as "thresholds" or "tipping points" (Lenton and Schellnhuber, 2007; Rockström et al., 2009; Russill and Nyssa, 2009). As these terms are not often concretely distinguished, we suggest a differentiation between them. A threshold is distinct from a "tipping point" in that it does not inherently imply a system that has lost crucial regulatory elements. A tipping point can be a form of a threshold, but thresholds exist which are not "tipping points." For example, quantification of sprat population size dynamics has revealed distinct thresholds at which the Baltic Sea ecosystem transitions to an alternate stable state (Casini et al., 2009) but the overall ecosystem dynamics remain unchanged. In contrast, extractivist policies based on research generated under SBS is a cultural characteristic that is causing our oceans to hurtle toward global fisheries collapse and near-total ecosystem degradation (Pauly, 1995; Jackson et al., 2001; Roberts, 2007; Bindoff et al., 2019), therefore is key to crossing the tipping point. Crossing this threshold would be nothing short of catastrophic, which only serves to highlight the importance of deepening our understanding of the other three thresholds. Establishing how thresholds in culture, ecology, and evolution are crossed is therefore of crucial importance to better inform ecosystem management efforts and foster novel approaches to avoid tipping points.

Although seemingly straightforward in definition, these thresholds are in practice entwined in complex, interdependent relationships. For example, advances in fishing technology constitute not only a cultural threshold, but also ecological and evolutionary thresholds as selective fishing and its ecological impact will act as new elements in the marine environment, e.g., with the advent of deep-sea trawling. Given the complexity of human behavior, ecosystem dynamics, and species evolution, it is critical to explore these thresholds thoroughly both in conjunction and as separate phenomena. It can be difficult to determine at what point a threshold has been crossed or what should be considered a "threshold." The threshold concept is not one-size-fits-all, rather it is a flexible approach that can be scaled across time and space.

Determining Thresholds: A Generalized Approach

Here, we lay out the ideal workflow for addressing the issue of identifying thresholds in marine ecosystems in the context of marine resource exploitation. First, context must be grounded in historical, paleontological, archeological, and anthropological research. This includes consultation of historical records, archeological site reports, and, where applicable,

ethnographies. Critically, what this means for ecologists is directly searching for answers to ecological/evolutionary questions outside the field of ecology. A strong background in the relevant historical and archeological contexts of the time period and/or biological system in question shifts ecological inquiry away from assumptions that long-term change must be caused by abiotic factors, such as climate, whereas only recent changes can be due to human activity in the ecosystem. Concurrently, a relevant background in the natural history of the biological system in question is crucial for framing the historical and archeological context. Biomolecular analysis can then be carried out. This work must be situated in archeology, history, indigenous knowledge, and ecology in order to interpret results in a nuanced approach based in systems-level thinking.

Archeology, anthropology, and historical research are key methodological approaches with which to explore cultural thresholds. The application of these methods, however, are not stand-alone modes of inquiry into the problem of identifying sustainable practices. Research must be framed in the context of archeology, history and, wherever applicable, indigenous knowledge. Incorporating all types of knowledge is the only way we will be able to balance issues such as food security with the pressing problems of declining biodiversity, climate change, and social justice.

Many studies of marine resource extraction set their baseline as the mid- or early twentieth century, citing a drastic change in technology that allowed for increased fishing capacity in hitherto inaccessible regions of the globe (Pinnegar and Engelhard, 2008). This is, of course, an important cultural threshold in marine resource extraction and one that merits attention. It is likely, however, that industrial-scale exploitation was occurring, for at least some populations, much earlier than the twentieth century. In the Atlantic, archeological and historical research has revealed fisheries on an enormous scale occurring up to 1,000 years ago (Barrett, 2019). Indeed, Barrett et al. (2004) propose that a cultural threshold was crossed at this time in the form of the "Fish Event Horizon," in which the English proto-industrial fishing operations reached a high enough level that the surrounding North Sea ecosystems were forever changed. These conclusions were reached based on zooarchaeological analysis in combination with analysis of historical documents, suggesting that the mid- or early twentieth century baselines established for many marine populations are again subject to SBS. This illustrates the capacity of archeological and historical research to exhibit thresholds in human culture that indicate likely parallel ecological thresholds. While providing significant insights, these sources remain limited for generating a comprehensive understanding of the marine ecosystem. Archeological and historical research efforts are adept at determining thresholds in human behavior such as major societal changes or shifts in scale of exploitation, yet both are less well-equipped for informing us about species or ecosystem dynamics (Barrett, 2019; Oosting et al., 2019).

Molecular analysis of archeological assemblages, undertaken in collaboration with archeologists and historians, can provide novel insight into broader ecological questions. These approaches include stable isotope analysis, proteomics, and genomics, often in conjunction with archeology and paleontology to form the discipline of biomolecular archaeo/paleontology. The research aims that can be addressed include: species identification (Biard et al., 2017); establishing trade, migration, and population continuity (Star et al., 2017); reconstructing demographic history and evolutionary change (De Bruyn et al., 2009; Foote et al., 2013); and analyzing past ecological and climatic conditions (Gokhman et al., 2017). Recent advances in ancient biomolecular techniques have created a novel arena for investigating thresholds, one that is already beginning to take shape in bioarchaeology and marine ecology (Martínez-García

et al., 2021; Ólafsdóttir et al., 2021). By reconstructing past ecosystems using ancient DNA and stable isotope analysis, it may be possible to observe periods of stability and flux, providing a better understanding of what constitutes a balanced and sustainable ecosystem in support of future-oriented conservation efforts. Indeed, ecological principles identified through molecular approaches could provide policymakers and scientists with the knowledge necessary to not only recover damaged ecosystems but to help foster novel ecosystems in the coming years (Alagona et al., 2012; Máñez et al., 2014).

We recognize that such an approach to inquiry requires generalized knowledge and a strong collaborative research network that draws on expertise in each of these fields. In recent years, such networks have been established in marine ecology (e.g. Oceans Past Initiative, 2021; Sea Change Project, 2021; SeaChanges ITN, 2021). We anticipate that as the field turns toward incorporating social science and humanities, we will see an influx of networks of this type increasing.

Determining Thresholds: Molecular Methods

Molecular methods in thresholds analysis often take the form of biomolecular archeology. This typically involves collecting remains from archeological sites, such as bones or other preserved tissue, to extract informative molecules from these samples. Although traditional morphological studies are typically used to understand faunal assemblages from archeological sites, molecular approaches can provide novel insight into broader ecological questions. To illustrate the potential of biomolecular archeology for research on marine thresholds, we highlight the fields of palaeogenomics and stable isotope analysis as well as the emerging field of palaeoproteomics to illustrate the power of biomolecular archeology to provide crucial information for research into marine thresholds.

Palaeogenomics

The field of palaeogenomics has come into its own in the last decade with the accessibility of ancient DNA laboratories and genomic sequencing techniques (Dabney et al., 2013). This has allowed the field to conduct molecular analysis to a scope that was previously not possible with the prior limited capacity for ancient genome sequencing, opening up new applications for ancient DNA (Rawlence et al., 2021 and references therein). Since then, ancient DNA has been extensively used to explore the relationship between humans and animal species, both wild and domesticated, mainly focused on large terrestrial land mammals and hominids (Meyer, 1992; Dabney et al., 2013; Druzhkova et al., 2013; Woods et al., 2018). However, remains from fish, marine mammals, and other aquatic species are often recovered from archeological sites and included in zooarchaeological analyses. This large corpus of predominantly unanalyzed material from marine animals should not be overlooked.

Molecular approaches can bypass many of the limitations of traditional zooarchaeological analysis and provide key insight on issues such as species identification (Biard et al., 2017; Rodrigues et al., 2018), establishing origin (Star et al., 2017), and demographic history (Nye et al., 2020). Similar methodologies have been extensively employed to research domesticates, large prehistoric mammals, and humans (Green et al., 2010; Lorenzen et al., 2011; Meyer et al., 2012; Librado et al., 2017; MacHugh et al., 2017). However, palaeogenomic analysis is far less frequently applied to marine populations, particularly those heavily exploited by humans, both past and present (Oosting et al., 2019). In the last few years, the use of ancient marine samples has begun to increase as laboratory techniques for such samples have improved

(Boessenkool et al., 2017; Der Sarkissian et al., 2020; Ferrari et al., 2021b; Martínez-García et al., 2021).

The use of aDNA and reconstructive genomics can provide a deeper time scale than historical catch records. With new bioinformatic techniques, ancient genetic data integrate seamlessly with modern data (Ferrari et al., 2021a; Speidel et al., 2021), for instance allowing temporal assessments of overall population size or population of origin, something that is challenging to achieve using traditional archeological and historical methods (Star et al., 2017; Smith B.T. et al., 2021; van der Valk et al., 2021). By reconstructing past ecosystems using ancient DNA, it may be possible to see changing relationships between human society and the marine ecosystem, thereby providing a better understanding of what constitutes a sustainable ecosystem and key tools for future-oriented conservation efforts. For example, Welch et al. (2012) successfully applied ancient DNA analysis to identify local extirpations in the endemic Hawaiian petrel, illustrating range contraction of the species over time and providing guidance for future conservation efforts. Similar approaches have been used to identify possible source populations for reintroducing Eurasian beavers to the United Kingdom (Marr et al., 2018). By establishing a time series of population dynamics that is hundreds to thousands of years old, it is, for the first time, possible to clarify long-term evolutionary trends for exploited populations and their likely drivers, whether those drivers be anthropogenic or otherwise.

Ancient DNA can also be used to identify cultural patterns, which could help elucidate past cultural thresholds. For example, studies of dental calculus—calcified plaque on tooth remains—is a treasure trove of information on the ancient oral microbiome (Warinner et al., 2015). Analysis of dental calculus in human remains has revealed information on past diets, medicinal use, and ancient dental practices and associated cultural shifts (Blatt et al., 2011; Adler et al., 2013; Warinner et al., 2014; Sawafuji et al., 2020). As of yet no studies specifically link dental calculus to marine exploitation, but that does not preclude its potential applicability to understanding changing marine resources. Ancient genomes can also be used to trace patterns of human migrations and associated changes in land use and subsistence (Racimo et al., 2020). Past patterns of human migration are often linked to cultural and/or biological replacement of pre-existing populations (Li et al., 2014), indicating large cultural thresholds could be crossed during periods of mass migration. Modern genomic sequences are often used to study past migrations to great effect (Leslie et al., 2015). Yet, the addition of ancient DNA can provide time-calibrations and an extended window into the past for some of these migrations that greatly alters our understanding of past cultures (Margaryan et al., 2020). These examples, which document Viking expansion into the islands that include the present-day United Kingdom and Ireland, can be used to assess past cultural relationships with the sea, as Viking culture is well-known to have been dependent in large part on marine subsistence, in contrast to some of the populations they conquered (Naumann et al., 2014).

It is often assumed that humans entering a new environment irrevocably change the ecosystem and, thus, efforts for “rewilding” or restoration rely on the premise that whatever existed prior to human interaction is the “natural” state of the ecosystem. Emerging evidence from palaeogenomic studies shows that, while humans have of course changed marine environments, the narrative that drives the human-ecosystem binary is over-simplifying the true state of ecosystem dynamics. For example, the Grand Banks Atlantic cod population famously collapsed in the 1990s, to the devastation of local communities (Myers et al., 1997). The cod population has still not rebounded, resulting in a trophic cascade around the Newfoundland coast (Frank et al., 2005; Neuenhoff et al., 2019). Yet, recent analysis has shown that, despite the severe population bottleneck the Atlantic cod population has suffered in the last few

decades, their genetic diversity remains stable and, thus, old phenotypes and ecosystem dynamics may still be recovered (Pinsky et al., 2021). Studies such as this challenge the long-held assumption that intense exploitation of fisheries must inherently result in evolutionary threshold-crossing on the species-level and emphasize that there are pathways forward for maintaining human-marine relationships that allow both sustainable interaction and exploitation of marine resources and balancing ecosystem dynamics. Similar studies have shown that genetic diversity is robust to periods of intense human exploitation in various species (Welch et al., 2012; Paijmans et al., 2020; Martínez-García et al., 2021).

These studies highlight the importance of not conflating different thresholds. Ecological thresholds were crossed in the Grand Banks as the ecosystem shifted to an alternate stable state, yet genomic results show that molecular evolutionary thresholds have not yet been crossed. Further, they illustrate that while cultural thresholds in exploitation may be informative for identifying ecological and evolutionary thresholds, one must be sure not to project cultural thresholds onto the environment as points of no return. The example of the Grand Banks cod industry also highlights the importance of scale in determining thresholds. At the regional level, the Grand Banks cod population seems to be on the cusp of recovery, as illustrated above. Yet, in some locations an ecological tipping point threshold has, indeed, been passed even if a molecular extinction threshold has not been shown, with local populations in some areas heading quickly toward extirpation (Swain et al., 2015, 2019). This illustrates the necessity of identifying thresholds across different geographic and ecological scales and the interplay between thresholds at various levels. There could, for instance, be an evolutionary or ecological threshold that is crossed at the species or regional level when enough local thresholds have been crossed. This is in line with the traditional emphasis in conservation genomics to preserve local populations for species-wide diversity and adaptive potential through techniques like genetic rescue (Supple and Shapiro, 2018).

Stable Isotopes

Stable isotopes analysis has, much like DNA, been an increasingly accessible tool for archeologists and ecologists alike over the last several decades. Stable isotopes of animal tissue are typically used for analysis of trophic level, dietary reconstruction and foraging ecology, migration, and habitat use. In archeology, stable isotope analysis has been primarily applied to human bone samples. The majority of these studies rely on carbon and nitrogen stable isotope analysis to reconstruct diet, often focused on identifying agricultural transitions (Lee-Thorp, 2008; Sponheimer et al., 2013; Hu, 2018). Other stable isotopic systems including sulfur and oxygen have also been applied to studies of human remains to examine diet (Nehlich et al., 2012; Rand and Nehlich, 2018) and migration (Prowse et al., 2007; Leach et al., 2009; Guo et al., 2018). Applications to past human cultures, particularly those that highlight changing diets and migration, can provide important insights into past cultural thresholds, such as changing religious practices, political regimes, or technological advance that resulted in altered subsistence patterns (Kosiba et al., 2007; Ventresca Miller et al., 2014; Alexander et al., 2019; Cheung et al., 2019). Dietary reconstruction of human populations can identify the incorporation (or lack thereof) of marine resources in the diet (typically requiring a consistent level of consumption over a sustained period) (Goude et al., 2017; McConnan Borstad et al., 2018; Tung and Knudson, 2018). Stable isotopic analysis is increasingly being applied to zooarchaeological remains providing a unique window into the past which can be used to compare to modern ecological studies (Pilaar Birch, 2013). These studies often focus on animal domestication (Hu et al., 2014), seasonality of birth (Frémondeau et al., 2015; Tornero et al., 2016), and husbandry practices (Cucchi et al., 2016; Manin et al., 2018; Bishop et al., 2020).

In marine systems, stable isotopes have provided proxies for changes in water temperature, salinity, nutrient sources, and food-web complexity (Geffen et al., 2011; Barrett, 2019). Carbon and nitrogen stable isotope analysis have been used to understand foraging ecology (McClellan et al., 2010; Newsome et al., 2010; Szpak et al., 2018, 2019). Due to differences in isotopic discrimination, there are benthic-pelagic differences in $\delta^{13}C$ values, which results in inshore foragers exhibiting higher $\delta^{13}C$ values than offshore species (Hobson et al., 1997; Cherel and Hobson, 2007; Andersen et al., 2021). For example, Ólafsdóttir et al. (2021) used zooarchaeological remains associated with Icelandic demersal fisheries to explore the impact of increasing fishing pressure on the trophic levels of Atlantic cod, haddock, and wolffish. By explicitly linking this research to historical and archeological research into the development of the proto-industrial cod fishery in the Medieval era, they were able to compare isotopic signatures over the last 700 years to modern signatures, revealing a strong sign that fishing pressures have both lowered the trophic level for species like cod and resulted in convergence on a single trophic niche for demersal fishes in this environment. Other isotopic systems have also been used, including sulfur as a proxy for foraging ecology (Szpak and Buckley, 2020) and zinc for trophic level in archeological arctic marine mammal bone (Jaouen et al., 2016; McCormack et al., 2021). Oxygen isotopes in marine systems have been used to identify habitat use (Clementz and Koch, 2001; Drago et al., 2020). Guy et al. (2018) used oxygen isotopes to identify fish from a hypersaline lagoon environment in northern Egypt and are able to identify a period of intense exploitation, and trade based on the isotopic signature.

Stable isotope analysis often relies on proxies for data collection. For example, using an innovative approach based on extracting isotopes from ocean quahog, Estrella-Martínez et al. (2019) were able to reconstruct recruitment estimates for the North Sea herring population. Using a marine historical ecology approach, they then cross-checked these estimates with historical resources regarding fishing pressure and catch-per-unit-effort over time. This sort of approach highlights the current ability of molecular research to transform our understanding of the interplay between humans and the marine environment. Further, it emphasizes the ability to conduct systems-level analysis (they primarily wanted to investigate primary productivity) from a single isotopic signature when this signature is fully grounded in historical and archeological context.

Palaeoproteomics

Ancient DNA is an incredibly powerful tool for illuminating past evolutionary dynamics. However, it is dependent on well-preserved specimens, often restricting research both temporally and geographically (Hofreiter et al., 2014). In contrast to fragile DNA sequences, which begin to degrade immediately post-mortem, proteins are more stable molecules that are less susceptible to degradation over time (Collins et al., 2010). Palaeoproteomics, the extraction of ancient protein molecules from paleontological and archeological remains, is an alternative, minimally destructive method that can also be used on older and less-well-preserved specimens (Welker, 2018). Emerging techniques based on well-established proteomics methods, such as Zooarchaeology by Mass-Spectrometry (ZooMS) can provide taxonomic identification of poorly preserved specimens at relatively low costs without destructive sampling (Buckley et al., 2009; Collins et al., 2010; van Doorn et al., 2011) and provide information for phylogenetic analysis (Welker et al., 2016; Welker, 2018).

Compared to the previously mentioned methods, palaeoproteomic methods are under-developed. Yet, they are still able to shed light on past societal trends of marine resource use, and biological and ecological trends, e.g., shifts in species distributions and changes in resource

use (Le Meillour et al., 2020). For example, cetaceans are morphologically hard to identify from traditional zooarchaeological analysis. ZooMS has become an alternative method for more accurate taxonomic identification of these specimens, which might otherwise not be identified (Kitchener et al., 2004; Speller et al., 2016; Rodrigues et al., 2018; van den Hurk et al., 2020, 2021; Wagner et al., 2020). Based on palaeoproteomic data, scientists were able to suggest an expanded distribution range for gray whales in the Mediterranean (Rodrigues et al., 2018), providing information that could be used to define ecological and evolutionary thresholds. When placed in an archeological and historical context, key aspects regarding cultural thresholds have been also explored, such as the dietary use of cetaceans for humans in the Roman and Medieval periods (van den Hurk et al., 2021), and by the American hunter-gatherer–fisher communities of Tierra del Fuego (Evans et al., 2016). Palaeoproteomics can be a stand-alone useful tool for marine areas where preservation of zooarchaeological remains are scarce—e.g., for remains in tropical and subtropical zones (Hofreiter et al., 2014; Speller et al., 2016)—as well as a supportive tool when integrated with other techniques (Evans et al., 2016; Rodrigues et al., 2018).

Determining Thresholds: Practical Limitations

The ancient biomolecular techniques discussed above are all established avenues of research for better understanding the human and beyond-human past. Yet, each of these techniques is also subject to particular limitations that stem from working with damaged, degraded, and/or partial material. Access to paleontological and archeological materials from which these molecules can be extracted is also a constraint on ancient biomolecular research. As each of these techniques necessitates some form of destructive analysis, research must be carried out painstakingly to minimize the risk of wasteful destruction of unique paleontological and/or archeological materials. Each approach is further limited in scope by the availability of archeological and paleontological remains, which are often biased toward particular regions (terrestrial ecosystems and Europe in particular). There are ongoing efforts to expand these fields into less "traditional" regions of the world (for example, the ERC-funded 4-Oceans project), but this will continue to be limited by both differential preservation in different climates and by problems of access, including funding and training opportunities. Each of these biomolecular techniques additionally has an upper limit on the time depth to which they can be used. Thus far, the oldest ancient DNA ever retrieved was 1 million years old (van der Valk et al., 2021), but the majority of ancient DNA is significantly younger. Both stable isotopes and proteomics can go much further back in time, but are still limited by access to suitable remains for analysis. We here highlight additional limitations that must be considered for each of the proposed biomolecular approaches.

Ancient DNA

Ancient DNA, while providing a wealth of information, can be costly. Ancient DNA laboratory work requires access to specialized equipment and high-level clean lab protocols to minimize the risk of contamination by modern biomolecules. Ancient DNA has a high risk of contamination, therefore all laboratory work must be conducted by experts in specialized clean facilities for working with old, fragmented DNA. In the past, the sheer cost of conducting ancient DNA lab work (not to mention the associated costs and computational requirements for analysis) has been prohibitive for many labs. However, as high-throughput genomic sequencing has become more prevalent in molecular ecology, population genetics, and medicine, the associated cost of ancient DNA sequencing and analysis has been driven down in conjunction (Der Sarkissian et al., 2015). As a result, the number of ancient genomic

sequences being produced has increased exponentially in recent years (Marciniak and Perry, 2017; Skoglund and Mathieson, 2018; Brunson and Reich, 2019). Recent advances in laboratory protocols and bioinformatic techniques have also reduced the impact of bias from post-mortem damage (Jónsson et al., 2013; Schubert et al., 2014; Prüfer, 2018) and expanded the capacity to conduct research on extremely poor-quality data (Ferrari et al., 2021a). The uncertainty associated with analyzing damaged DNA sequences can impact evolutionary analysis, but recent work on methodological development has done much to address this (Prüfer et al., 2010; Martiniano et al., 2020; Orlando et al., 2021). By using ancient DNA in conjunction with modern genomes, some of the limitations of ancient DNA can be addressed and ancient DNA can provide additional insight into deeper evolutionary history than modern genomes alone (for more detailed discussion of ancient DNA applications and limitations, see Slatkin and Racimo, 2016; Pont et al., 2019; Spyrou et al., 2019; Dehasque et al., 2020; Smith A. D. et al., 2021).

Stable Isotopes

Stable isotope analysis of archeological material faces similar problems to the other biomolecular techniques. Sufficiently preserved material must be obtained and destructive analysis is required for collagen extraction (Hoke et al., 2018). The quality of stable isotope results can also be impacted by contamination (Vaiglova et al., 2014) and diagenetic processes that result in isotopic degradation, both of which have been addressed in recently published guidelines for quality-control stable isotope analysis (Guiry and Szpak, 2021). It can also be difficult to determine the comparability of environments across time. For example, whether differences in isotopic signatures across time are merely a reflection of chronology or actually indicate a significant change in environment or diet. This is an issue for $\delta^{15}N$ analysis, which is often used in marine ecology and historical ecology to conduct trophic web analysis (Jennings and van der Molen, 2015; Guiry, 2019). In order to account for chronological change, it is often necessary to find $\delta^{15}N$ values for a species that would have been close to the baseline of the trophic web both in the past and in the present to provide accurate trophic level estimation for the target species (Post, 2002). It is not always possible to access baseline trophic web species from past ecosystems, thereby limiting the power of trophic web analysis in the past. However, past dietary analysis using carbon and nitrogen signatures is an established and successful field in historical ecology and biomolecular archeology (Miller et al., 2020; Bird et al., 2021), as well as various other applications, including elucidating cultural thresholds in resource use (Lewis and Sealy, 2018; Nord and Billström, 2018; Miller et al., 2020) and geographic region of origin (Hobson, 1999; Lightfoot and O'Connell, 2016).

Palaeoproteomics

The advantages of palaeoproteomics include being putatively less destructive than stable isotopes and palaeogenomics, and that proteins can be extracted from very old material. However, proteomics and palaeoproteomics cannot provide as fine-scale information as ancient DNA, as it is not yet possible to conduct more than rudimentary evolutionary analysis such as phylogenetic assignment and taxonomic identification (Welker, 2018; Horn et al., 2019). Even if less dependent on material preservation, palaeoproteomics still relies on material quality and protein evolution. Poor recovery of peptides in ancient samples, as well as protein similarities between close-related taxa, can restrict palaeoproteomic studies to identification on higher taxonomic levels (Speller et al., 2016; Buckley, 2018). Existing palaeoproteomics databases also show a strong geographic bias, as research efforts have been concentrated at a small number of institutions in Europe (Welker, 2018). Despite this, recent advances in

proteomics show promise for greatly expanding the evolutionary applicability of palaeoproteomics (e.g., Runge et al., 2021) and it will likely be an important field in the near future as more applications are explored.

Thresholds and Resource Management

All of the above analytic capacities of ancient biomolecular research are crucial for understanding our past relationship with the marine environment. To establish future sustainable measures for marine resource exploitation, it is necessary to contextualize today's sustainability and conservation efforts with knowledge of the long-term relationships between humans and these ecosystems. The data currently available on human impact are typically collected on decades-long bases, and almost never predates the beginning of the twentieth century. As discussed above, there is strong evidence for industrial-scale marine resource extraction that occurred up to 1,000 years ago, at least in the North Atlantic context and likely elsewhere. This understanding is based on historical records and the analysis of archeological sites and remains. These initial research efforts provide a platform for understanding the human relationship with the sea going far back in time. They also provide context for emerging biomolecular techniques and novel applications. The above methodologies should, therefore, always be conducted with as much ecosystem-wide information as is possible, acknowledging the limitations of working with damaged and/or partial datasets. By exploring the past population dynamics of one species, it is possible to gain long-term information on balanced ecosystem dynamics. This will provide us with tools for avoiding the catastrophic threshold in our near future: the tipping point.

It is too often that thresholds identified for sustainable human exploitation fall under the category of the tipping point, pushing population dynamics to the point of collapse. For example, the commonly used notion of "maximum sustainable yield" in many modern fisheries sets catch limits on exploited species based on the amount of spawning stock biomass that can be sustainably removed from the population (Tsikliras and Froese, 2019). In practice, maximum sustainable yield does not take into account ecosystem dynamics and fluidity, rather it is based mainly on the population size of the focal species, allowing extraction up to the point of population collapse (McEvoy, 1986). In the same way, whaling regulative measures, such as the potential biological removal level defined by the U.S. Marine Mammal Protection act, and the Strike Limit Algorithm by the International Whaling Commission, are also based on the minimum population estimates of the stocks and their carrying capacity (Wade, 1998; Givens, 2000). Application of these and similar approaches have led to marine population collapses around the world due to the combined effects of overexploitation and climate change (Guénette and Gascuel, 2012).

The marine historical ecology approach provides ecosystem-based measures for the determination of "sustainable yield" by broadening the definition of the threshold, establishing a conceptual space for ecosystem change that occurs outside of, and prior to, the tipping point. Broadening thresholds allows quantification of population dynamics in focal species that lead to ecosystem perturbation, providing threshold indicators that signal impending population or ecosystem collapse rather than driving the system to the breaking point (Duarte et al., 2020). It has been demonstrated that ecosystem complexity and controlled exploitation measures have overwhelmingly positive impacts on human and societal health, the marine environment, and the health of global fisheries (Hutchinson, 2008; Howarth et al., 2014).

By utilizing thresholds that act as boundaries for ecosystem dynamics rather than bringing the population to the tipping point each year, sustainable management measures are more likely to ensure the ecosystem remains stable. Further, FAO nations have pledged since 2003 to put in place ecosystem-based fisheries management (EBFM) practices in fisheries instead of devising policies that only apply to individual species, as was previously the case (FAO, 2003). In practice, EBFM has been difficult to implement due to the lack of consensus regarding issues of practical application and scale (Trochta et al., 2018), yet is demonstrably crucial to adapting to climate change (Holsman et al., 2020). The threshold concept provides a clear framework for identifying ecosystem, evolutionary, and cultural dynamics that can assist with developing EBFM practices. To effectively enact regulations such as ecosystem-based fisheries management, it is necessary to understand long-term ecosystem dynamics. To do so we must provide space for input from research that emphasizes long timescales and incorporates human society into our understanding of an "ecosystem," a perspective that has long been held by many indigenous communities (Salomon et al., 2014 and references therein).

Case Study—Sea Otters in British Columbia

We here highlight the example of sea otter conservation management in British Columbia as a case study in how this research approach can be practically applied to policy. Sea otters (*Enhydra lustris*) are native to the Pacific coasts of North America and northern Asia (Kenyon, 1969). During the North American fur trade of the eighteenth and nineteenth centuries, sea otters were a prized trade item due to their dense, soft fur (Ravalli, 2009; Berg, 2019). By 1929, sea otters were extirpated in British Columbia (BC) (DFO, 2019), and there was near complete extinction of the species (Ravalli, 2009). Sea otters are a critical species for maintaining the kelp forest habitat that used to spread from BC to Baja California (Estes and Palmisano, 1974), a high-productivity environment that likely assisted human migration to the continent ~20,000 years ago (Erlandson et al., 2007). Sea otters consume invertebrates such as mussels and, most notably, sea urchins; grazing herbivores that can devastate kelp forests when left unchecked (Estes et al., 2016). In the absence of sea otters keeping the invertebrate population low, there have been two main effects. First, the kelp forest habitat along the North American coast has been threatened as a result of the trophic cascade initiated by extirpation of the sea otter (Szpak et al., 2013). Second, a large shellfish industry sprang up in British Columbia, Alaska, and the Pacific coast of the United States to take advantage of the increasing numbers of desirable food species, such as abalones and clams (Gamble, 2021).

In BC, there has been a campaign to reintroduce sea otters to the region in an effort to save kelp forests from devastation by sea urchins. Beginning in the 1960s and 1970s, this campaign has successfully reintroduced sea otters to various places along the coast of BC (DFO, 2019). A 2009 Department of Fisheries and Oceans (DFO) Canada report showed a population increase rate of 19% per year from 1977 to 1995, before slowing to a rate of 8.4% per year from 1995 to 2008, with associated patterns in range expansion (Nichol et al., 2009). With increasing population density and ongoing range expansion, BC sea otters have begun to impact shellfisheries in the region, as shellfish are a shared prey among humans and otters. While otters are not the only factor resulting in diminishing returns from shellfisheries (other factors such as pollution and climate change are crucial components as well), they have become a point of contention among fishers and indigenous communities (Gregr et al., 2020; Gamble, 2021).

Szpak et al. (2012) examined the isotopic signatures of otter remains on Haida Gwaii, an island near Vancouver Island with a long history of occupation (Salomon et al., 2014, 2018). They

found that over the course of the last 12,000 years, the $\delta^{15}N$ and $\delta^{13}C$ signatures of sea otters around the island indicated the otters consumed a diet of primarily benthic invertebrates rather than benthic fish. Contextualizing these results with historical and ethnographic work, Szpak et al. (2012) concluded that these isotopic signatures reflect the long-term management strategies indigenous communities practiced to keep the sea otter population low. This conclusion was supported by further research efforts in ethnography, archeology, ecology, and history (Salomon et al., 2014 and references therein; Stevenson et al., 2015) not to mention indigenous activism and participation in sea otter conservation efforts (Salomon et al., 2018).

Slade et al. (2021) further analyzed this pattern by showing that mussel size over the course of occupation on Haida Gwaii was consistent with low levels of predation by sea otters. They go on to argue that current conservation DFO efforts, which are based on estimations of environmental carrying capacity in an abstract world in which humans are not present, are actually increasing otter population density beyond what the environment would have experienced over the last 12,000 years, thereby causing reductions in invertebrate populations that are threatening shellfisheries (Gamble, 2021; Slade et al., 2021). Recent historical ecology work has shown that the coastal ecosystems of BC were intensively managed by indigenous communities (Salomon et al., 2014), including selective culling of sea otter populations. This ultimately led to a localized system of mosaic micro-environments in which areas near human settlements were comparable to today's sea urchin barrens and areas further away from humans were thick with kelp forests and sea otters, a pattern which is mirrored in research on sea otters and ecosystem stability today (Smith J. G. et al., 2021). While there were likely fluctuations in local environmental stability, the regional environment remained stable over the course of the late Holocene.

Sea otters are an animal of great importance to the indigenous communities of BC (Salomon et al., 2018). Under current DFO regulations, indigenous communities are only allowed to take 500 sea otters from the total population (Salomon et al., 2018; Gamble, 2021). First Nations activists have argued recently that in order to provide balance between sea otters and people, and by extension invertebrates and kelp forests, they should be able to practice the traditional management techniques of the past, including increasing the amount of sea otters that can be removed from the ocean each year (Salomon et al., 2018). This is just one critical aspect of first nations' perspectives on resource management in the BC coastal region (Lee et al., 2019). The recent biomolecular archeology work cited above has strengthened the argument that sea otter management cannot be based purely on false assumptions of "pristine" environments that existed prior to humans.

We highlight this case study not to argue for or against a particular mode of sea otter conservation management, but to illustrate the utility of our research approach. Through the use of ancient biomolecules, archeology, history, and ethnography, research on sea otter management in BC has revealed key thresholds for sea otter management that can be directly applied through policy: thresholds in sea otter population density (*ecological*) and thresholds in sea otter hunting (*cultural*). This research has provided new evidence showing localized, mosaic management techniques that persisted over thousands of years, resulting in long-term ecological and cultural stability in the region that endured through the late Holocene. Further research in this area could incorporate more direct applications toward threshold identification, including using ancient DNA to model sea otter population sizes in the past, that could be directly applied to management strategies and inform sea otter conservation.

The authors would like to note that sea otter conservation is a controversial issue. This case study is merely meant to illustrate the applicability of the thresholds research approach. For more information on the issue of sea otter conservation in BC, please see articles published in Hakai magazine (such as Salomon et al., 2014, 2018; Gamble, 2021, and references therein, Gregr et al., 2020; Slade et al., 2021).

Conclusion

To effectively conduct research on changing marine ecosystems, common frameworks and terminology are required. For compelling communication between researchers, this framework must be flexible enough to encompass a myriad of different fields and scales, and requires a shift to incorporate humans and human society into our understanding of the environment. We have introduced a new theoretical language and a generalized approach that incorporates commonly used ecological ideas to address these issues. The thresholds theory is a way to conceptualize ecosystems and ecosystem change that is both flexible and scalable to the research questions being addressed, and lends itself well to practical applications.

We will never return to the oceans of old. It is not the aim of this research to provide pathways for doing so. Rather, it will likely be required to foster new ecosystems as we move forward into the future (Alagona et al., 2012; Duarte et al., 2020), therefore it is crucial to understand what constitutes a balanced ecosystem, the issue of ecosystem complexity, and the role humans might play in these ecosystems. In addition to establishing regulation and oversight to provide future sustainable marine management, consideration must be given to long-term evolution that has occurred between people and marine environments around the globe, such as the interplay between indigenous communities and sea otters in BC during the late Holocene. Funding efforts for marine historical ecology should be directed toward archeology and population genomics analysis for the marine ecosystems on which people most directly depend, including a targeted effort to fund research occurring outside the traditional spheres of Europe and North America. To provide food security in a changing world, the species people depend on need to be understood from an evolutionary standpoint; how have they adapted to past climate change, anthropogenic exploitation, and different actors in their ecosystems? Are balanced ecosystem dynamics in a north Atlantic context the same as in a Pacific island context? What are the local boundaries management programs must navigate to ensure ecosystem balance? Through the thresholds framework, marine historical ecology is poised to answer these questions and provide crucial information for establishing sustainable marine ecosystems for future generations of humans and the species living with them.

Data Availability Statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author Contributions

LMA and MA wrote the manuscript with contributions from FF. All authors contributed to the development of the theory and revised, and accepted the manuscript.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chapter 2:

How to use small bones for ancient DNA


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Highlights:

- Whole-genome ancient DNA (aDNA) sequences have been successfully recovered from bones weighing less than 10mg
- aDNA sequence quality is not significantly associated with starting bone weight
- Using small amounts of bone material can mitigate some ethical issues posed by destructive sampling
- Researchers should interrogate the amount of material and species investigated for aDNA research

Abstract:
The field of ancient DNA (aDNA) typically uses between 50 to 200mg of minimum input weight of bone material for the extraction of DNA from archaeological remains. While laboratory and analysis techniques have focused on improved efficiency of extracting usable sequence data from older and poorer quality remains, bone material input requirements have rarely been critically evaluated. Here, we present the aDNA analysis of 121 size-constrained Atlantic herring remains – weighing between <10 and 70mg – that were individually sequenced to explore the capacity of successful aDNA retrieval from small archaeological remains. We statistically evaluate the relationship between bone weight and several response variables, including library success, endogenous DNA content, and library complexity, i.e., the number of unique molecules that are obtained. Remarkably, we find no relationship between bone weight and library success, levels of endogenous DNA, or library complexity. Our results imply that – at least in the case of fish bone – even minute bones can yield positive results and that the presumed minimum sample size required should be re-evaluated. Archaeological site, instead of bone size, is the primary driver of DNA sequence quality. Our work expands the number of specimens considered suitable for aDNA analyses, and therefore facilitates efforts to minimize the destructive impact of aDNA research and mediate some of the ethical concerns surrounding destructive analysis.

Introduction

Ancient DNA (aDNA) analysis is constrained by access to suitable archaeological and/or palaeontological material. Research is further hampered by the inherently destructive nature of DNA extraction, meaning archaeological remains are destroyed or damaged for successful recovery of sequences. Successful DNA recovery is often unpredictable (Ferrari et al. 2021; Tin, Economo, and Mikheyev 2014; Keighley et al. 2021), therefore workflows often involve screening large numbers of specimens from which only a small subset ultimately yields usable DNA for analysis (e.g. Star et al. 2018; van der Valk et al. 2021). Thus, the typical aDNA workflow is costly, in laboratory expenses, use of materials, time used and in unnecessary destruction of archaeological material. Responsible destruction and sampling of archaeological remains therefore continues to be a pressing ethical issue for the aDNA field (Pálsdóttir et al., 2019; Wagner et al. 2020).

Recent efforts in improving the aDNA workflow have focused on minimizing destruction of archaeological remains (e.g. Sirak et al. 2017; Scarsbrook et al. 2022) and improving available analytical tools for using poor quality sequences (e.g., Ferrari et al. 2022; Boessenkool et al. 2017; Dabney and Meyer 2019; Parker et al. 2021; Damgaard et al. 2015). Yet, little research has critically evaluated the amount of archaeological material that is commonly used in aDNA workflows. Those protocols that have been developed for minimally-destructive DNA extraction have so far been focused exclusively on specific bones from human and large mammal remains (Pinhasi et al. 2015; Sirak et al. 2017; Dabney and Meyer 2019), and are not always applicable to other species.

The field of aDNA typically uses 50 to 200mg of minimum input weight of bone material for the extraction of DNA from archaeological remains (Dabney et al. 2013; Dabney and Meyer 2019; Dalén et al. 2007; Palkopoulou et al. 2015). It has been shown that more material – crushed bone with some upper limit, e.g. 200 mg – can improve complexity, and help successful extraction of DNA from archaeological bones (Boessenkool et al. 2017; Sirak et al. 2017); although DNA from some mammal bones (e.g. petrous bone) has been successfully extracted from smaller quantities of bone powder (Dabney and Meyer 2019; Parker et al. 2021). Moreover, given the unpredictability of success and often time-limited access to samples, researchers often take sufficient material from a bone to be able to run multiple extractions, resulting in significant quantities that are removed from individual remains.

Nonetheless, for some species, there are no bones large enough to yield such amounts of bone, leading researchers to resort to bulk bone approaches (e.g., Grealy et al. 2015; Seersholm et al. 2021), or to avoid these species altogether. For example, small fish bones may often be assumed to be of insufficient quantity for ancient DNA extraction for high-throughput sequencing even if uncovering the historical ecology of the oceans could retrieve a wealth of information (Oosting et al. 2019; Atmore et al. 2021). Given the ethical issues surrounding the destruction of irreplaceable archaeological remains and the possibility of sequencing previously-unexplored ancient specimens, the quantity of bone material required for successful aDNA retrieval must be interrogated, particularly for species which are not human and receive less research attention and ethical consideration (Pálsdóttir et al., 2019).

Here, we evaluate the impact of bone quantity on the success of ancient DNA retrieval of Atlantic herring (*Clupea harengus*), a species with particularly small bones, which is prevalent in many European archaeological sites. Although DNA has been successfully amplified from ancient herring bone before (Speller et al. 2012; Moss et al. 2016), their suitability for whole genome sequencing has yet not been systematically explored. We sampled 121 individual

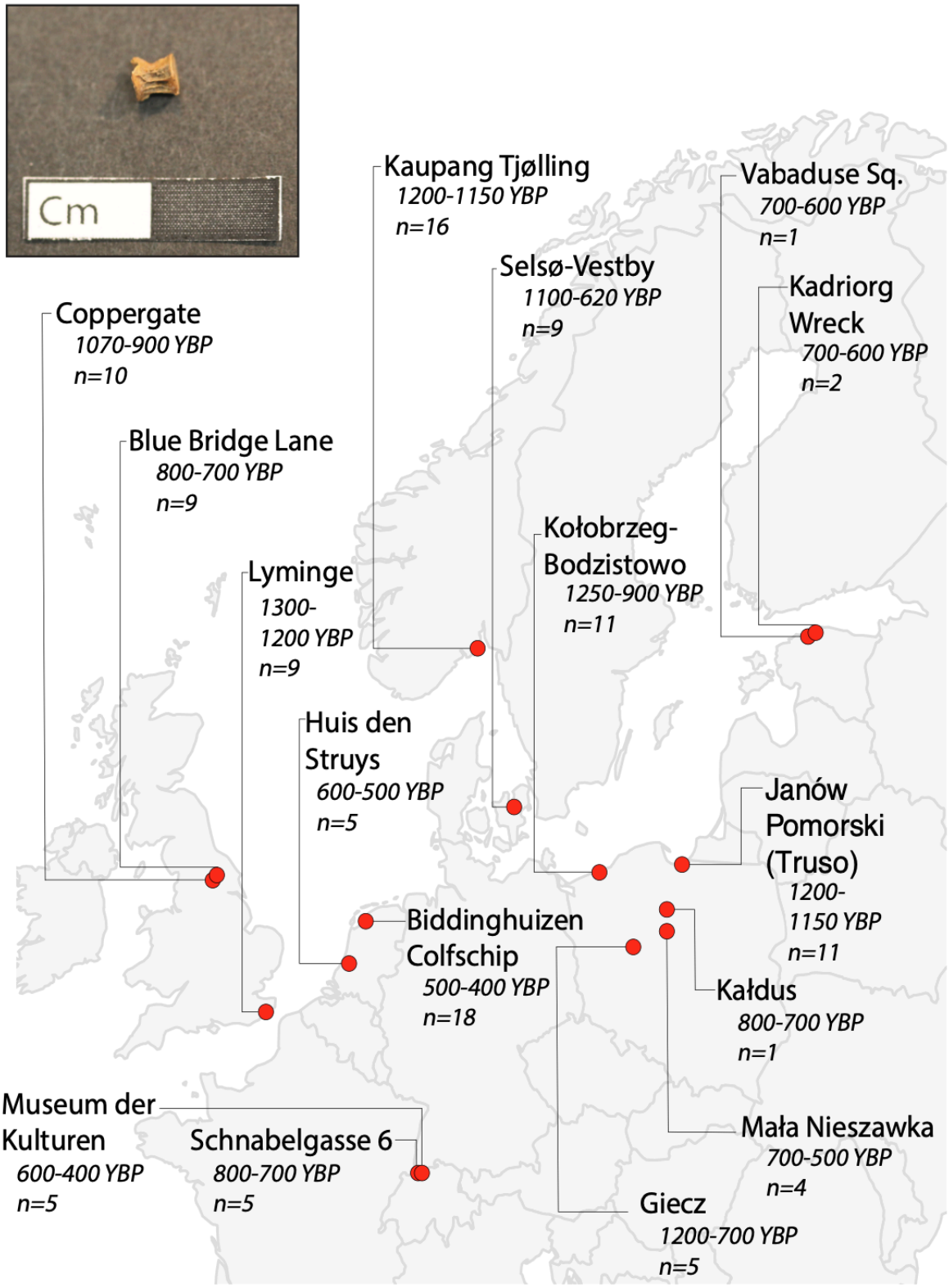

Figure 1 – Sampling distribution of archaeological Atlantic herring bones. Sampling was conducted across 16 sites throughout Europe, ranging from 1300-400 YBP. A total of 121 herring bones were processed in the ancient DNA lab. The inset photo shows the small size of herring vertebrae, which were the most commonly sampled skeletal elements.

Table 1 – Archaeological sites with taphonomy and sampling data

Site	*Country*	*Age (YBP)*	*N*	*Skeletal Elements*
Lyminge	UK	1300-1200	9	vertebrae
Janów Pomorski (Truso)	Poland	1200-1150	11	ceratohyale
Kaupang Tjølling	Norway	1200-1150	16	vertebrae, prootic, unidentified
Kołobrzeg- Bodzistowo	Poland	1250-1000	11	dentaries, ceratohyale, unidentified
Coppergate	UK	1070-900	10	vertebrae
Giecz	Poland	1200-700	5	prootica, vertebrae
Schnabelgasse 6	Switzerland	800-700	5	vertebrae
Kałdus	Poland	800-700	1	unidentified
Selsø-Vestby	Denmark	1100-620	9	cleithra, dentaries
Blue Bridge Lane	UK	800-700	9	vertebrae
Vabaduse Sq.	Estonia	700-600	1	prootic
Mała Nieszawka	Poland	700-500	4	dentaries
Huis den Struys	Netherlands	600-500	5	dentaries, prootics, maxillae
Kadriorg Wreck	Estonia	700-600	2	dentaries
Museum der Kulturen	Switzerland	600-400	5	vertebrae
Biddinghuizen Colfschip	Netherlands	500-400	18	prootics, dentaries

Context and Taphonomy	*Original Citation*
Anglo-Saxon monastery midden, clay sediment	*Reynolds 2013*
Sunken cellar, fish salted and barreled, the craft and trade settlement located near the estuary of Vistula; mentioned by Alfred the Great in the Old English Orosius (ca 890 CE or later)	*Makowiecki 2012; Jagodziński, 2009*
Larvikite monzonitic bedrock with high phosphate levels, mixture of peat bog and sand layers, elements from this site show signs of burning, site waterlogged, stored in museum storage	*Skre 2007*
Stronghold settlement (preurban centre), one of the most important trade centres, among others specialised in herring catches located on the southern Baltic coast	*Leciejewic 2007a; 2007b*
Urban site near a river with moist, peaty conditions; Stored in an archaeological depot	*Bond and O'Connor 1999*
Stronghold settlement, in the 9th-11th century the residence of the Piast dynasty, founder and ruler of Poland, located in the core of the state on Wielkopolska Lakeland	*Makowiecki, Orton, and Barrett 2016; Kurnatowska 2004*
Dryland urban site, craftmen's quarter,cesspit with mineralised preservation and waterlogged condition, dry stored	*Häberle and Plogmann 2019*
Stronghold settlement, located on Vistula river, one of the most important centers in the Chełmno Land, a commercial centre, the seat of the Piast state administration	*Makowiecki, Orton, and Barrett 2016; Chudziak et al., 2016*
Coastal fjørd site	*Enghoff 1996*
Urban site near a river with moist, peaty conditions; Stored in an archaeological depot	*Harland et al. 2016; Keaveney 2005*
Suburban soil layer near road to larger urban settlement	*Kadakas et al. 2010*
Teutonic Castle, the seat of the commander and the convent, located on the left bank of the Vistula, the fish assemblage recovered from an area in the near the castle kitchen	*Iwaszkiewicz 1991; Makowiecki 2003; Józwiak 2003*
Urban cesspit, cooked fish remains, fish salted and packed in barrels	*Laarman and Lauwerier 1996*
Underwater shipwreck, fish salted and packed in barrels, excavated on reclaimed land	*Roio et al. 2016*
Dryland urban site, rich/clerical context, cesspit with mineralised preservation, probably not permanently waterlogged, dry stored	*Häberle & Plogmann 2019*
Underwater shipwreck excavated on reclaimed land, fish salted and packed in barrels; Stored in climate-controlled conditions in an archaeological depot away from UV light; One barrel (M11/58): specimens used for educational purposes, exposed to high temperatures	*Lauwerier and Laarman 2008*

skeletal elements found in 16 archaeological sites around Europe dating from 700-1600 CE (see Figure 1). Each sampled herring bone weighed between <10 and 70mg. The smallest amount used for mammal bones in recommended protocols has so far been limited to a minimum of 10mg and then only in cases with uniquely well-preserved bone (Dabney & Meyer, 2019). DNA was extracted from each bone separately, sequenced, and assessed for quality using the proxy measures of endogenous DNA content, percent clonal reads, total reads, mapped nuclear reads, and DNA extract concentration.

Materials and Methods

Archaeological material

Individual herring bones were sampled from 16 archaeological sites around Europe dated between 1300 and 400 years ago (YBP) (Figure 1, Table 1). 121 bones were collected with weights ranging from <10 to 70mg. Each bone was photographed and initially weighed on a scale with precision of 0.01g. All ancient DNA lab work was conducted in the designated ancient DNA clean lab at the University of Oslo following established protocols for minimizing contamination (Gilbert et al. 2005; Llamas et al. 2017).

Ancient DNA extraction

Bones were bathed in UV light for 20 minutes on each side to remove external contamination, but were too small for further surface cleaning with chemicals or mechanical methods such as sandblasting. After the UV wash, samples were placed in 1.5ml Eppendorf tubes with 100µl of digestion buffer (0.5 M EDTA, 0.5 mg/ml proteinase K, and 0.5% N-Lauryl sarcosine) then crushed with single-use, UV-sterilized plastic micro-pestles. An additional 900µl of extraction buffer was then added to each tube and extraction proceeded following the double-digest protocol from Damgaard et al. (2015). DNA was extracted following overnight digestion using a PB buffer (Qiagen), after which samples were purified through MinElute columns using a QIAvac 24 Plus vacuum manifold system (Qiagen) for a final volume of 65µl. DNA concentrations after extraction were measured using a Qubit Fluorometer (ThermoFisher Scientific).

Ancient DNA library prep

Libraries were built following the Santa Cruz Reaction protocol for single-stranded DNA (Kapp et al., 2021) using the Tier 4 dilution modification. Two unique PCR indexes were added to each library. Each library was amplified with 12-15 cycles of PCR, then purified using the Agencourt AMPure XP PCR purification kit (Bronner et al. 2009) with a 1:1 bead:template ratio for a final volume of 30µl. Libraries were then assessed for quality using a Fragment Analyzer™ (Advanced Analytical) with the DNF-474 High Sensitivity Fragment Analysis Kit. Libraries with no dimers and library fragment length concentrated between 150-250bp, a typical length for aDNA including adapter sequences (Jónsson et al. 2013), were deemed successful and selected for sequencing.

Ancient DNA sequencing

Libraries that were deemed of high enough quality for sequencing were then pooled into one of four total sequencing lanes for screening. These specimens were submitted in pools with samples from other sequencing experiments with between 20 and 50 samples per pool. All pools were balanced so each individual's concentration ratio within the pool was as close as possible to one. 8 million reads were requested per individual for screening purposes. Each sample was sequenced using paired-end sequencing on an Illumina NovaSeq 6000 at the Norwegian Sequencing Centre.

Library success

Bone weights were binned into 10mg categories, with weights rounded to the nearest 10mg. All bones weighing less than 10mg (n=20) were too small to register on the scale. These samples were coded as weighing "1 mg," although this category contains samples weighing between 0-9mg. We then assessed the relationship between weight and library success using percent failed libraries per bin as a response variable using a Fisher's exact test (Fisher 1934), where a "failed library" refers to those libraries that were not selected for sequencing after assessment with the Fragment Analyzer™. We statistically compared the average weight of all specimens and those successfully sequenced using a student's t-test. The same tests were then run using site, age, and bone element as explanatory variables.

Analysis of raw sequencing data

Raw sequence data were aligned to the Atlantic herring reference genome Ch_v2.0.2 (Pettersson et al. 2019) with PALEOMIX (Schubert et al. 2012) using *bwa-aln*. aDNA authenticity based on expected degradation patterns was investigated using mapDamage2.0 (Jónsson et al. 2013). Nuclear sequence quality was assessed from the alignment summary statistics using percent endogenous DNA content (here referring to DNA that belongs to the individual rather than bacterial or other DNA that has contaminated the sample over time) and percent clonal reads as proxies for library quality. As measures of sequence complexity, we further assessed DNA concentration from the extract, total number of reads retained after quality filtering in PALEOMIX, and the total number of reads mapped to the nuclear genome per specimen. We analyzed these proxies as response variables using differential initial bone weight as a categorical explanatory variable.

We further analyzed the relationship between site, age, and bone element using multiple linear regression and sequential regression analysis. Variance inflation factors (VIFs) were used to determine the presence of multicollinearity in the dataset. Chi-squared tests (Pearson 1900) were used to assess interrelatedness between the explanatory variables site, age, element, and weight. All statistical analysis was carried out in RStudio with R version v4.1.2 "Bird Hippie" (R Core Team 2021). Scripts used for statistical analysis can be found on GitHub (https://github.com/laneatmore/small_bones_analysis). A complete dataset can be found in the supplementary materials.

Results

Library success

All sites yielded at least one sample with a mappable DNA sequence. Of the 121 samples in the dataset, 90 yielded successful libraries (Table 2). Endogenous DNA content ranged from 0.0028-43%, with an average endogenous content of 10.1% (Supplement S1) and a large standard deviation of 12.28%. Clonality was low overall, with a range of 0-15% and an average of 9% (Supplement S1). No significant association was obtained between weight bin and percentage of samples that yielded successful libraries (Fisher's exact test, p=1). Similarly, there was no significant difference in the mean weight of samples contained in the whole dataset versus the mean weight of samples in the dataset containing only successful libraries (Student's t-test, t=0.82, df=13.48, p=0.43).

Given the small sample sizes in the largest weight bins (50, 60, and 70 mg), these three weight classes were further binned into a single group of 50+ mg. The above tests were repeated with minimal change in results (Fisher's exact test for weight, p=1; Student's t-test, t=1.49, df=9.97, p=0.17). Thus, weight does not appear to explain difference in successful DNA recovery. No

significant association was found between library success and site (Fisher's exact test, p=1), element (Fisher's exact test, p=1), or age (Fisher's exact test, p=1).

Table 2 – High-throughput aDNA library success based on weight of archaeological Atlantic herring bones. Bone weights were binned into 10mg categories, with weights rounded to the nearest 10mg. All bones weighing < 10mg were categorized as weighing 1 mg. Libraries were considered a success in absence of dimers and fragment lengths that are typical for aDNA.

Weight (mg)	*Total Count*	*Successful Libraries*	*Percent Failed*
1	20	9	55%
10	26	22	15.4%
20	18	12	33.3%
30	27	22	18.5%
40	19	16	15.8%
50	9	6	33.3%
60	1	1	0%
70	1	1	0%

Determination of sequence quality

The impact of weight on sequence quality showed no significant relationship to percent endogenous DNA content (*lm(nu_end ~ Weight)*, p=0.69, df=88, *adjusted* r^2=-0.01) (Figure 2a). This pattern was maintained when all larger specimens were grouped into a single 50+ mg bin (*lm(nu_end ~ Weight_grouped)*, p=0.81, df=88, *adjusted* r^2=-0.01). We assessed the ability of all explanatory variables to explain variation in endogenous DNA content using multiple linear regression (*nu_end ~ Site + element + age_ybp + Weight_mg*). This model significantly explained 49.1% of the data (*adjusted* r^2=0.49, df=67, p=8.8e-9). However, the only independent variables that had p-values within the range of significance were three of the archaeological sites (Coppergate, Giecz, and Mała Nieszawka). Nearly identical results were obtained when grouping the larger weight classes (*adjusted* r^2=0.49, df=67, p=2.3e-7). To ensure the result of this model was not artifactual, further analysis was required.

We assessed if multicollinearity affected the efficiency of this model, given the likely interrelatedness between some of the variables (e.g., the relationship between weight and element or site and age). The vif() function from the *car* package (Fox et al., 2022) showed a variance inflation factor (VIF) of 1.9 for weight, 1.4 for site, 2.18 for element, and 11.2 for age. The dataset with grouped 50+ mg weight class showed similar or higher VIFs: grouped weight, 2.13; site, 1.4; element, 2.19; and age, 11.2. Given the similarity between the non-grouped and grouped weight datasets and the higher VIF for grouped weight, this dataset was discarded and subsequent analysis was conducted only on the dataset containing the original weight classes in 10mg increments. Age was the only explanatory variable with a VIF over the accepted threshold of 5, indicating it is not independent from one or more of the other variables.

To explore further the relationships between the variables, chi-squared tests were used. Weight was found to be significantly correlated with site (Chi-squared test, p=0.047) and bone element (Chi-squared test, p=0.003). Element and site were also correlated (p=0.0005) (Figure S3) as were site and age (p=0.0005). Despite their interrelatedness, the VIF analysis did not show strong multicollinearity between these variables. Therefore, to determine the individual

contribution of these variables to endogenous DNA content, sequential regression analysis was used with each explanatory variable isolated. Age was not significantly correlated with endogenous DNA content (*adjusted* r^2=0.032, *df*=88, *p*=0.051), neither was element (*adjusted* r^2=0.055, *df*=84, *p*=0.083). In contrast, a linear regression of the relationship between archaeological site and percent endogenous DNA explained 49% (*adjusted* r^2 = 0.49) of the overall variation with a p-value of 0.88e-9 (Figure 2c; *df*=74, residuals reported in Fig S4). Site is therefore likely driving the majority of variation in percent endogenous DNA content.

Given the significant interaction between weight, site, and element, we investigated if non-random distributions of weight throughout each site (Figure 2b) confounded the impact of site on percent endogenous DNA. To determine if there is a significant interaction between weight and site impacting the regression analysis results, we allowed for an interaction between the two variables (*nu_end ~ Site * weight*). This model was then compared to an additive regression model (*nu_end ~ Site + weight*) using a chi-squared test (*anova(model2, method = 'Chisq')*). We obtained no significant relationship between site and weight (p=0.079), indicating it is not necessary to stratify the weight regression by archaeological site.

Finally, we determined whether there was an interaction between site and skeletal element, which also revealed that only site was significantly associated with percent endogenous site only is the most appropriate model for understanding the variance in percent endogenous DNA content per sample. Percent clonality was then assessed in a similar fashion. There was no significant relationship between weight and clonality (*adjusted* r^2=-0.011, *df*=88, *p*=0.91). Further, no variables showed significant association with clonality except site, which explained 19.9% of the variation in clonality (*adjusted* r^2=0.199, *df*=74, *p*=0.0052; residuals reported in Figure S5). This result strengthens the conclusion that sequence quality variation depends on differences in site preservation.

Assessing library complexity

To better assess the question of library quality and complexity, we then evaluated the relationship between bone weight and site and several additional factors, including: DNA concentration as measured by the Qubit Fluorometer (ng/µl), total number of reads (after quality and duplicate filtering) and total number of reads mapped to the nuclear genome per specimen (see Supplementary Data S1). These last two values were obtained from the summary output files from PALEOMIX after sequence alignment.

DNA concentration as measured by the Qubit Fluorometer (ng/µl) were significantly correlated with bone weight (*p*=0.0006), yet this explained only 11.7% of the variation in DNA concentration (*adjusted* r^2=0.117, *df*=88). While raw concentration was associated with weight, total retained reads per sample was not (*adjusted* r^2=0.016, *df*=87, *p*=0.12), DNA (significance of interaction model: *p*=0.95), nor was the number of mapped hits on the nuclear genome (*adjusted* r^2=-0.0009, *df*=88, *p*=0.34). Quality and complexity factors are plotted against weight class in Figure S6. Given the shown interaction between skeletal element and weight, element was used as an explanatory variable, which gave similar results (DNA concentration: *adjusted* r^2=0.15, *df*=84, *p*=0.002; Total reads: *adjusted* r^2=-0.007, *df*=83, *p*=0.51; Mapped nuclear reads: *adjusted* r^2=0.006, *df*=84, *p*=0.36). Again, DNA concentration was the only significantly-correlated variable. Further analysis of the relationship between DNA concentration and complexity (here only using mapped nuclear reads) showed no significant result (*adjusted* r^2=0.004, *df*=88, *p*=0.24). This indicates that bone weight may have something to do with DNA concentration, but not ultimate sequence quality or complexity.

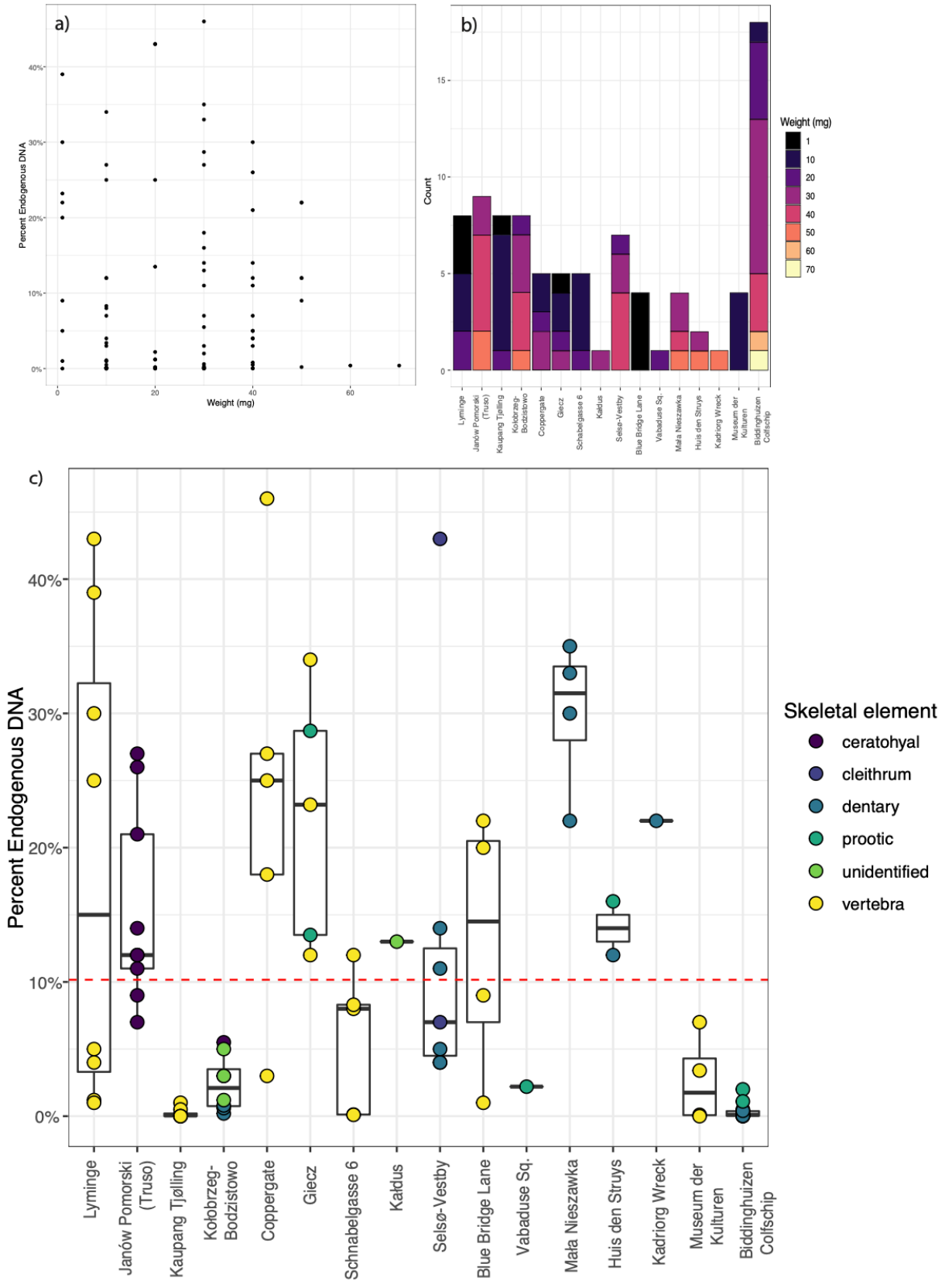
a)
Percent Endogenous DNA
Weight (mg)
b)
Count
Weight (mg)
1
10
20
30
40
50
60
70
c)
Percent Endogenous DNA
Skeletal element
ceratohyal
cleithrum
dentary
prootic
unidentified
vertebra
Lyminge
Janów Pomorski (Truso)
Kaupang Tjølling
Kołobrzeg-Bodzistowo
Coppergate
Giecz
Schnabelgasse 6
Kałdus
Selsø-Vestby
Blue Bridge Lane
Vabaduse Sq.
Mała Nieszawka
Huis den Struys
Kadriorg Wreck
Museum der Kulturen
Biddinghuizen Colfschip

Figure 2 – Relationship between archaeological context and percent endogenous DNA. a) Distribution of percent endogenous DNA content by bone weight. Each dot represents a DNA sequence extracted from a single archaeological herring bone element. The x axis denotes the different weight class bins in 10mg increments. The weight class <10mg is here classified as "1 mg". There appears to be no relationship between weight and endogenous DNA content, which is supported by linear regression analysis (p=0.69, adjusted r^2=-0.01). Only those samples that were suitable for sequencing are represented here. b) Distribution of bone weight bins by site. c) Distribution of percent endogenous DNA content per archaeological site. Each dot represents a DNA sequence extracted from a single archaeological herring bone element. Sites are arranged according to age from oldest (Lyminge, 1250 YBP) to youngest (Biddinghuizen Colfschip, 500 YBP). Linear regression showed a significant relationship between archaeological site and endogenous DNA content (p=0.88e-9, adjusted r^2 = 0.488). The red dashed line indicates the average percent endogenous DNA across all sites and samples. Color represents skeletal element. Only those samples that were suitable for sequencing are represented here.

In contrast, site was significantly associated with DNA concentration (*adjusted* r^2=0.28, *df*=74, *p*=0.0003) and total retained reads (*adjusted* r^2=0.21, *df*=73, *p*=0.004), although not mapped nuclear reads (*adjusted* r^2=0.1, *df*=74, *p*=0.08). However, site does not appear to explain all the variation in library complexity, given that a minimum of 70% of the variation in these measures is unexplained by site differences. The models were marginally improved by includimg age as an explanatory variable (DNA concentration: *adjusted* r^2=0.3, *df*=73, *p*=0.0.0002; Total reads: *adjusted* r^2=0.22, *df*=72, *p*=0.004; Mapped nuclear reads: *adjusted* r^2=0.12, *df*=73, *p*=0.06).

Discussion

Here, we investigated the potential of whole genome, aDNA retrieval from small, archaeological herring bones. We assessed the relationship between various contextual variables including archaeological site, bone weight, skeletal element, and age. We make several observations.

First, 90 of 121 samples (73%) yielded successful sequence libraries, with a wide range of percent endogenous DNA content and percent clonality. Given the rough scale of our weight measurements, it is possible that there is some impact on library success from extremely small samples that was not observed due to a lack of resolution in our analysis. Although less than half of the samples in the smallest bin (<10mg) yielded libraries, we found no significant relationship between bone weight and library success, neither was site or any of the other explanatory variables. It must be noted, however, that we were successful with 45% of the 20 bones in the smallest size bin, indicating that even the tiniest bones can yield useable DNA sequence data.

Further, we sampled entire bones with minimally-destructive cleaning beforehand; no bleach or surface removal was used to clean the surface of the bone, techniques which have been shown to increase endogenous DNA content (Boessenkool et al. 2017; Pinhasi et al. 2015). These techniques are typical components of the aDNA lab pipeline, but are too destructive for such small bones. Therefore, we expect that the endogenous DNA content of our samples was likely lower than it could have been, yet we were able to achieve an average endogenous DNA content of 10.1%. These results therefore show the viability of using archaeological small fish bones for providing historical aDNA data that can be applied in historical ecology studies (e.g., Oosting et al., 2019; Atmore et al., 2021).

Second, we found that the level of endogenous DNA retrieved from our small samples is comparable to studies using larger quantities of bone powder (Ferrari et al. 2021; Martínez-

García et al., 2022). Previous studies have shown that fish bone may be particularly suited to ancient DNA analysis given the lack of bone remodeling (Ferrari et al., 2022), a developmental process that is shown to have an impact on endogenous DNA preservation (Kontopoulos et al. 2019; K. Sirak et al. 2020). However, no studies have thus far explored the impact of bone size on DNA library success, instead largely focusing on differences in laboratory protocols, variables that are here held constant (Ferrari et al., 2021; Sirak et al. 2017; Sandoval-Velasco et al. 2017).

Moreover, we found no relationship between bone weight and endogenous DNA content or library complexity, and only a weak relationship between complexity and archaeological site. While the DNA concentration of each extract was weakly correlated with skeletal element and weight, neither of these were correlated with quality or complexity measures in the end, indicating that the increase in DNA concentration is likely from exogenous sources. Library complexity does not have to be particularly high to sequence a genome to low coverage, and even ultra-low-coverage genomes can yield meaningful biological results (Ferrari et al., 2022; Atmore et al., 2022). However, for some analyses, such as demographic inference, a greater amount of coverage and complexity is required (Schiffels & Wang 2020). We here found that weight is not related to complexity when sequencing to low effort. To further support this result, future analysis should be conducted on samples that have been exhaustively sequenced, allowing better assessment of the library complexity that can be attained from each specimen.

One limitation this analysis faces is the lack of ability to sample a single bone multiple times with different volumes. Previous analysis has shown multiple extractions can dramatically increase library complexity (e.g., Boessenkool et al., 2016), and by sampling multiple times we would have been able to control for individual variation and/or within-site preservation differences. Unfortunately, given the small size of herring bones (see inset photo on Figure 1), this approach was impractical. We therefore rely on the distribution of weights across the sample dataset to serve as an imperfect proxy. Future research efforts should be focused on using larger bones that can be sequentially sampled in decreasing quantities to further expand upon our results.

We found that the most significant relationship in determining DNA sequence quality was archaeological site rather than bone weight, bone element, or age. Archaeological sites can have different taphonomic histories, therefore preservation of molecules between different sites can be drastically different (Ferrari et al., 2021). For example, colder and drier climates with stable temperatures lend themselves to DNA preservation, whereas ancient DNA recovery from tropical climates is possible but much more time-restricted (Reed et al. 2003; Bollongino, Tresset, and Vigne 2008; Willerslev, Hansen, and Poinar 2004; Kistler et al. 2017; Dommain et al. 2020; Woods et al. 2018). While general site conditions are known for these specimens (see Table 1), regression analysis of specific site characteristics is not possible here, as measurements on important variables (e.g., soil pH, humidity, temperature, storage temperature and UV exposure) were not available for all sites under consideration.

Each site also had a different history relating to sample storage. Exact storage conditions and full storage history are not available for all specimens in this study therefore the impact of storage temperature and UV exposure could not be explored systematically for this dataset. Where available, storage conditions are listed under “Taphonomy” in Table 1. These factors indicate that explanatory variables beyond the scope of this study likely have some relationship with sequencing success. Given the controlled variables of laboratory protocols and sequencing effort, which were the same for all samples, it is likely something not included in the dataset.

This could be an as-of-yet unknown component of preservation or methodological bias, such as within-site variation (Massilani et al. 2022), storage history and taphonomy, and/or manual variation in laboratory processing.

While this study is focused on the question of bone size, future research should focus on issues such as soil condition and pH, storage history, and within-site variation in determining the impact of site and taphonomy on molecular preservation. Further, our samples stem from relatively young sites in northern Europe, which typically results in better DNA preservation. In order to determine whether such micro-sampling is feasible in various contexts, future research efforts should attempt to replicate our results for sites in regions with poorer preservation and/or sites that are older than 1500 years.

Previous studies have indicated the potential for archaeological herring bone weighing >10mg in ancient DNA research (Speller et al. 2012; Moss et al. 2016). These studies successfully amplified mitochondrial DNA, as well as generated microsatellites and SNP assay data from Pacific herring (*Clupea pallasii*) bones dating up to 10,000 YBP. However, whole-genome sequencing of ancient herring bones has not been successful until very recently (Ferrari et al., 2022). Combined with our results that DNA library quality is not determined by bone size, but by archaeological context, these studies illustrate the high potential of small bones in ancient DNA analysis.

Due to the high potential for fish bone to yield successful ancient DNA libraries despite its brittle, porous nature and lack of petrous bone (Ferrari et al. 2021), these results may not hold true for other types of bone, such as mammal remains. Prior to 2017, the majority of micromammal ancient DNA studies focused on sequencing segments of the mitogenome, such as the *cytB* sequence (Woods et al. 2017). However, recent studies have shown that micromammal remains can also yield whole-genome sequences (Cucchi et al. 2020; Yu et al. 2022). Thus, smaller quantities of bone powder and small-boned specimens should be considered viable options for ancient DNA sequence analysis rather than discarded or immediately pooled into bulk-bone sequencing.

Conclusion

We have here shown that successful ancient DNA whole-genome sequence recovery is possible from individual archaeological fishbone weighing less than 10mg. This supports the growing consensus that fish bone is an excellent material for DNA preservation. We show that there is no significant relationship between bone weight and DNA sequence quality or suitability for sequencing. Instead, our results support that the most important factor to consider in destructive analysis for ancient DNA extraction is taphonomy and site preservation rather than the quantity of retrievable bone powder.

Our results therefore provide novel evidence that site preservation and taphonomic history are also the crucial determining factors in DNA sequence recovery for small fishbones. Importantly they show that archaeological context is more important in determining whether or not to sequence a site rather than the amount of bone powder that can be recovered. This, in combination with bioinformatic approaches specially designed for low-coverage and poor-quality sequences have also reduced the DNA quantity and quality required for meaningful analysis (Ferrari & Atmore et al., 2021), thereby further increasing the ability to use those samples that may have otherwise been discarded.

Author contributions:
LMA, GF, and LMG conducted the lab work. LMA and GF designed the laboratory modifications for small bone samples. IJ, RB, JG, SH, KD, LQ, LL, DM, AKH, and JHB provided archaeological material and contextual information. LMA and BS designed the study. LMA conducted the statistical analysis and wrote the manuscript with input from BS and GF. All authors revised and agreed to the final version of the manuscript.

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The authors declare no conflicts of interest.

Data Availability
Sequences used for analysis are available on the European Nucleotide Archive at the accession PRJEB54658.
Additional raw data for this chapter can be found on the associated Github repository. They were not included here due to the size of the tables.

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Supplementary material

Ancient DNA sequence quality is independent of fish bone weight

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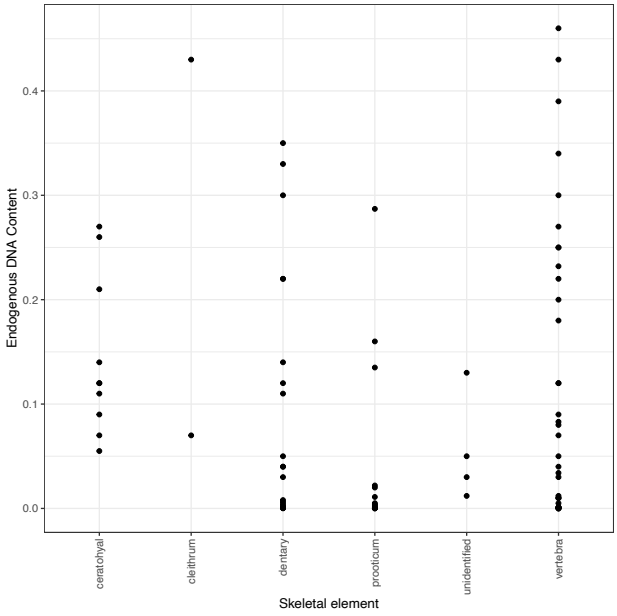


Figure S1 – Percent endogenous DNA content by skeletal element across all sites

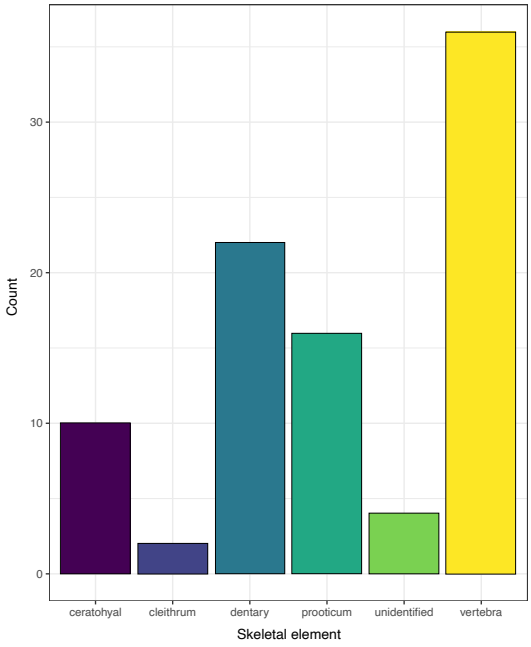


Fig S2 – Number of samples per element across all sites.

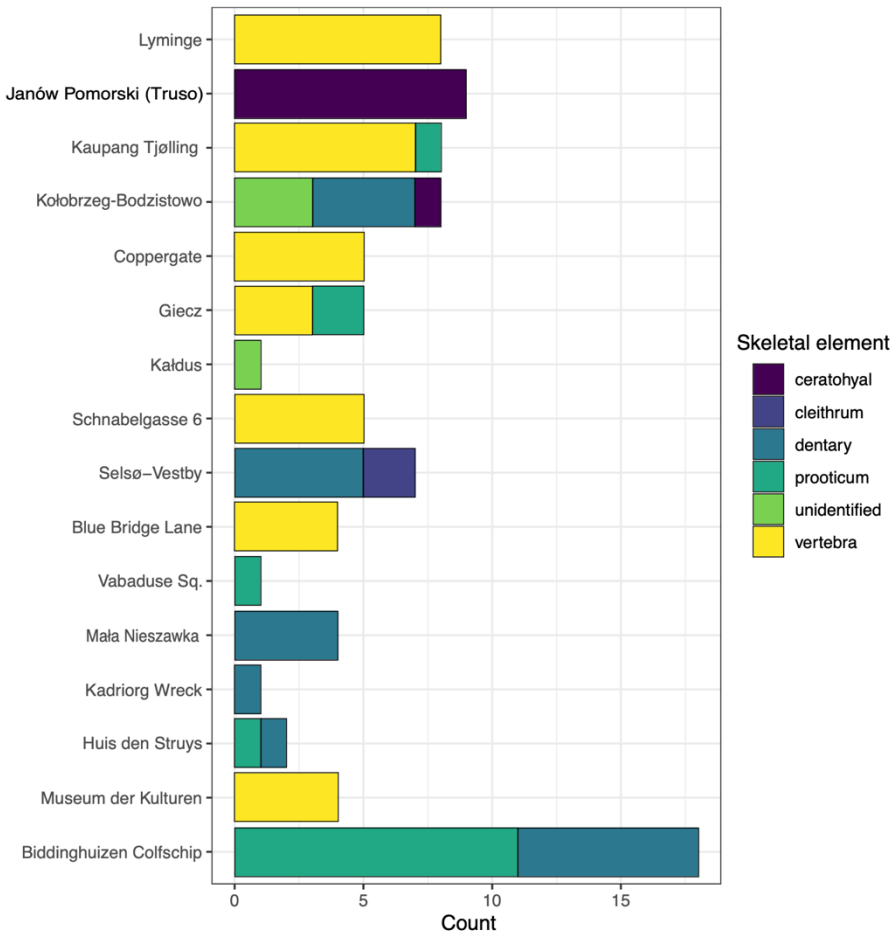


Fig S3 – Distribution of skeletal elements per site.

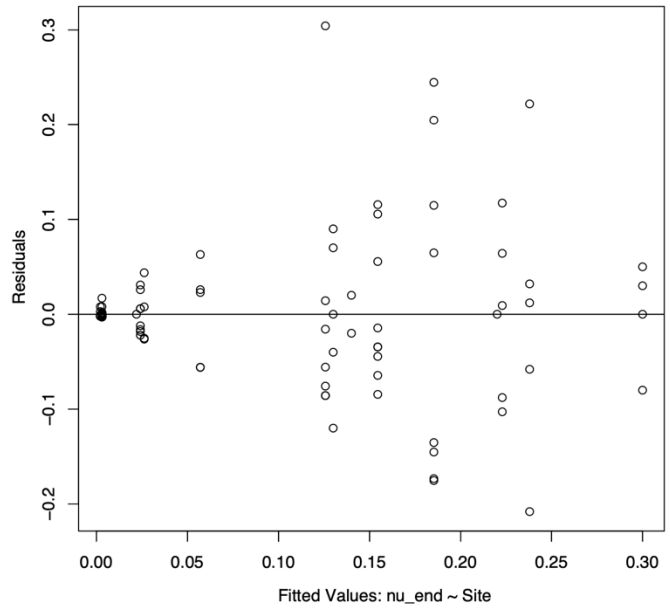


Fig S4 – Residuals for endogenous DNA content as explained by archaeological site

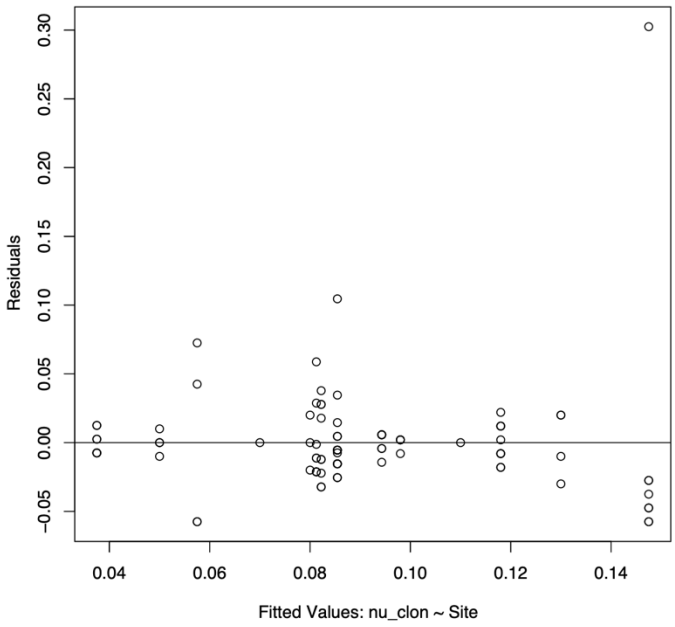


Fig S5 – Residuals for nuclear clonality as explained by archaeological site

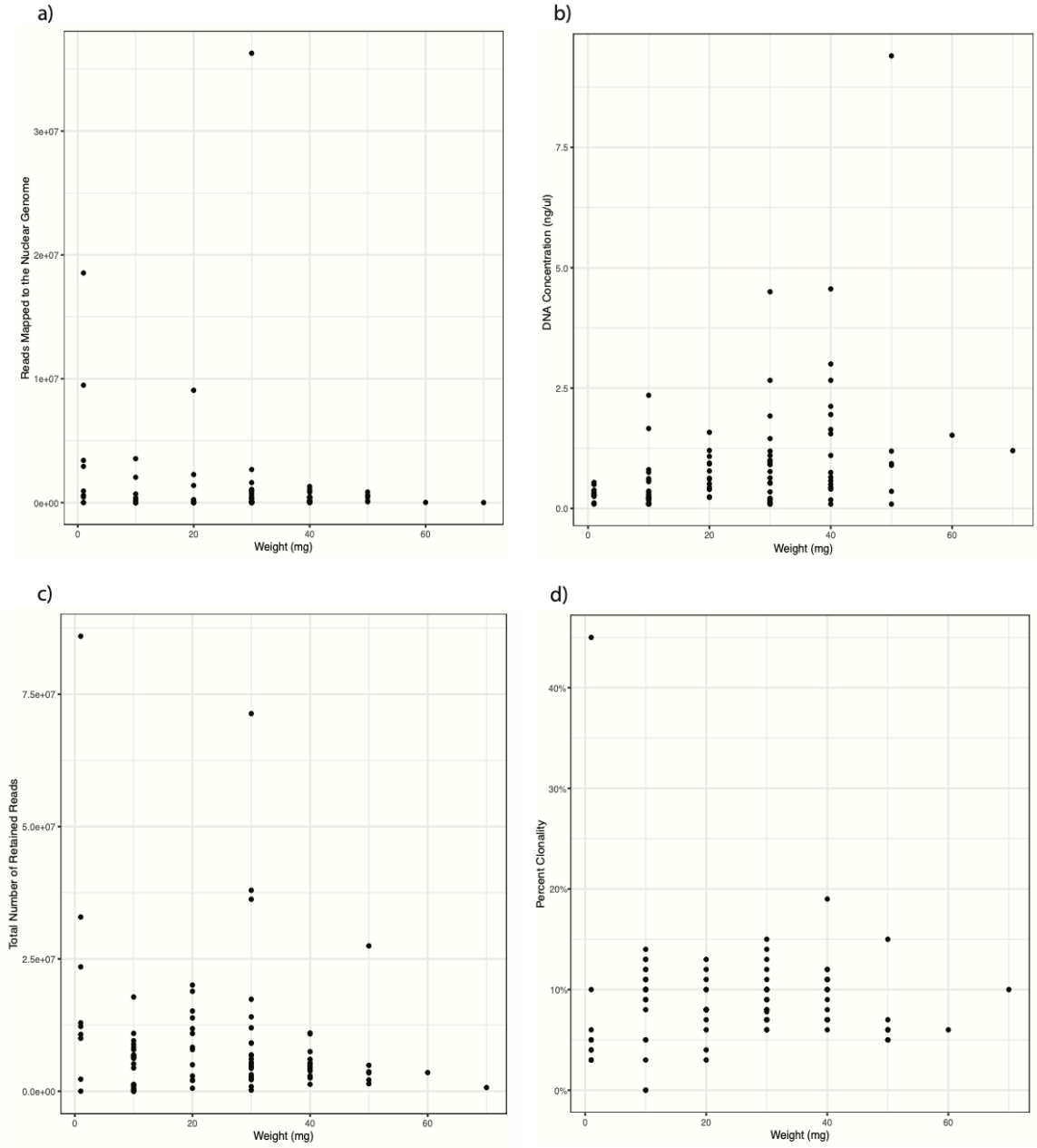
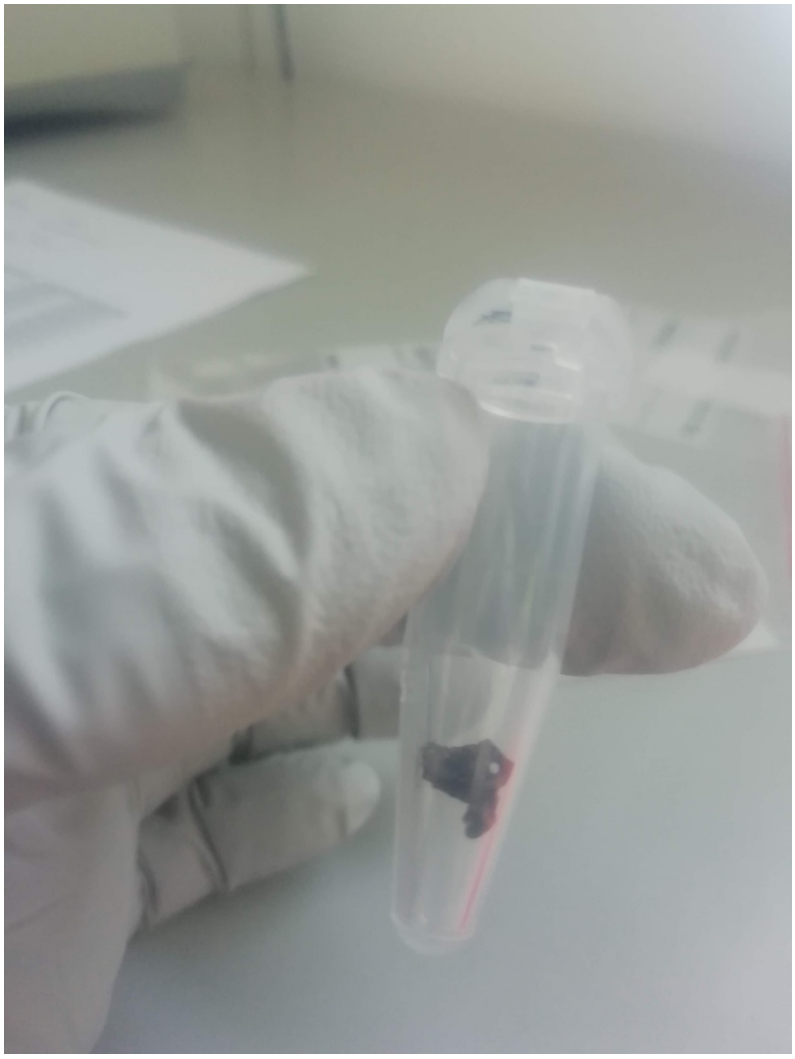


Fig S6 – Indicators of library complexity and quality by weight bin. a) reads mapping to the nuclear genome; b) DNA concentration as measured on the Qubit Fluorometer; c) total number of retained reads after PALEOMIX quality control; d) percent nuclear clonality.

Chapter 3:

How to use terrible data



MOLECULAR ECOLOGY RESOURCES

RESOURCE ARTICLE

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An accurate assignment test for extremely low-coverage whole-genome sequence data

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Abstract

Genomic assignment tests can provide important diagnostic biological characteristics, such as population of origin or ecotype. Yet, assignment tests often rely on moderate- to high-coverage sequence data that can be difficult to obtain for fields such as molecular ecology and ancient DNA. We have developed a novel approach that efficiently assigns biologically relevant information (i.e., population identity or structural variants such as inversions) in extremely low-coverage sequence data. First, we generate databases from existing reference data using a subset of diagnostic single nucleotide polymorphisms (SNPs) associated with a biological characteristic. Low-coverage alignment files are subsequently compared to these databases to ascertain allelic state, yielding a joint probability for each association. To assess the efficacy of this approach, we assigned haplotypes and population identity in *Heliconius* butterflies, Atlantic herring, and Atlantic cod using chromosomal inversion sites and whole-genome data. We scored both modern and ancient specimens, including the first whole-genome sequence data recovered from ancient Atlantic herring bones. The method accurately assigns biological characteristics, including population membership, using extremely low-coverage data (as low as 0.0001x) based on genome-wide SNPs. This approach will therefore increase the number of samples in evolutionary, ecological and archaeological research for which relevant biological information can be obtained.

1 Introduction

Continuous advances in sequencing technology have resulted in an exponential increase in coverage and genomic resources for many species (Reuter et al., 2015; Wetterstrand, 2021). Nonetheless, although high-coverage whole-genome data allow a plethora of detailed bioinformatic analyses, low-coverage data remains common in fields that cannot always generate large amounts of high-coverage sequences, such as molecular ecology and ancient DNA (Bohmann et al., 2020; Malé et al., 2014; Peterson et al., 2012; Ripma et al., 2014; Suchan et al., 2016). Such low coverage data may arise for various reasons. First, whole genome population-level analyses in ecological research may remain cost-prohibitive, leading researchers to turn to techniques such as reduced-representation sequencing (e.g., Dodsworth, 2015; Marcus, 2021; Nevill et al., 2020; Zeng et al., 2018). Second, recent strategies targeting organelle (mitochondrial or chloroplast) reference databases increasingly use genome skimming sequencing approaches (Bohmann et al., 2020). Finally, DNA preservation in subfossil, archaeological, historical, or degraded biological material remains variable and is often context-specific (Ferrari et al., 2021; Keighley et al., 2021; Tin et al., 2014). In order to account for such unpredictability, ancient DNA sequencing studies typically screen many specimens, from which a subset with the best DNA preservation is selected for deeper sequencing (e.g., Martínez-García et al., 2021; Star et al., 2018; van der Valk et al., 2021). These practices result in a proliferation of specimens for which (extremely) sparse genome-wide data is obtained.

Low-coverage sequence data are difficult to jointly analyse with specimens that have obtained higher coverage without introducing various types of statistical bias (e.g., François & Jay, 2020; Lee et al.,2010; Patterson et al., 2006; Skoglund et al., 2014). Researchers who use low-coverage sequence data are therefore restricted in the analyses they can conduct and are often forced to discard specimens that do not yield sufficient coverage. For any investigation within the field of molecular ecology, the discarding of specimens due to low coverage results in wasted resources, and a reduced number of specimens for downstream analyses. Moreover, for ancient DNA studies this leads to the destruction of limited and unique zooarchaeological material for which no meaningful information is obtained. Efforts to obtain as much relevant information as possible from such specimens are therefore particularly important, from a biological and an ethical perspective (Pálsdóttir et al., 2019).

Genomic assignment tests can provide important biological information in ecological research and in several cases, low-coverage data have been effectively used for the determination of a range of basic biological characteristics (e.g., Grossen et al., 2018). For instance, the genetic sex of ancient mammals can easily be assigned from sparse sequencing data due to its association with extensive genomic differentiation on a chromosomal scale. Sexing has been applied to ancient low-coverage sequences to infer burial practices (Fages et al., 2020; Nistelberger et al., 2019), the impact of historic hunting (Barrett et al., 2020), and the behaviour of extinct species (Pečnerová et al., 2017). Aside from sex determination, other relevant biological characteristics may also be associated with large-scale genomic differentiation. In particular, structural variants (e.g., chromosomal inversions, haploblocks, or supergenes) have been increasingly identified as major drivers of evolutionary and ecological processes (Wellenreuther & Bernatchez, 2018), playing important roles in population structure and evolution. For instance, inversions are involved in the evolution of sex chromosomes (Hughes et al., 2010; Lemaitre et al., 2009) and speciation (Noor et al., 2001), and are critical for within-species adaptation to local environments (Ayala et al., 2013; Barth et al., 2017; Berg et al., 2016; Jones et al., 2012; Leitwein et al., 2017; Lowry & Willis, 2010; Morales et al., 2019;

Nadeau et al., 2016; Pettersson et al., 2019; Todesco et al., 2020; Twyford & Friedman, 2015). Chromosomal inversions can affect megabase-sized genomic regions (e.g., Berg et al., 2017; Fang et al., 2012; Twyford & Friedman, 2015), and are often characterized by high levels of linkage disequilibrium (LD; Hoffmann & Rieseberg, 2008) due to inhibited recombination between noncollinear inversion haplotypes. Genotyping of such haplotypes using a subset of segregating genetic markers is feasible using whole genome sequencing data (Donnelly et al., 2010; Salm et al., 2012). Therefore, low-coverage data can retrieve relevant biological characteristics, potentially yielding useful insights on population continuity, species migration and distributions, hunting, historic trade, and burial practices, depending on archaeological context or ecological setting.

Several methods have been developed for assigning inversion haplotypes in order to facilitate GWAS analysis for SNPs within inversions in the human genome (*scoreInvHap*, Ruiz-Arenas et al., 2019; *pfido*, Salm et al., 2012; *InvClust*, Cáceres & González, 2015; *inveRsion*, Cáceres et al., 2012; and methods proposed by Bansal et al., 2007; Sindi & Raphael, 2010). These methods rely on LD break-points and structural variation (e.g., *InvClust*, *inveRsion*, and *scoreInvHap*, as well as the methods proposed by Bansal et al. and Sindi et al.) or haplotype tagging (*inveRsion*) to identify inversion sites and then conduct various types of SNP calling within those sites. All of these methods are specifically developed for identifying inversions in human genomes (e.g., Ma & Amos, 2012) and their use in disease- and other phenotype-association studies (Ruiz-Arenas et al., 2019; Salm et al., 2012). They have not been tested with sparse genomic data and are specific to use with inversions; indeed, *pfido* was designed for just one inversion in the human genome (Salm et al., 2012). Because of their reliance on signatures of structural variation, they cannot be applied to other types of variation, such as genome-wide population differentiation. There is currently no approach specifically designed to classify extremely low-coverage data with a broad applicability to score different types of large-scale genomic differentiation in a range of species.

Here, we developed a new method that allows efficient assignment of different biological characteristics using extremely low-coverage sequence data. First, a database is created that contains the allele frequency association of individual SNP loci with a specific biological characteristic (e.g., an inversion type or population membership). These databases are based on moderate- to high-coverage sequences of a subset of specimens (Figure 1a). Second, sequence alignment data of (ancient) specimens are compared to this database and a joint probability (e.g., see Star et al., 2017) is calculated based on the binomial distribution of their frequency association (Figure 1b). This two-step approach is analogous to *scoreInvHap* (Ruiz-Arenas et al., 2019), yet there are some key differences. Importantly, in contrast to earlier approaches, this probability calculation does not make any assumptions regarding specific signatures of structural variation and can therefore be applied to different types of genetic differentiation. For instance, our approach includes differentiation between inversion haplotypes or genome-wide differences associated with ecotype or population structure. Our program depends solely on freely available, commonly used software and file formats, and is freely available for download at: https://github.com/laneatmore/BAMscorer.

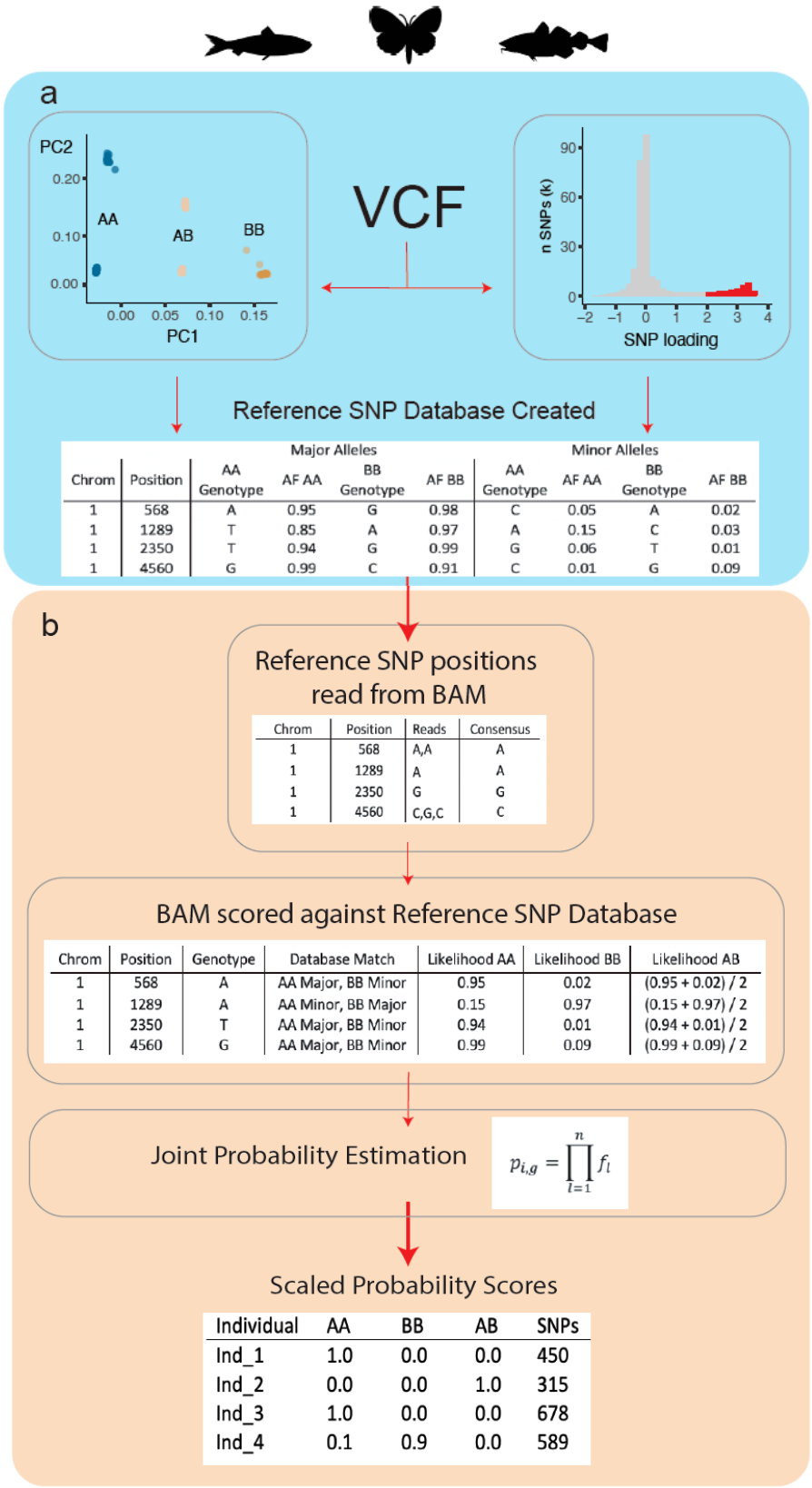
a
PC2
0.20
0.10
0.00
AA
AB
BB
0.00 0.05 0.10 0.15
PC1
VCF
n SNPs (k)
90
60
30
0
−2 −1 0 1 2 3 4
SNP loading
Reference SNP Database Created
Major Alleles
Minor Alleles
Chrom | Position | AA Genotype | AF AA | BB Genotype | AF BB | AA Genotype | AF AA | BB Genotype | AF BB
1 | 568 | A | 0.95 | G | 0.98 | C | 0.05 | A | 0.02
1 | 1289 | T | 0.85 | A | 0.97 | A | 0.15 | C | 0.03
1 | 2350 | T | 0.94 | G | 0.99 | G | 0.06 | T | 0.01
1 | 4560 | G | 0.99 | C | 0.91 | C | 0.01 | G | 0.09
b
Reference SNP positions read from BAM
Chrom | Position | Reads | Consensus
1 | 568 | A,A | A
1 | 1289 | A | A
1 | 2350 | G | G
1 | 4560 | C,G,C | C
BAM scored against Reference SNP Database
Chrom | Position | Genotype | Database Match | Likelihood AA | Likelihood BB | Likelihood AB
1 | 568 | A | AA Major, BB Minor | 0.95 | 0.02 | (0.95 + 0.02) / 2
1 | 1289 | A | AA Minor, BB Major | 0.15 | 0.97 | (0.15 + 0.97) / 2
1 | 2350 | T | AA Major, BB Minor | 0.94 | 0.01 | (0.94 + 0.01) / 2
1 | 4560 | G | AA Major, BB Minor | 0.99 | 0.09 | (0.99 + 0.09) / 2
Joint Probability Estimation
$p_{i,g} = \prod_{l=1}^{n} f_l$
Scaled Probability Scores
Individual | AA | BB | AB | SNPs
Ind_1 | 1.0 | 0.0 | 0.0 | 450
Ind_2 | 0.0 | 0.0 | 1.0 | 315
Ind_3 | 1.0 | 0.0 | 0.0 | 678
Ind_4 | 0.1 | 0.9 | 0.0 | 589

Figure 2.1– The BAMscorer pipeline

The BAMscorer pipeline has two main modules—reference database creation and alignment file scoring. (a) Sequence data must be pre-processed and input into the pipeline as a VCF file. smartPCA (Patterson et al., 2006; Price et al., 2006) is used to generate eigenvalues and SNP loading weights, which are then used to assign population groups or inversion types in the reference database and create a database of highly-divergent loci in a given region of interest. (b) These positions are called from the alignment files to be scored. The positions are then compared to the database for allelic similarity. The likelihood of a given allele at a locus belonging to a haplotype is coded as the frequency of that allele at the locus in each database. AB allele frequencies are calculated as the average of frequencies present in AA and BB haplotypes. A joint probability is estimated for each alignment file belonging to each of the three haplotypes (for genome-wide assignment only AA and BB are used) and these values are scaled to one, outputting a probability index of genomic assignment for each individual

We investigated the efficiency of our approach in assigning haplotypes for three chromosomal inversions in species that differ in their availability of reference specimens (P3 on Chr15, *Heliconius numata*, $n = 20$; Chr12, *Clupea harengus*, $n = 19$; and LG01, *Gadus morhua*, $n = 276$). These inversions display clinal distributions that are associated with biological characters such as wing pattern phenotypes (Joron et al., 2006, 2011; Nadeau, 2016), adaptation to water temperature and salinity (Pettersson et al., 2019), and migratory behaviour (Berg et al., 2016). Finally, we investigated the accuracy of this approach for the genome-wide population assignment of Atlantic cod specimens (Barth et al., 2019; Pinsky et al., 2021). To assess the program, we first built reference databases for each species and then used these databases to identify biological characteristics in nonreference alignment files. We used both ancient and modern sequences for scoring, including the first ancient whole-genome sequences recovered from Atlantic herring bones.

2 Materials and Methods

2.1 Reference and scoring databases

For each species investigated, two different data sets were used. The first data set was used to create the reference SNPs database (hereafter referred to as the 'reference database') and the second data set (hereafter referred to as the 'scoring database'), containing individuals not found in the reference database that were scored utilizing the BAMscorer program. Reference databases were processed and filtered as described below to be input to the BAMscorer program as VCF files. Scoring databases were aligned to appropriate reference genomes and left as unfiltered BAM files for scoring. All the data used in this manuscript—including the newly generated archaeological Atlantic herring data—are publicly available. Below we describe each data set in terms of composition, sample size and biological characteristics.

2.1.1 Heliconius butterflies

Heliconius numata butterflies are known to exhibit distinct wing-pattern morphs that are associated with different genomic haplotypes (Joron et al., 2006). These wing-pattern morphs are generally thought to be mimicry adaptations to predation and signalling between butterflies, therefore are probably under strong selective pressures (Chouteau et al., 2017; Joron et al., 2011). Research has suggested a supergene on chromosome 15 that determines the wing pattern morph of a particular butterfly (Joron et al., 2006, 2011). There are three inversion sites within the *P* supergene—P1, P2, and P3—which are associated with *Heliconius* wing-pattern morphs (Jay et al., 2018; Joron et al., 2011). We chose to focus on P3, which can be used to discriminate between the major types of wing-pattern morphs (Jay et al., 2021). Two databases were obtained for *Heliconius* butterflies, one as a reference database and one as a scoring database.

The reference database was obtained from Nadeau et al. (2016), which contains 20 individual genomes from various *H. numata* subspecies. This reference database encompasses several different wing morphs, which are associated with inversions at the *P* supergene (Chouteau et al., 2017; Jay et al., 2021). The scoring database consisted of 40 individual genomes obtained from Jay et al. (2021). This database, which has no overlap with the reference database from Nadeau et al. (2016), contains several different *H. numata* subspecies and encompasses various wing pattern morphs, therefore different P3 inversion types. The reference database was well-balanced between the three possible inversion types, with seven individuals belonging to types AA and BB and six individuals belonging to the AB heterozygous type.

Both the reference and scoring databases were aligned to the *Heliconius melpomene* Hmel2.5 reference assembly (http://ensembl.lepbase.org) using PALEOMIX v.1.2.13 (Schubert et al., 2014) with BWA-mem. The ~1.1 Mb P3 inversion is found on reference scaffold Hmel215003o (between 2,000,001 and 3,100,000 bp). Genotypes for the reference database were called using the GATK4 pipeline (Van der Auwera & O'Connor, 2020) and the following filtering parameters: FS<60.0 && SOR<4 && MQ>30.0 && QD >2.0 && INFO/DP<5500, SnpGap 10, minGQ 15 minDP 3, maf 0.001, with indels removed and biallelic variants selected. The reference database, therefore, is contained in a single VCF file, whereas the scoring database is a collection of unfiltered alignment files.

2.1.2 Atlantic herring

Atlantic herring show a high degree of genomic structure that is probably associated with environmental characteristics such as salinity and sea surface temperature and behaviours, such as spawning season (Lamichhaney et al., 2017; Martinez Barrio et al., 2016). Several inversion sites have been identified that are thought to be linked with some of these adaptations (Han et al., 2020; Pettersson et al., 2019). These sites show structure between various populations of Atlantic herring as well as between the true Atlantic herring (*Clupea harengus*) and the Baltic herring (*Clupea harengus membras*), a slightly smaller subspecies living in the Baltic Sea (Han et al., 2020).

For Atlantic herring, an ~8Mb inversion on chromosome 12 was investigated, which may be associated with a 'supergene' denoting different Atlantic herring ecotypes associated with salinity (Han et al., 2020; Pettersson et al., 2019). The inversion is located at chr12:17,900,000–25,600,000 bp and is linked to salinity adaptation for autumn-spawning herring populations (Han et al., 2020). A modern reference database was obtained from Han et al. (2020), which consisted of 20 individual genome sequences. These sequences encompassed all major populations of Atlantic and Baltic herring identified by Pettersson et al. (2019) and Han et al. (2020), with the exception of the spring-spawning Baltic herring. There were 6 Baltic individuals and 14 Atlantic individuals in the reference database.

To assess the applicability of BAMscorer for conducting assignment tests on ancient genomes, the scoring database was created from nine newly sequenced ancient Atlantic herring genomes (see following section for DNA extraction and sequencing methodology). Both modern and ancient herring reads were aligned to the Atlantic herring reference genome (GCA_900700415.1, Pettersson et al., 2019). The modern reads were aligned as described above for the *Heliconius* butterflies. Ancient herring reads were aligned as described in Ferrari et al. (2021), using BWA-aln, which is commonly held to be the most appropriate mode for aligning ancient genome sequences (Schubert et al., 2012).

Genotypes for the reference database were called and filtered following the same protocol as for the *Heliconius*, while the scoring database was not processed further. Two individual outliers in the reference database were observed and subsequently checked for relatedness using KING (Manichaikul et al., 2010; Note S1). These individuals appeared to be duplicates and were removed from the database to ensure accuracy of metadata.

In the scoring database of ancient individuals, most specimens have excellent DNA preservation (Table S1) and all show the typical fragmentation and misincorporation patterns of authentic ancient DNA data (Jónsson et al., 2013; Figure S1). The aligned sequences were down-sampled to 100,000 reads and are now available on ENA (accession number

PRJEB45393). Similar as for *Heliconius*, the reference database for Atlantic herring thus consisted of a single VCF file and the scoring database of a collection of alignment files.

2.1.3 Atlantic cod

The Atlantic cod reference database was created using 276 Atlantic cod individuals representing most major geographical locations (western Atlantic, eastern Atlantic, and Baltic Sea) in the species' range (Barth et al., 2019; Pinsky et al., 2021). In Atlantic cod, four large chromosomal inversions have been identified that are associated with differences in migratory behaviour and environmental adaptations (Berg et al., 2016; Kirubakaran et al., 2016; Sodeland et al., 2016). These chromosomal inversions originated independently, between 0.40 and 1.66 million years ago (Matschiner et al., 2021). A data set of 15 unrelated archaeological specimens were obtained from Star et al. (2017) to use for the scoring. Modern and ancient reads were aligned to the gadMor2 reference genome (Star et al., 2011; Tørresen et al., 2017) as above for *Heliconius* and Atlantic herring. SNP calling and filtering for the reference was performed as described in Barth et al. (2019) using the GATK haplotype caller v.3.4.46 (McKenna et al., 2010), bcftools v.1.3 (Li, 2011), VCFtools v.0.1.14 (Danecek et al., 2011), again mirroring methods used for Heliconius and Atlantic herring. For Atlantic cod, we investigated both an inversion locus, as well as genome-wide patterns of divergence. First, we targeted an ~16 Mb double chromosomal inversion on LG01 which is associated with differences in migratory behaviour (Berg et al., 2016; Kirubakaran et al., 2016; Sodeland et al., 2016). This inversion is located at LG01:9,100,000–26,200,000 bp, and the reference database contained 217 nonmigratory and 30 migratory specimens. Second, we analysed genome-wide data separating 24 western Atlantic from 252 eastern Atlantic cod specimens and genome-wide data separating 23 Baltic from 229 eastern Atlantic cod specimens. For the whole genome analyses, we excluded the location of four major inversions (on LG01, LG02, LG07, and LG12, as described in Berg et al., 2016, 2017) following the coordinates used in Star et al. (2017).

2.2 Ancient DNA extraction and sequencing

Nine Atlantic herring bones from two Polish sites, dated between the 9th and 15th century CE (Domagała & Franczuk, 1992; Iwaszkiewicz, 1991; Makowiecki, 2003; Makowiecki et al., 2016; Table S1), were UV-treated for 10 min per side and cleaned with ultra-pure water. DNA was extracted including a predigestion step, following Damgaard et al. (2015). Then, 10–40 mg of bone were pulverized with micro pestles in the digestion buffer (1 ml 0.5 M EDTA, 0.5 mg/ml proteinase K, and 0.5% N-Lauryl sarcosine). Following overnight digestion, DNA was extracted with nine volumes of a 3:2 mixture of QG buffer (QIAGEN) and isopropanol. MinElute purification was carried out using the QIAvac 24 Plus vacuum manifold system (Qiagen) in a final elution volume of 65 µl. Parallel nontemplate controls were included. Single-indexed blunt-end sequencing libraries were built from 16 µl of DNA extract or nontemplate extraction blank, following the single-tube (BEST) protocol (Carøe et al., 2018) with the modifications described in Mak et al. (2017). All laboratory protocols up to indexing of sequencing libraries were carried out in a dedicated ancient DNA clean laboratory at the University of Oslo following standard anti-contamination and authentication protocols (Cooper & Poinar, 2000; Gilbert et al., 2005; Llamas et al., 2017). Library quality and concentration were inspected with a High Sensitivity DNA Assay on the Bioanalyser 2100 (Agilent) and sequenced on an Illumina HiSeq 2500 platform at the Norwegian Sequencing Centre with paired-end 125 bp reads, demultiplexed allowing zero mismatches in the index tag.

2.3 The BAMscorer Pipeline

2.3.1 Module 1: creation of SNP reference databases

The initial step of the BAMscorer pipeline is to create a reference database of divergent SNPs associated with each haplotype or population in a set of focal individuals (Figure 1a). These divergent SNPs are referred to as belonging to 'AA', 'BB', or 'AB' genotypes (or groups). The BAMscorer program does not conduct SNP calling, but takes a preprepared VCF file as input. SNP calling methods and filtering parameters are therefore at the discretion of the user and can be done using a reference genome as we have done with our species or with de novo SNP calling techniques as is often used for reduced-representation sequencing. As long as the reference data is input to BAMscorer as a VCF file, a reference database can be created.

Reference SNP databases are created as follows:

1. The VCF file is first prepared with VCFtools v.0.1.16 (Danecek et al., 2011) and PLINK v.1.9 (Purcell et al., 2007), selecting only those regions of interest (i.e., where inversions are located, or genome-wide).
2. A Principal component analysis (PCA) is run as implemented in smartPCA (EIGENSOFT v.7.2.1; Patterson et al., 2006; Price et al., 2006) to calculate axes of differentiation and individual SNP loadings between homozygote inversion haplotypes or populations. As a default, the BAMscorer pipeline selects diagnostic loci in the top and bottom 5% of the SNP loading distribution, although the optimal SNP loading cutoff value should be determined by the user. Visualization of the SNP loading profile can help decide such cutoffs (see further below). The BAMscorer program is capable of filtering SNPs in the reference database based on both symmetrical and asymmetrical distribution cutoffs.
3. SNPs that pass cut-off filters form the divergent SNPs database for each haplotype or population. To assist the user in the selection of individuals to represent each haplotype, heterozygosity is calculated per individual based on SNPs in the divergent database.
4. Individuals from the reference database VCF file are scored for PC1 and heterozygosity values, and manually classified into types: when whole genome data are investigated, individuals are separated into groups called AA and BB; when inversions are investigated, individuals are separated into three clusters, representing genotypes that comprise homozygous AA and BB and heterozygous AB haplotypes. Inversion genotypes are known to fall into specific clusters in PCA analysis (see Figure 1a), which allow for easy identification using separation on PC1 and assessing levels of individual heterozygosity.
5. For individuals in the homozygote AA and BB haplotypes/groups, allele frequencies of the divergent SNPs are calculated. Two databases are created, containing the allelic state (i.e., A, C, G, T) and allele frequencies of the major (first database) and minor (second database) alleles in the AA and BB haplotypes. Databases containing few individuals often contain fixed alleles due to limited sampling. The uncertainty associated with sampling fixed alleles is addressed in the BAMscorer program by calculating a minimum expected frequency of $(1/((2*N)+1))$ for the minor allele, where N is the number of individuals in the reference database for fixed alleles in the region of interest. When scoring inversions, the sample probability of obtaining alleles from heterozygous AB genotypes are calculated by averaging the observed allele frequencies in the AA and BB haplotypes. We note that due to the nature of inversions, it is highly likely that a heterozygous genotype will fall directly in between the homozygous

genotypes (see Figure 2). This may not be the case for all genomic regions and is certainly not always the case for whole-genome variation. We therefore recommend running BAMscorer with the—wg flag for genomic regions that do not follow such a pattern and/or whole-genome analysis, as this will provide an assignment of either type AA or type BB without attempting to estimate the average allele frequencies between the two.

Once optimal database parameters have been identified (a full list of parameters can be found at https://github.com/laneatmore/BAMscorer), the SNP database can be reused for BAM scoring on many different data sets of the same species.

2.3.2 Module 2: BAM scoring

The reference database can then be used to assess the scoring database (Figure 1b). The scoring database consists of unfiltered alignment files in BAM format. BAM files can be generated at the user's discretion as long as the coordinate system matches that of the reference database. Rather than conducting SNP calling on the scoring database, BAMscorer takes the position of each SNP in the reference database and pulls these loci from each BAM file in the scoring database. A consensus read for each locus is determined via a simplified genotype calling process, and then the alleles in the alignment files are compared to the reference database to determine the probability of a given individual belonging to each genotype or genetic cluster.

A detailed overview of the BAM scoring process is as follows:

1. The divergent SNPs databases are used to score alignment files (BAM format) for a given set of (low-coverage) individuals. For each locus in the divergent SNPs database, matching reads are pulled from the BAM file using the python module pysam (https://github.com/pysam-developers/pysam). The ‘consensus' read is determined based on the most highly-represented allele in all reads for each position. In the event that there are equal numbers of reads for multiple alleles at a given locus, one allele is then chosen at random, although these instances were extremely rare in the low-coverage data analysed here. This process provides a subset of observed alleles at divergent loci in each inversion or population for each individual BAM file. Using pysam for genotype calling directly from BAM files is the most accurate method when dealing with low-coverage data (Ros-Freixedes et al., 2018).
2. The probability of observing a variant associated with a specific haplotype is calculated using the allele frequencies of matching positions in the reference databases. For example, if the position in the BAM file matches the dominant allele in haplotype group AA, the probability for that locus belonging to the AA genotype is coded as the allele frequency of the dominant allele in haplotype group AA. If the allele also matches the major or minor allele in haplotype group BB, the probability for that locus belonging to the BB genotype is coded as the allele frequency of that allele in the BB reference set. This allows a proxy calculation for heterozygous sites in the BAM file without requiring the extensive computational requirements it would take to determine genotype likelihood directly for each position. Both the dominant and minor allele frequencies for each genotype in the reference database for alleles in homozygote AA and BB haplotypes are used, thereby providing a likelihood estimation that the consensus read pulled from the BAM file is any one of the following: AA dominant, AA minor, BB dominant, BB minor. For inversions, three probabilities are recorded for

each position—the frequency of that allele in haplotype groups AA, BB, and AB (only AA and BB for genome-wide analysis).

3. Joint probabilities of all observed alleles belonging to a particular haplotype group or population are calculated for each individual using the following equation:

Whereby the probability (p) of the scored individual (i) and genotype (g) is the product of allele frequencies (f) of the number (n) of observed SNP loci (l) in each database. Finally, the joint probability scores for all genotypes are scaled to one to provide a final probability estimate of an individual belonging to a certain haplotype or population. We also provide the number of SNPs in the reference database that were recovered from each individual BAM file to inform on how well scored a specific individual is.

2.4 Analyses

We ran the above pipeline on each of the four databases outlined above: *Heliconius* P3 inversion, Atlantic herring chr12 inversion, Atlantic cod LG01 inversion, and the whole-genome data set for Atlantic cod (in two different scenarios). For each of these data sets, we also tested program parameters to assess the impact of noise and filtering in the reference database on BAMscorer accuracy. We created separate reference databases for each inversion using SNP loading cutoff values between 1% and 25% and an additional set of reference databases for the genome-wide analysis of Atlantic cod with SNP loading cutoff weights between 1% and 5%. We further assessed the impact of SNP filtering in reference database creation by limiting analysis to asymmetrical tails of the SNP loading distribution (e.g., taking only SNPs in the top 5% of loading weights).

2.5 Assessing scoring certainty

To investigate the sensitivity of the BAMscorer pipeline, we down-sampled each BAM file in the five databases (P3 from *Heliconius*, Jay et al., 2021; chr12 from Atlantic herring; and LG01 and two whole-genome population comparisons from Atlantic cod, Star et al., 2017). Following an approach described in Nistelberger et al. (2019), BAM files containing whole-genome shotgun data were downsampled to contain between 1000 and 40,000 reads (in most instances this is a mere fraction of the available data). At each read interval, and for each individual, the downsampling was randomly iterated 20 times. We compared accuracy of the scoring results of the extremely downsampled *Heliconius* data using BAMscorer by comparing these results to a separate PCA analysis using all data for the individuals in both databases. All of the individuals from the scoring database clustered with one of the three inversions in the reference database (Figure S2) and this clustering fully agreed with the assessment using BAMscorer. For Atlantic herring and Atlantic cod, accuracy of results was confirmed by prior knowledge of the inversion types or geographic origin of specimens. We assume that the ancient Polish herring have a Baltic origin given the archaeological context and age, and should therefore more closely match with the chr12 BB haplotype group, which is associated with the Baltic individuals in our reference database.

TABLE 1. Inversion and genome characteristics of *Heliconius*, Atlantic herring, and Atlantic cod. Each comparison differs in terms of size of inversion, overall genome size and relative size of inversion in regards to species-specific genome size, as well as in terms of the optimum number of divergent SNPs (see methods) and individuals used for the reference databases and scoring

Species	Inversion name, location	Inversion size (Mbp)	Genome size (Mbp)	Relative size (%)	Divergent SNPs (n)	Database individuals (n)	Scored individuals (n)
H. numata	P3, Chr15	1.1	273	0.4	1701	20	40
C. harengus	Chr12	8	726	1.1	28205	19	9
G. morhua	LG01	16	643	2.5	47564	276	15
G. morhua	WG	na	643	na	221790	276	15

3 Results

We investigated three chromosomal inversions and one genome-wide analysis using BAMscorer. The *Heliconius* P3 inversion is the smallest (1.1 Mb) inversion, followed by the Atlantic herring Chr12 (8 Mb) and Atlantic cod LG01 inversion (16 Mb, Table 1). Principal component analysis (PCA) as implemented through BAMscorer *select_snps* separates the three main inversion genotypes along PC1 for the *Heliconius* P3, Atlantic herring Chr12, and Atlantic cod LG01 databases (Figure 2a), reproducing earlier observations (Barth et al., 2019; Han et al., 2020; Nadeau et al., 2016; Pinsky et al., 2021). Similarly, the whole genome analysis separates western from eastern Atlantic cod specimens along PC1 (Figure 2a, Pinsky et al., 2021) and Baltic from other eastern Atlantic cod (Figure S3, Barth et al., 2019). For the data analysed here, BAMscorer *select_snps* typically runs within 15 min when using the test scoring database provided on a MacBook Pro (running MacOS Catalina with a 1.4 GHz Quad-Core Intel Core i5 and 16GB RAM). The SNP weight loading distribution underlying genetic divergence between inversion haplotypes of populations is either approximately symmetrical (*Heliconius* and Atlantic herring) or asymmetrical (Atlantic cod, Figure 2b). SNP weights are proportional to the correlation (across samples) between each SNP and each PC (Patterson et al., 2006; Price et al., 2006). SNPs that are strongly associated with divergence will have the highest SNP weight loading values and are therefore biologically informative.

An important consideration of our approach therefore lies in the selection of loci based on their SNP loading distribution patterns. In order to maximize the probability of observing loci in low-coverage sequencing data, as many loci as possible should be included in the database. Yet, adding those loci that are not significantly associated with either inversion haplotype or specific population will add noise and uncertainty. We therefore tested the accuracy of our approach using a range of SNP loading filtering parameters. For inversions, databases were created using cutoff values between 1% and 25%, depending on the species under investigation. For our genome-wide analyses, we set the SNP loading cut-off weights between 1% and 5%. The default parameter in the BAMscorer pipeline is to take symmetrical portions from each side of the SNP loading distribution (the 5% cutoff value takes the top and bottom 5% of SNPs), yet we also noticed asymmetrical SNP loading distribution values. We therefore also investigated the effect of selecting SNPs from either the top or bottom of the SNP loading distribution.

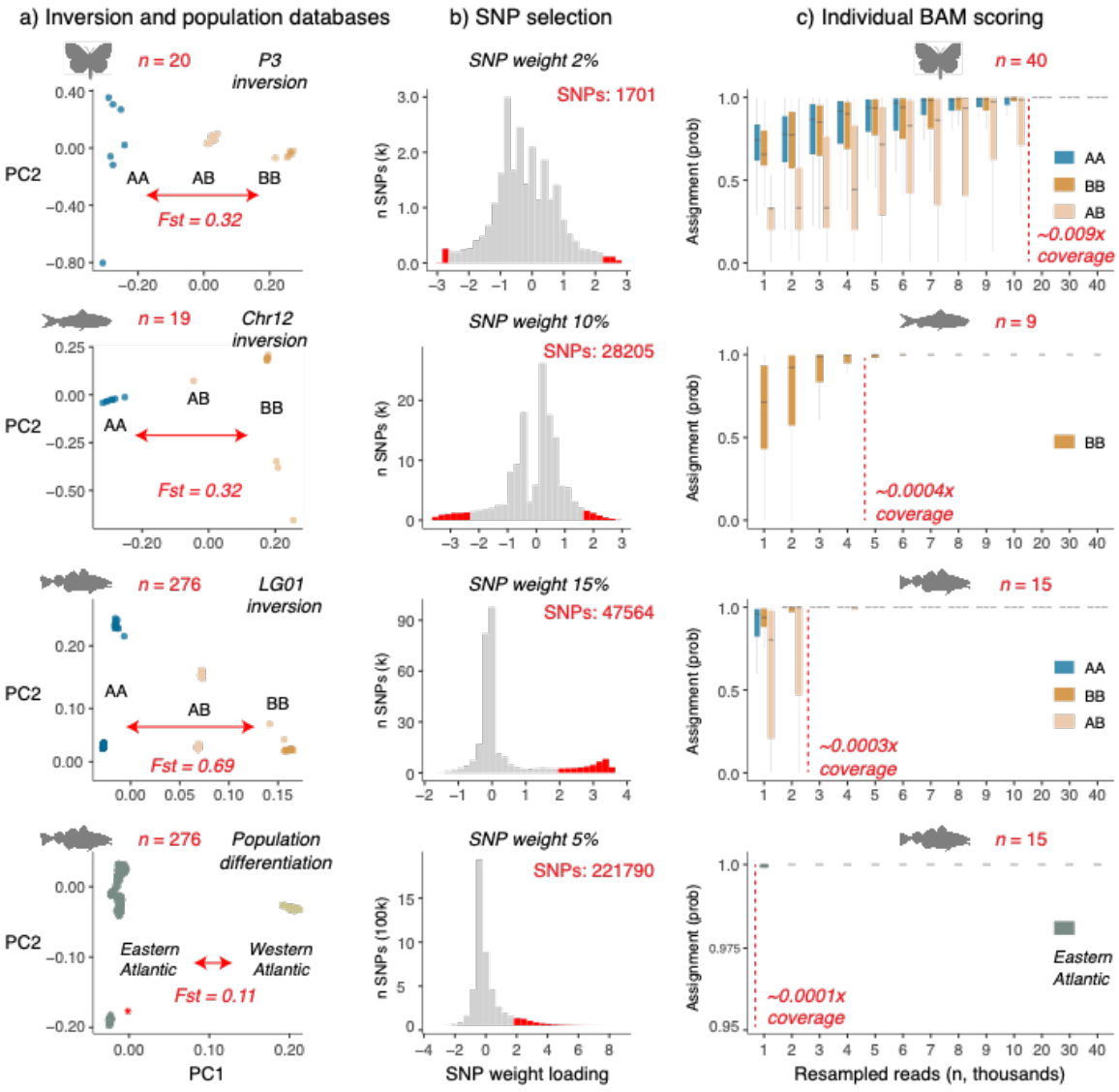

FIGURE 2 - Inversion and population assignment for *H. numata* (P3 inversion), *C. harengus* (Chr12 inversion) and *G. morhua* (LG01 inversion, population differentiation) using extremely low-coverage data. (a) Inversion and population PCA plots generated for the three species (silhouettes) using smartPCA (Patterson et al., 2006; Price et al., 2006). Haplotype clusters (AA; blue, AB; beige, BB; sepia) and main population clusters (eastern Atlantic; dark green, western Atlantic; light green) are named. The number of individuals (red) and the weighted F_{ST} differentiation between inversion-loci (between AA and BB) or genome-wide (red arrow) is indicated. The Baltic population is indicated by a red '*' amongst the eastern Atlantic populations. (b) SNPs most associated with either inversion (A or B) haplotype or large-scale population differentiation (western or eastern Atlantic) are selected based on their SNP weight loading distribution along PC1. Those with lowest and highest loadings are most associated with differentiation along PC1. SNP weight indicates the percentage of SNPs selected from the most extreme end(s) of the distribution (red). (c) Assignment probability for individual specimens generated by downsampling BAM files 1000–40,000 reads. At each interval, and for each individual, the downsampling is iterated 20 times in order to generate box plots. Probabilities are calculated based on the joint binomial distribution of observing divergent SNPs associated with either haplotype group or population. Also indicated is the number of individuals scored (red, note these are not the same individuals used to create the original databases) and fold coverage (red dotted line, *x* coverage) at which more than 0.99 median assignment probability is obtained.

For the *Heliconius* P3 inversion, the ability to confidently score heterozygous individuals (Jay et al., 2021) erodes with increasing SNP weight values (Figure 3), and the optimal cutoff to simultaneously score all possible genotypes lies at 2% and 1701 SNPs. For Atlantic herring Chr12, not all inversion types are observed in the ancient read data, yet no major increase in ability of scoring is obtained after a SNP weight of 10% and 28,205 SNPs (Figure S4). For Atlantic cod, best separation of ancient data (Star et al., 2017) was obtained by selecting SNPs from the single, most extreme end of the SNP weight loading distribution (Figure 2b, Figures S5–S7). For Atlantic cod LG01, SNP selection is similar to the *Heliconius* P3, in that the optimal cutoff is a trade-off in scoring homozygotes and heterozygotes, which for cod lies at 15% and 47,564 SNPs (Figure S6). Finally, best population separation for Atlantic cod using whole genome data is obtained at 5% and 221,790 SNPs for the trans-Atlantic separation (Figure S7), and 5% and 217,328 SNPs for the Baltic separation (Figure S3).

After deciding the best-possible cut-off values, several observations can be made regarding the scoring accuracy of BAMscorer *score_bams* depending on the number of reads for each of the comparisons. First, accurate scoring is obtained in extremely low-coverage data for all comparisons (Figure 2c). For *Heliconius*, accurate haplotype determination is obtained with 20,000 reads and 0.009x nuclear coverage. For all other comparisons, even less reads—by an order of magnitude—are required. Second, the scoring accuracy of heterozygous genotypes requires more reads compared to homozygous genotypes (see *Heliconius* P3 and Atlantic cod LG01, Figure 2c). Thus, different levels of accuracy are obtained depending on each sample's haplotype or population of origin. Third, an increase in scoring accuracy at lower numbers of reads is observed for those comparisons for which more SNPs could be obtained (Table1, Figure 2c). Best scoring accuracy is obtained for the population comparison of Atlantic cod, for which population of origin can be determined with 1000 reads or less than 0.0001x nuclear coverage (Figure 2c). The Baltic cod population (separated from other eastern Atlantic populations on PC2 indicated with red '*', Figure 2a), can be identified by iteratively investigating a smaller subset containing the eastern Atlantic specimens only (Figure S3). Finally, BAMscorer *score_bams* takes—on average—approximately 5 min to complete each comparison using a single Intel Xeon-Gold 6138 2.0 GHz CPU with 10 Gb of ram. It takes a similar amount of time to run the test scoring database on the MacBook Pro system as described above.

4 Discussion

The BAMscorer program allows genomic assignment on extremely low-coverage sequence data, thereby increasing the capacity for conducting population genomics analysis on sparse genome-wide data. Sequence data with low coverage is often discarded as there is little usable information that can be reliably recovered in comparative analyses with higher coverage specimens (e.g., François & Jay, 2020; Lee et al., 2010; Patterson et al., 2006; Skoglund et al., 2014). Applying our method will allow samples with sparse genome-wide data to be used. The method is, additionally, fast and can be applied to large quantities of data at one time, providing an efficient overview of the biological characteristics of a large data set. This approach will therefore expand the amount of information that can be gleaned from sparse genome-wide data (Bohmann et al., 2020), and reduce sample dropout in the ancient DNA analyses pipeline where destructive sampling is wide-spread (Pálsdóttir et al., 2019).

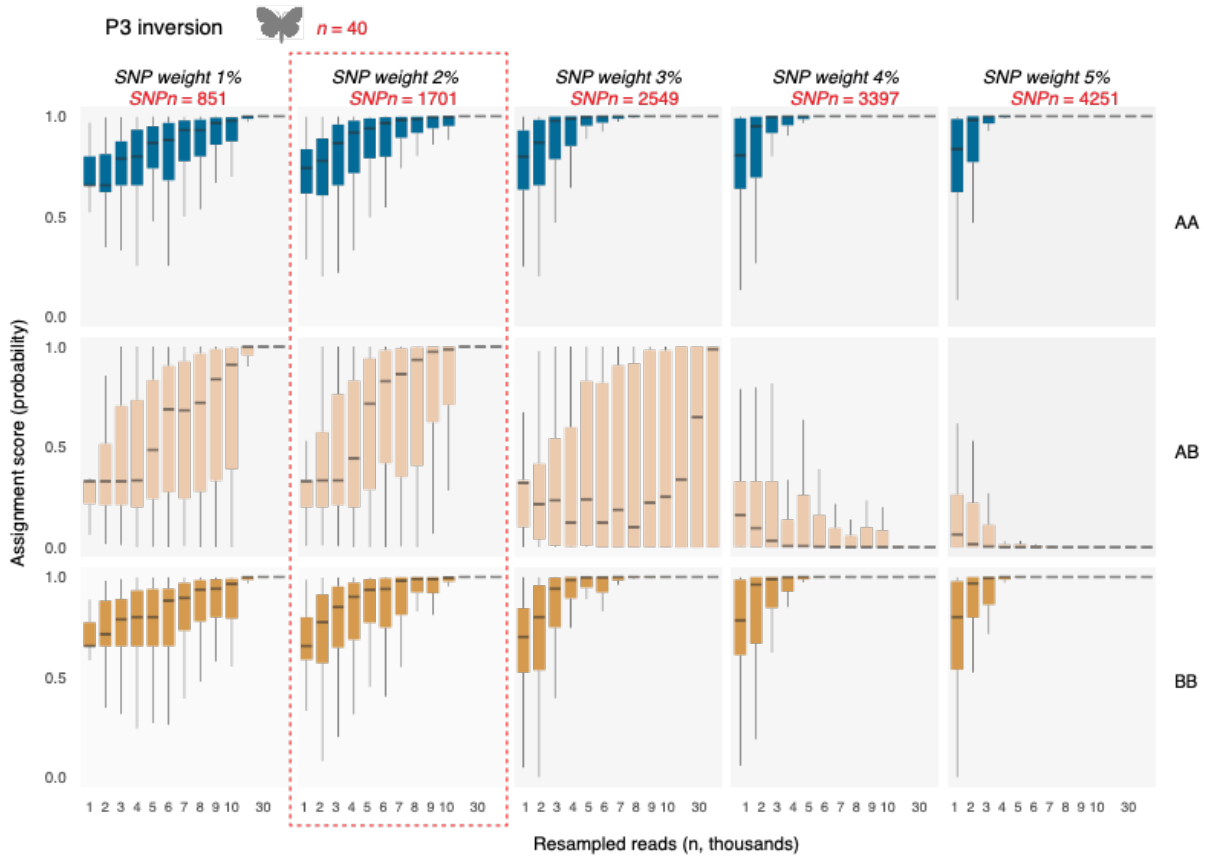

FIGURE 3 – SNP selection by varying SNP weight in *H. numata* (P3 inversion). SNP weight is here defined as the percentage of SNPs with the most extreme values at both sides of the SNP loading distribution. Confidence in probability assignment is obtained by down-sampling BAM files 1000–40,000 reads. At each interval, and for each individual, the downsampling is iterated 20 times in order to generate box plots. Probabilities are calculated based on the joint binomial distribution of observing divergent SNPs associated with either haplotype group. Also indicated is the number of individuals (*n*, red) and number of SNPs (SNP*n*, red) and the chosen cutoff value (red dotted lines) at which all three haplotype groups can be efficiently separated

We applied our method to three biological examples that have different levels of genomic differentiation. In *Heliconius* butterflies, inversions have not been found to be sympatric barriers to interspecific gene flow (Davey et al., 2017), and there is a high degree of interbreeding between the seven different *H. numata* wing pattern morphs (Chouteau et al., 2017). Within the wing pattern morph supergene on chromosome 15, there is incomplete genetic segregation between several of the *P* locus inversion types, including the P3 inversion site (Jay et al., 2021). This could suggest incomplete lineage sorting—a phenomenon known to be highly prevalent in mimetic species such as *H. numata* (Kozak et al., 2015; Savage & Mullen, 2009)—and/or introgression among *H. numata* morphs. Although BAMscorer exhibited less power in distinguishing this complex pattern of genomic divergence between *H. numata* morphs than between fish ecotypes, the approach still showed a high degree of efficiency. Within the *Heliconius* we were able to correctly identify all morphs without error at just 0.009x coverage, including heterozygous individuals, for which identification becomes significantly more challenging at low sequencing effort.

Both Atlantic herring and Atlantic cod exhibit temporally and geographically isolated spawning reproductive behaviour, although overall levels of genetic differentiation are relatively low (e.g., F_{ST} ~0.1 or less; Barth et al., 2017; Berg et al., 2016, 2017; Martinez Barrio et al., 2016; van Damme et al., 2009). Nonetheless, the divergence time of the LG01 inversion haplotypes of Atlantic cod is estimated to be around 600,000 years ago (Matschiner et al., 2021), driving divergence in many thousands of SNPs, which explains the success of BAMscorer at extremely low-coverage for the LG01 inversion. Similarly, strong selective pressures on Baltic herring have driven differentiation between these populations and their Atlantic conspecifics, as herring populations entering the Baltic Sea ~8000 years ago would have had to adapt to new, brackish conditions. Even though divergence time between Atlantic and Baltic herring cannot be older than 8000 years, herring exhibit distinct signals of adaptation to low salinity conditions at several thousand loci (Han et al., 2020; Lamichhaney et al., 2012). Our results therefore indicate a high degree of accuracy in determining inversion types even for subspecies and haplotypes with a range of divergence times.

We obtain the highest power in scoring accuracy for the whole genome analyses of Atlantic cod. Both comparisons investigate populations that have diverged relatively recently: the western and eastern Atlantic populations around 65,000 years ago (Matschiner et al., 2021), and have genome-wide nuclear genetic divergence measured at F_{ST} ~0.11 (Pinsky et al., 2021). The Baltic was colonized between 8000 and 6000 years ago (Berg et al., 2015) and has genome-wide nuclear genetic divergence at F_{ST} ~0.04. Neither of these populations or areas exhibits fixed mitogenomic differentiation (Martínez-García et al., 2021). The high scoring accuracy that is obtained at a low number of reads in this whole-genome analysis—despite low overall genetic divergence—suggests a wide range of applicability in different biological settings.

Additionally, the accuracy of our results indicate that the method is robust to deamination damage, a common feature of ancient genomic sequences. By sampling ancient alignment files from different places in the genome, the scoring is robust to the noise created by deamination damage, which typically occurs at the ends of reads. Our ancient sequences for cod and herring exhibited up to 17% deamination damage (Figure S1), yet a high scoring accuracy was obtained despite the presence of such damage.

There are several practical considerations and limitations to take into account while using the BAMscorer program. First, each example provided here is associated with different levels of genomic divergence and size of genomic regions under investigation. We find that there are no optimal program settings that apply to all cases. Each of our three species required different filtering parameters, such as optimum SNP loading weight cut-off value, and required different minimum numbers of reads to obtain high scoring accuracy. Similarly, differences in these parameters were also required within species, such as when analysing the inversion on LG01 as compared to the genome-wide data in Atlantic cod. It is thus recommended that users explore the filtering parameters as we have done above to ascertain the appropriate parameters, as an understanding of the biological system in question is important for assessing the efficacy of BAMscorer.

Second, the whole-genome (population) application of BAMscorer is currently limited to assigning two clusters or populations simultaneously. We are in the process of developing a more generic approach that will allow scoring of an undefined number of populations and aim to make this available in future versions of the BAMscorer. The current version of BAMscorer, however, can be applied iteratively to sequentially score finer scales of genomic differentiation

within data sets containing multiple clusters (see Figure S3). Moreover, BAMscorer is reliant on existing reference data to create the database from which alignment files are scored. This is a limiting factor in any assignment test, and probably unavoidable. However, we found that even with a relatively low number of reference genomes (our reference databases ranged from 19 to 276 individuals), we were still able to efficiently identify haplotypes in low-coverage data. The requirements for the reference databases are therefore not especially demanding in order for BAMscorer to be used efficiently.

Finally, an assignment test only evaluates the scenario as given by the user. It is therefore important to use BAMscorer with an understanding of the biological system in question and with these limitations in mind. We further recommend that users assess the impact of filtering parameters for creating the BAMscorer reference SNP database for each biological system. To provide an understanding of how much data BAMscorer is actually getting from each BAM file, we provide a read-out of the number of SNPs in the reference database that were read from the BAM file in question.

We have here introduced a novel software program that can be used to increase the information gleaned from extremely low-coverage sequence data. We have found that biological characteristics and genomic assignment can be recovered from sequences with as little as 1000 aligned reads (at ~0.0001x coverage in the case of Atlantic cod). The method is flexible and can be used on various types of genomic data. Because all SNP calling and alignment takes place prior to using the BAMscorer program, the program itself is not dependent on a reference genome and can therefore be used with SNPs calling generated de novo, as is often the case for reduced-representation sequencing. The program is further scalable for BAM files from 1000 to 50 M reads and can handle up to hundreds of thousands of SNPs without sacrificing computational efficiency.

We have shown that BAMscorer can differentiate between subspecies, populations, ecotypes, and genomic inversions and can successfully recover relevant biological information from extremely low-coverage data. As the underlying methodology is general in design, it can be applied to any low-quality samples and reduced representation sequence data such as ddRAD (Peterson et al., 2012) or hyRAD (Suchan et al., 2016), two common methods for cost-efficient sequencing used in ecology and evolution studies for modern and historic specimens. We expect that the capacity to quickly identify population of origin, determine between domestic and wild types (e.g., farmed vs. wild salmon; Glover et al., 2013, 2017), assess ecotype distribution, and to identify hybrids will be a useful additional tool in the fields of wildlife forensics and conservation genomics.

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Author Contributions

Giada Ferrari, Lane M. Atmore, and Bastiaan Star wrote the manuscript. Bastiaan Star conceived the project. Giada Ferrari, Lane M. Atmore, and Bastiaan Star developed the method and statistical framework. Lane M. Atmore wrote the code for the software program. Giada Ferrari, Lane M. Atmore, and Bastiaan Star conducted data analysis and visualization. James H. Barrett and Daniel Makowiecki provided archaeological material for sequencing. Sissel Jentoft and Kjetill S. Jakobsen provided early access to genomic sequence data. All authors have read and approved the manuscript.

Open Research Badges

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://github.com/laneatmore/BAMscorer.

Data Availability Statement

Reference data for all species have been publicly released earlier and are available from the European Nucleotide Archive (ENA) with the following accession numbers: *Heliconius*; PRJEB12740 and PRJEB40136, Atlantic herring; PRJNA642736, Atlantic cod; PRJEB29231 and PRJEB41431. The nine ancient Atlantic herring sequences are available at ENA under accession number PRJEB45393. The full software package is available for download at: https://github.com/laneatmore/BAMscorer.

Chapter heading photo taken by Lane M. Atmore

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Supplementary Information:

An accurate assignment test for extremely low-coverage whole-genome sequence data

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Supplementary Note 1 – Relatedness/duplicates in modern herring reference data

21 modern genomes were accessed from publicly available datasets (Han et al., 2020). When these specimens were run through the first BAMscorer module to select divergent SNPs and identify reference genotypes, two individuals had PC1 eigenvalues that were distinct outliers from the rest of the data. Subsequent smartPCA of the whole-genome –rather than just the inversion on chr12– showed again that these individuals created a unique cluster away from the rest of the group.

Table SN1a – *select_snps* output for modern herring reference data for the inversion site on chr12. Eigenvalues and heterozygosity for each individual in the modern herring reference dataset are sorted by PC1. Two individuals (Gavle54 and Gavle98) here form a distinct cluster from the rest of the reference data.

Individual	PC1 Eigenvalue	Heterozygosity
Gavle54	-0.2701	0.67803
Gavle98	-0.2701	0.67805
NSSH34	-0.2598	0.51112
NSSH33	-0.2533	0.69821
Gavle100	-0.249	0.67549
Fehmarn44	-0.2401	0.55478
NSSH36	-0.2388	0.64941
Fehmarn3	-0.2286	0.6297
Fehmarn6	-0.2022	0.37827
AAL1_CelticSea	-0.0142	-0.37773
Z14_IsleOfMan	0.1863	0.43059
AK2_Downs	0.1882	0.45048
AAL2_CelticSea	0.1889	0.45951
AK1_Downs	0.1889	0.47169
AK3_Downs	0.1889	0.46896
AAL3_CelticSea	0.1902	0.44224
NorthSea13	0.1911	0.42011
Z4_IsleOfMan	0.1915	0.41241
NorthSea34	0.2155	0.39734
NorthSea19	0.2193	0.44939
Z12_IsleOfMan	0.2669	0.00079

TableSN1b–*Select_snps* output for modern herring reference data, whole-genome. The two individuals identified in the chr12 analysis again formed a distinct cluster.

Individual	PC1 Eigenvalue	PC2 Eigenvalue
NorthSea34	-0.3680	0.1668
NorthSea19	-0.3866	0.1832
Z12_IsleOfMan	-0.6399	0.3155
NorthSea13	0.0234	-0.1005
AK3_Downs	0.0246	-0.1034
AAL2_CelticSea	0.0250	-0.1054
Z4_IsleOfMan	0.0257	-0.1029
AAL3_CelticSea	0.0261	-0.1005
AK1_Downs	0.0267	-0.1021
Z14_IsleOfMan	0.0270	-0.1018
AK2_Downs	0.0289	-0.1086
AAL1_CelticSea	0.0324	-0.0963
NSSH36	0.0399	-0.0920
Fehmarn44	0.0533	-0.0785
Fehmarn3	0.0549	-0.0831
Fehmarn6	0.0566	-0.0825
NSSH33	0.0663	-0.2189
NSSH34	0.0672	-0.2620
Gavle100	0.0691	-0.0768
Gavle54	0.3707	0.5448
Gavle98	0.3707	0.5448

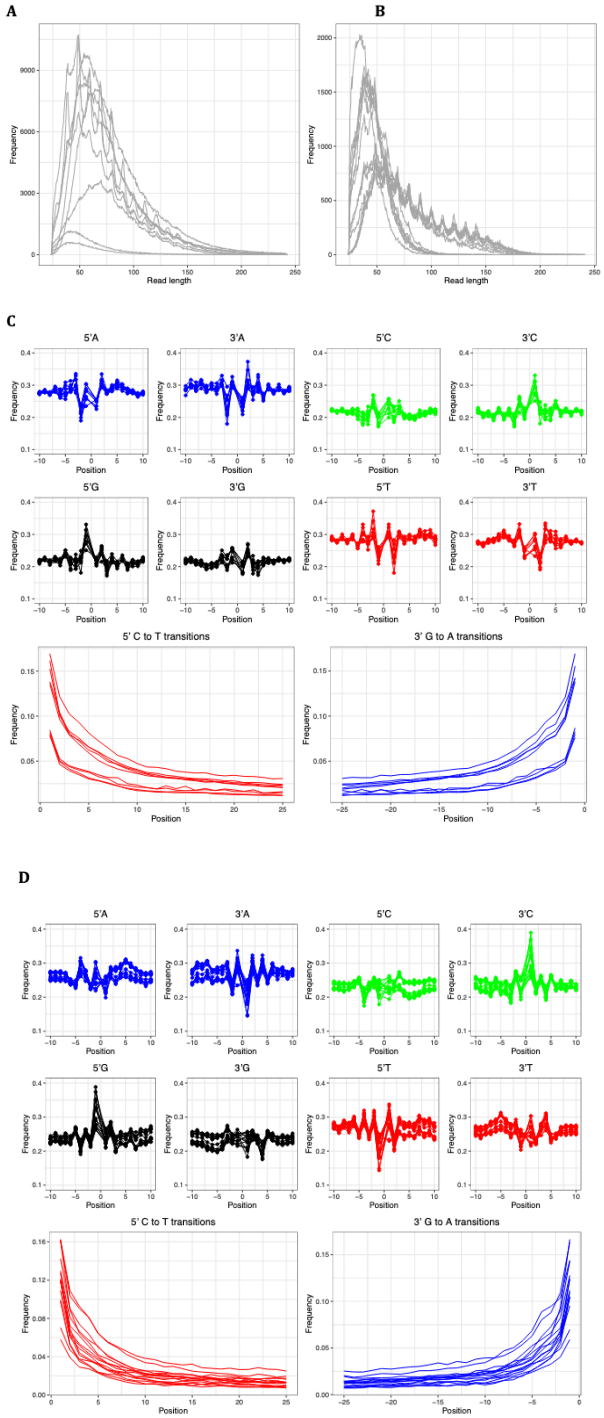
While it is uncommon for fish samples to be related given the extremely large population sizes in such species, this pattern can sometimes be formed when there are related individuals in a dataset. We therefore applied the KING pipeline (Manichaikul et al., 2010) to check for duplications and relatedness. Two of the samples in the dataset were found to be either related or duplicates, both from the same Baltic Sea population.

TableSN1c–KING output for duplicates/relatedness in modern herring reference data. Concordance values above 0.8 are indicative of duplicates according to the KING documentation.

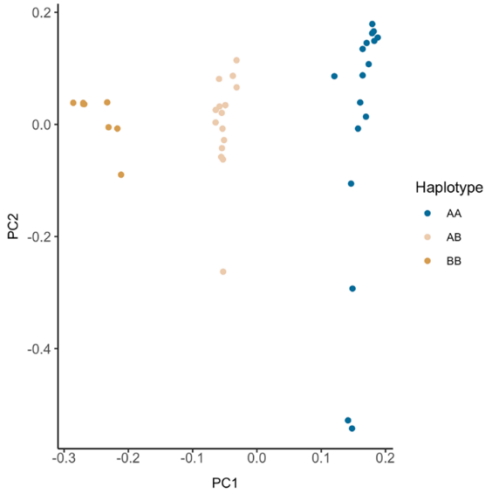
FID1	ID1	FID2	ID2	N	N_IBS0	N_IBS1	N_IBS2	Concord	HomConc	HetConc
Gavle54	Gavle54	Gavle98	Gavle98	11314407	187	4995	11309225	0.99954	0.99998	0.99674

The two individuals were subsequently removed from the reference dataset, leaving a total of 19 herring genomes for the reference data

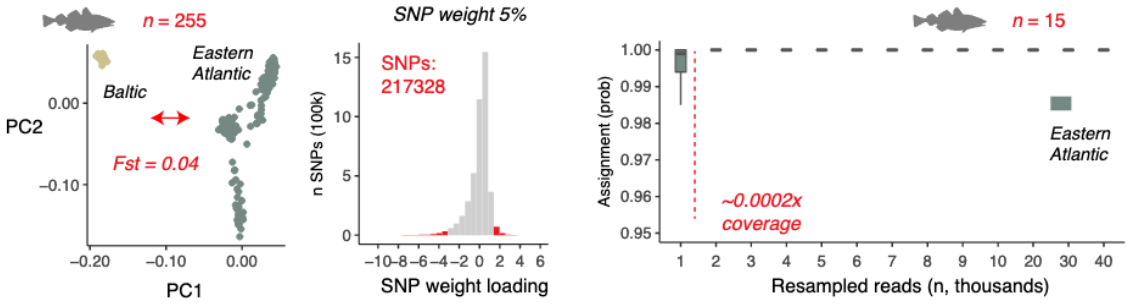
Supplementary figures



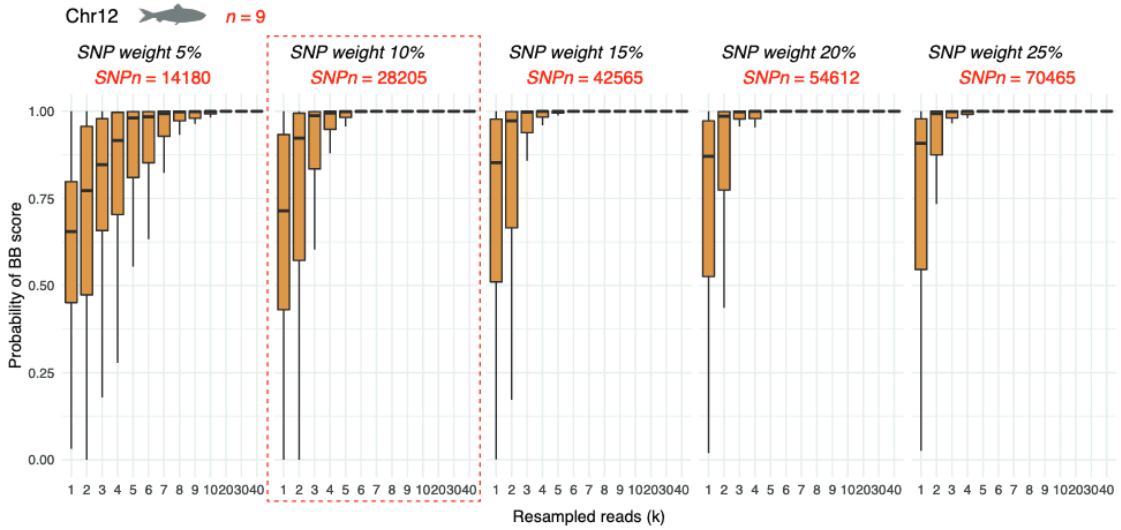
Supplementary Figure 1. aDNA fragmentation (A-B) and misincorporation (C-D) patterns of sequencing read data from nine Atlantic herring (A and C) and 15 Atlantic cod (B and D) specimens. Patterns were obtained by using MapDamage v. 2.0.6 (Jónsson et al., 2013). For visualization purposes, we only show the typical increase in C > T misincorporations due to cytosine deamination at the 5′-end of DNA fragments and the corresponding increase of G > A misincorporations at the 3′-end.



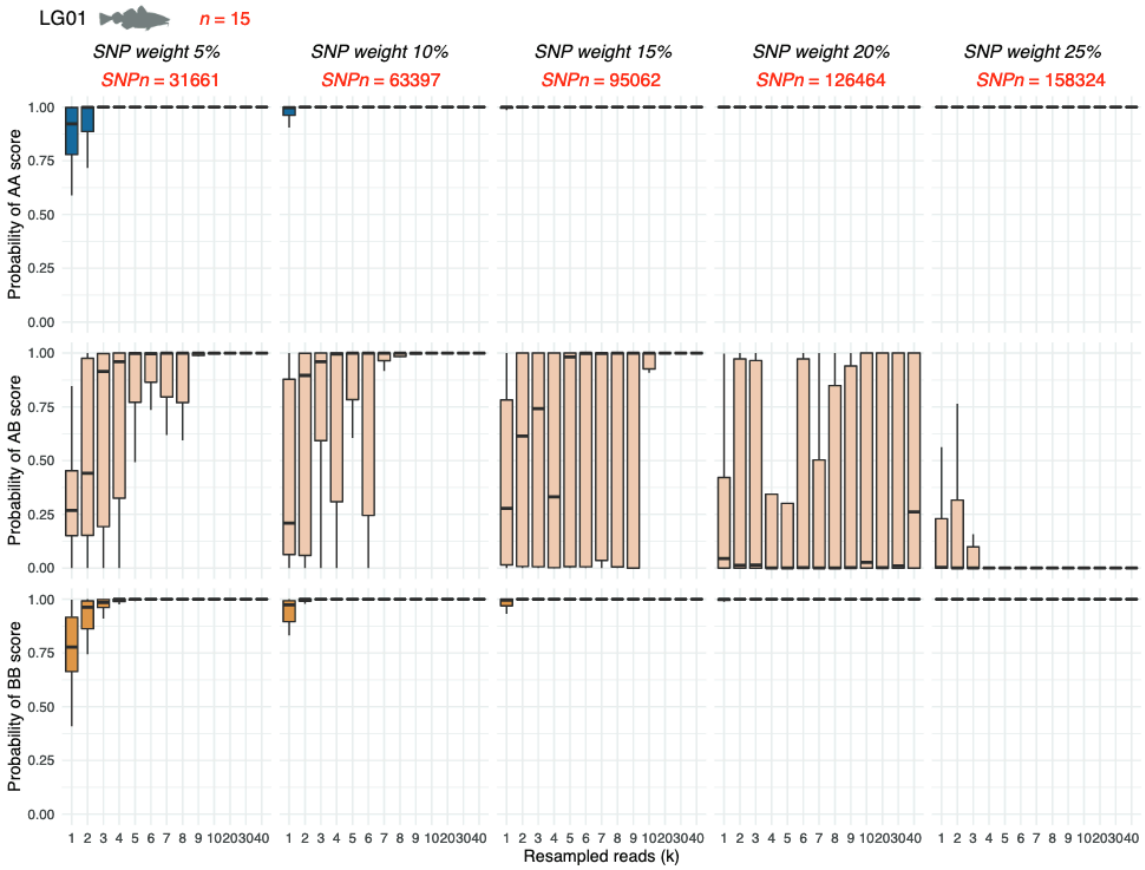
Supplementary Figure 2. Principle Component Analyses of the P3 inversion in 40 Heliconius butterflies. We compared the accuracy of the scoring results of the extremely down-sampled Heliconius data using BAMscorer with the classification obtained using all available genomic data for the individuals in both databases. Whole genome data were obtained from Jay et al. (2019). The individuals segregate in three distinct clusters along PC1 according to their inversion diploid genotypes (AA, AB, or BB). The classification using extremely down-sampled BAM files and the BAMscorer pipeline is 100% identical to the generated classification based upon the high coverage dataset.



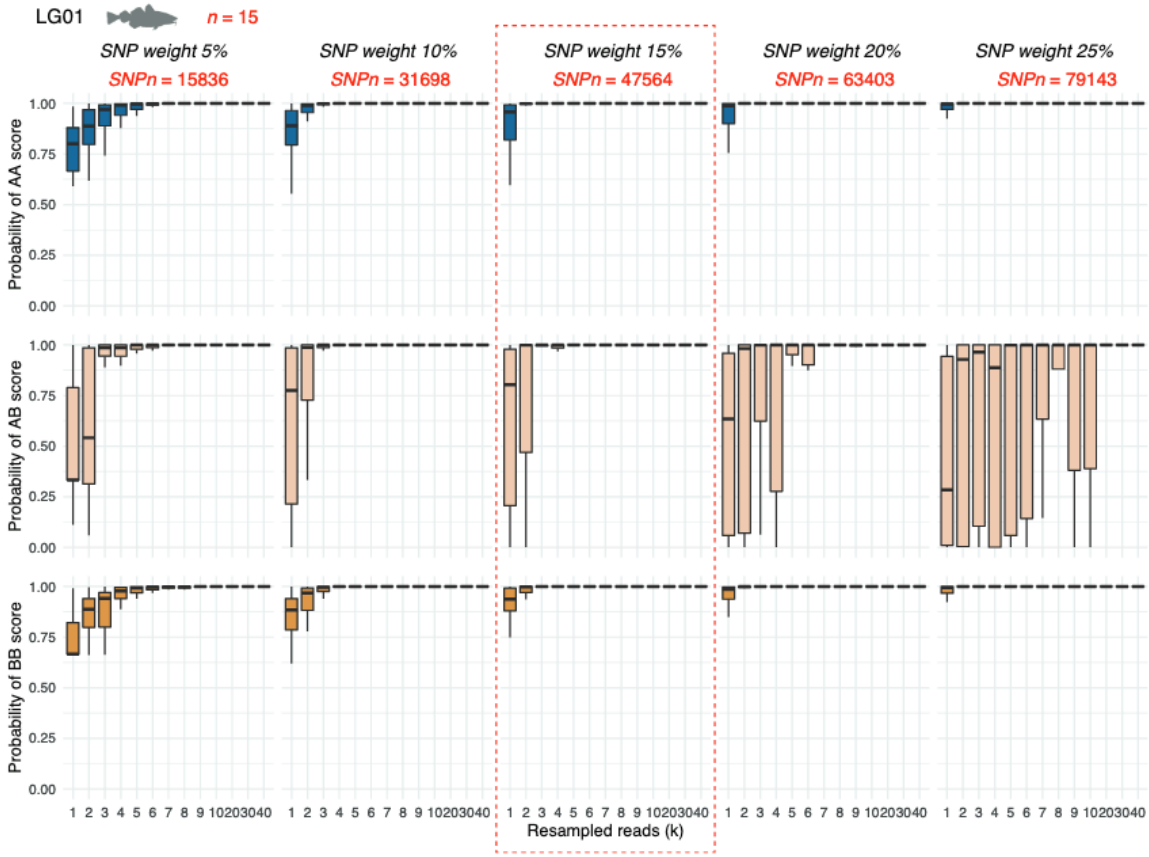
Supplementary Figure 3. Population assignment for G. morhua (population differentiation) using extremely low-coverage data. Left: population PCA plot generated using smartPCA (Patterson et al., 2006; Price et al., 2006). The number of individuals (red) and genome-wide weighted FST differentiation (red arrow) is indicated. Middle: SNPs most associated with large-scale population differentiation (Baltic Sea or eastern Atlantic) are selected based on their SNP weight loading distribution along PC1. Those with lowest and highest loadings are most associated with differentiation along PC1. SNP weight indicates the percentage of SNPs selected from the most extreme ends of the distribution (red). Right: assignment probability for individual specimens generated by down-sampling BAM files 1000 to 40,000 reads. At each interval, and for each individual, the down-sampling is iterated 20 times in order to generate box plots. Probabilities are calculated based on the joint binomial distribution of observing divergent SNPs associated with either population. Also indicated is the number of individuals scored (red, note these are not the same individuals used to create the original databases) and fold coverage (red dotted line, x coverage) at which more than 0.99 median assignment probability is obtained.



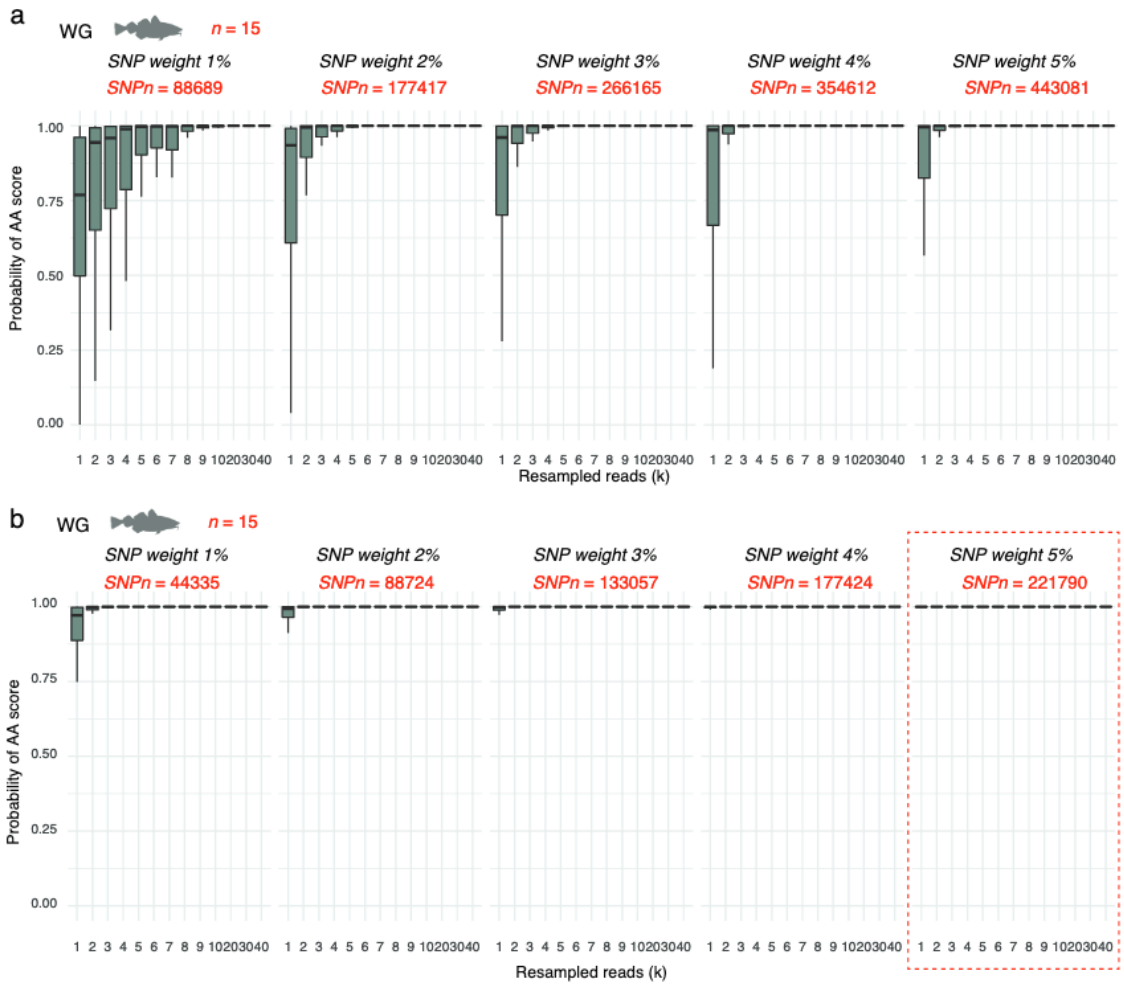
Supplementary Figure 4. SNP selection by varying SNP weight in Atlantic herring (Chr12 inversion). SNP weight is here defined as the percentage of SNPs with the most extreme values at both sides of the SNP loading distribution. Confidence in probability assignment is obtained by down-sampling BAM files to 1000-40,000 reads. At each interval, and for each individual, the down-sampling is iterated 20 times in order to generate box plots. Probabilities are calculated based on the joint binomial distribution of observing divergent SNPs associated with either genotype. Also indicated is the number of individuals (n, red) and number of SNPs (SNPn, red) and the chosen cut-off value (red dotted lines) at which genotypes can be efficiently separated.



Supplementary Figure 5. SNP selection by varying SNP weight at both sides in Atlantic cod (LG01 inversion). SNP weight is here defined as the percentage of SNPs with the most extreme values at both sides of the SNP loading distribution. Confidence in probability assignment is obtained by down-sampling BAM files to 1000-40,000 reads. At each interval, and for each individual, the down-sampling is iterated 20 times in order to generate box plots. Probabilities are calculated based on the joint binomial distribution of observing divergent SNPs associated with either genotype. Also indicated is the number of individuals (n, red) and number of SNPs (SNPn, red).



Supplementary Figure 6. SNP selection by varying SNP weight at the most extreme end in Atlantic cod (LG01 inversion). SNP weight is here defined as the percentage of SNPs with the most extreme values at the most extreme side of the SNP loading distribution. Confidence in probability assignment is obtained by down-sampling BAM files to 1000-40,000 reads. At each interval, and for each individual, the down- sampling is iterated 20 times in order to generate box plots. Probabilities are calculated based on the joint binomial distribution of observing divergent SNPs associated with either genotype. Also indicated is the number of individuals (n, red), number of SNPs (SNPn, red), and the chosen cut-off value (red dotted lines) at which genotypes can be efficiently separated.



Supplementary Figure 7. SNP selection by varying SNP weight in Atlantic cod (whole genome). SNP weight is here defined as the percentage of SNPs with (a) the most extreme values at both sides of the SNP loading distribution and (b) the most extreme values at the most extreme side of the SNP loading distribution. Confidence in probability assignment is obtained by down-sampling BAM files to 1000-40,000 reads. At each interval, and for each individual, the down-sampling is iterated 20 times in order to generate box plots. Probabilities are calculated based on the joint binomial distribution of observing divergent SNPs associated with either genotype. Also indicated is the number of individuals (n, red) and number of SNPs (SNPn, red) and the chosen cut-off value (red dotted lines) at which all three genotypes can be efficiently separated.

Supplementary tables

Supplementary Table 1 – Sample information and sequencing results from ancient Atlantic herring samples. All samples were sequenced on the Illumina HiSeq 2500 platform at the Norwegian Sequencing Centre. Paired-end reads were aligned to the Atlantic herring reference genome (GCA_900700415.1, Pettersson et al., 2019) as described in Ferrari et al. (2021).

Sample	ENA accession	Site	Date (century CE)	Element	Endogenous DNA	Fragment length	Clonality
HER001	ERR6003880	Giecz	9th-13th	Prootic	0.212	77.5	0.021
HER002	ERR6003881	Giecz	9th-13th	Prootic	0.422	67.9	0.046
HER003	ERR6003882	Giecz	9th-13th	Caudal vertebra	0.272	84	0.28
HER004	ERR6003883	Giecz	9th-13th	Caudal vertebra	0.163	75	0.23
HER005	ERR6003884	Giecz	9th-13th	Caudal vertebra	0.4	75.3	0.129
HER006	ERR6003885	Mała Nieszawka 1	14th-15th	Dentary	0.311	71.4	0.032
HER007	ERR6003886	Mała Nieszawka 1	14th-15th	Dentary	0.44	59.7	0.059
HER008	ERR6003887	Mała Nieszawka 1	14th-15th	Dentary	0.478	60	0.036
HER009	ERR6003888	Mała Nieszawka 1	14th-15th	Dentary	0.475	75	0.063

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Chapter 4:

1200 years of herring fishing in the Baltic

"They present themselves in such large numbers off shore that they not only burst the fishermen's nets, but, when they arrive in their shoals, an axe or halberd thrust into their midst sticks firmly upright."

- Olaus Magnus
1555

PNAS

Population dynamics of Baltic herring since the Viking Age revealed by ancient DNA and genomics

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Author Contributions: LL and DM provided archaeological material. CA provided modern tissue samples. DM and JHB provided archaeological and historical context. LMA and LMG conducted the lab work. LMA and BS designed the genomic analysis and LMA conducted the analysis. LMA, BS, and JHB conceptualized and designed the study. LMA wrote the manuscript with contributions from BS and JHB. All authors read, revised, and agreed to the final manuscript.

Classification: Biological Sciences, Sustainability Science

Keywords: ecology, history, ancient DNA, fisheries, sustainability

This PDF file includes:
Main Text
Figures 1 to 3

Abstract

The world's oceans are currently facing major stressors in the form of overexploitationand anthropogenic climate change. The Baltic Sea was home to the first "industrial" fishery ~800 y ago targeting the Baltic herring, a species that is still economically andculturally important today. Yet, the early origins of marine industries and the long-term ecological consequences of historical and contemporary fisheries remain debated. Here,we study long-term population dynamics of Baltic herring to evaluate the past impactsof humans on the marine environment. We combine modern whole-genome data withancient DNA (aDNA) to identify the earliest-known long-distance herring trade in theregion, illustrating that extensivefish trade began during the Viking Age. We further resolve population structure within the Baltic and observe demographic independencefor four local herring stocks over at least 200 generations. It has been suggested thatoverfishing atØresund in the 16th century resulted in a demographic shift from autumn-spawning to spring-spawning herring dominance in the Baltic. We show that while theØresundfishery had a negative impact on the western Baltic herring stock,the demographic shift to spring-spawning dominance did not occur until the 20th century. Instead, demographic reconstructions reveal population trajectories consistentwith expected impacts of environmental change and historical reports on shifting fishing targets over time. This study illustrates the joint impact of climate change andhuman exploitation on marine species as well as the role historical ecology can play inconservation and management policies.

Significance Statement

A growing body of research indicates that ocean ecologies have been more impacted by human exploitation and for longer than previously understood. Here, we evaluate human impact on Baltic herring, an ecologically, culturally, and economically important species with an iconic history of exploitation. We observe genomic evidence of the earliest long-distance trade for this species, providing evidence for a longer exploitation history than previously understood. Observations of serial exploitation are consistent with classic patterns of resource depletion. Our results illustrate the importance of including knowledge regarding long-term population dynamics, including differential stock responses to climate change, in sustainable management strategies, as efforts to achieve food security by aquaculture- and marine-based industries are demanding ever-increasing resources from the oceans.

Introduction

Atlantic herring, *Clupea harengus*, has made a dramatic mark on European history; the fate of nations and peoples has depended on the Atlantic herring trade (1–4). By the medieval era, herring was traded in urban areas across Europe, becoming one of the first true commodity goods and arguably marking the advent of the modern market economy (5). Today, Atlantic herring is one of the most important fisheries for several countries across the Atlantic and into the Baltic (6, 7). We here assess the demographic trajectories of four key Baltic herring stocks, relating these trajectories to historical data on changing fishing pressures and climate proxies. The Baltic Sea has long been under intense exploitation pressure (8) and is particularly vulnerable to both climate change (9) and intensified extraction (10). It therefore provides an excellent opportunity to evaluate shifting impacts of human exploitation and changing climate on marine species (11). We further explore evidence for earlier long-distance trade and exploitation in the Baltic than previously considered. This long, interconnected history between herring, humans, and the climate must be jointly considered when determining crucial stock assessment and fishing measures, such as maximum sustainable yield (MSY) and total allowable catch.

Historical Exploitation

During the Middle Ages, diets throughout Europe shifted to rely more heavily on marine fish (12). Increased demand for fish protein followed the rise of urbanism and the spread of Christian fasting practices in Europe (5, 12, 13). Herring was a particularly valued species at the time due to its ability to be preserved and sold in quantity at market, its massive spawning aggregations that provided easy capture, and its high fat content (14, 15). Long-distance herring trade, however, was limited by economical access to salt, which is required to keep fresh-caught herring from spoiling (16). One of the earliest fisheries that had access to both salt and coastal herring spawning aggregations occurred in the western Baltic. According to historical data, the earliest commercial herring fishing operations in the Baltic took place on the island of Rügen in the early 12th century (1), an operation that was succeeded by the first fishery in Europe to reach the level of a true industry: the Øresund fishery (8, 17, 18).

The Øresund fishery (here broadly defined) operated between Denmark and southern Sweden, corresponding to International Council for the Exploration of the Sea (ICES) subdivisions 22 to 24, with most activity concentrated in subdivisions 23 and 24 (7, 16) (Fig. 1*A*). During the peak of the Øresund fishery, herring were traded across Europe, reaching as far away as Italy and northern Norway (19). Yet, the nature, origin, and timing of onset of this commercial fishery remain unclear (4, 8). The famous account of the Anglo-Saxon Wulfstan (20) reports a voyage c. 880 CE between the trading ports Hedeby (in modern Schleswig-Holstein, Germany) and Truso (identified at the archaeological site of Janów Pomorski) near Elbląg in Poland. Zooarchaeological evidence from a herring bone assemblage dated c. 800 to 850 CE from Janów Pomorski might be indicative of such early medieval fish trade in the same direction due to the absence of cleithra—a bone element often removed during medieval commercial processing (4, 21)—yet the origin of these bones remains unknown. Furthermore, some have hypothesized that the Øresund fishery targeted an Atlantic herring population that had made its way into the transition zone between the Baltic and the Atlantic (13). These hypotheses can now be tested using ancient DNA (aDNA).

Under the control of Denmark and the Hanseatic League, the Øresund fishery was most active during the 13th to 16th centuries. Exact quantities of catch are still under debate (8, 16), but the Øresund fishery likely surpassed contemporary western Baltic fisheries, with catches estimated at up to 50,000 t per annum at its peak (8, 16, 17). In contrast, early–20th century

landings from the Øresund were between 100 and 10,000 t (18), and more recent landings have been on the order of 10,000 to 20,000 t (7). Unlike current Baltic fisheries, the Øresund fishery focused exclusively on autumn-spawning herring, with a restricted fishing season between August and October to target the spawning aggregation (16, 22). Today, there are autumn spawners extant in the Baltic, but none of these populations are large enough to support a commercial industry (7). Instead, contemporary Baltic herring fisheries target the smaller-bodied spring spawners, which are ecologically distinct from autumn spawners (23). Although both autumn- and spring-spawning populations coexist wherever herring are found, in each area only one of these distinct ecotypes appears to dominate numerically (e.g., spring spawners in the Norwegian Sea and autumn spawners in the North Sea) (24, 25). This alternating pattern of dominance in today's oceans, and the known historical shift toward targeting spring- instead of autumn-spawning stocks, indicates that a dramatic demographic and ecological shift has occurred in the Baltic ecosystem. Nonetheless, the timing and nature of this shift are currently poorly characterized.

The Øresund fishery collapsed in the late 16th century, with only marginal operations continuing into the 17th century (16). After this, commercial herring fishing operations were limited in the area until the 20th century (16, 26). European herring production moved to the North Sea (27), a transition that is generally linked to the decline in the Øresund fishery landings. Yet, whether this decline was due to ecological changes or driven by market and political factors is still under debate (28). A recent study argued that the impetus for the collapse of the Øresund fishery was the disappearance of the western Baltic autumn-spawning herring due to overfishing as the stock was pushed past modern MSY estimates (5, 7, 16). This study further proposed that such collapse may have led to a demographic dominance of spring-spawning herring as early as the 16th century (16). Yet, catch records indicate that autumn spawners constituted up to 90% of Baltic herring landings as late as 1927 (29), and autumn-spawning fisheries were supported in the gulfs of Finland, Riga, and Bothnia into the 1970s (30). Past research regarding herring stock collapses has shown that the species is vulnerable to overfishing despite its natural abundance (26, 30); the Baltic autumn spawners' collapse in the early 20th century has been definitively linked to fishing pressure rather than contemporaneous factors such as eutrophication (30). Thus, the medieval Øresund fishery could well have had a real ecological impact on the herring stock. However, whether this fishery caused a Baltic-wide demographic shift, a short-term local extirpation, or more lasting impacts remains unclear. The impacts of fisheries that succeeded the medieval Øresund industry are also uncharted. Finally, changing climate in the Baltic during periods such as the Medieval Climate Anomaly (MCA) and the Little Ice Age (LIA) likely contributed to herring population dynamics as well (31, 32). Therefore, a holistic evaluation of complex herring ecology, human impacts, and the dynamic Baltic ecosystem is merited.

The Baltic Ecosystem

The Baltic exhibits a stark salinity gradient, from near-ocean levels where it connects with the Kattegat to near-freshwater levels in the gulfs (33). Baltic herring is a schooling fish with enormous population sizes, with current stock estimates on the order of millions of tons of spawning stock biomass (7). Herring show strong genetic adaptation for spawning season and adaptation to differing salinity conditions (23, 34–37), ultimately segregating into two distinct metapopulations: spring spawners and autumn spawners (38, 39). Spring spawners are smaller and mature more quickly than autumn spawners, and prefer to spawn in coastal areas as opposed to the deeper waters used by the slow-growing, larger autumn spawners (30, 40).

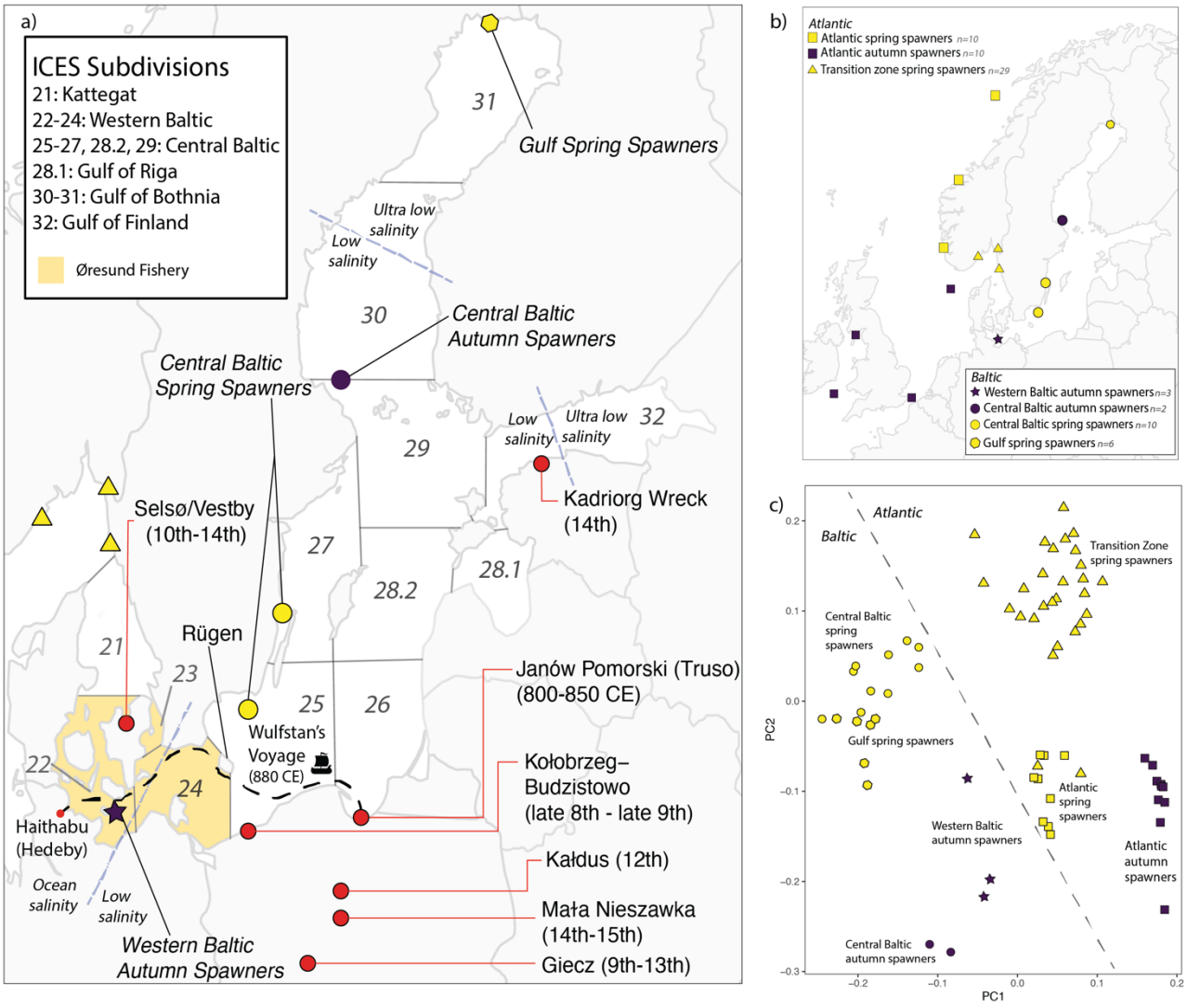

Figure 1 – Archaeological sites, sample locations, and population structure of Baltic herring. (*A*) Baltic Sea with ICES management subdivisions indicated. Named regions corresponding to ICES subdivisions are noted (*Inset*). Spring- and autumn-spawning herring occur in all subdivisions. Archaeological sites and their dates are noted as well as the sampling locations for modern genome data. The approximate area targeted by the Øresund fishery (broadly defined) in the Middle Ages is highlighted in yellow (ICES subdivisions 22 to 24), although it is concentrated in subdivisions 23 and 24. Approximate locations of salinity boundaries between high (ocean), low, and ultra-low salinity are marked by blue dashed lines. Wulfstan's voyage from Hedeby to Truso c. 880 CE is noted in black. (*B*) Sample locations of herring specimens used for this study. The final dataset contained genome sequences generated for this study *(n* = 53) and publicly available genome sequences (*n* = 22). Color denotes spawning season, indicating near-complete coverage of the major herring populations identified by Han et al. (**37**). (*C*) PCA of the entire modern nuclear genome using 10,368,446 SNPs. The modern genome shows segregation on PC1 based on Atlantic vs. Baltic, and spawning season on PC1 and PC2. The Atlantic spring spawners are divided into two groups, with the transition zone between the Baltic and Atlantic separating on PC2. This is likely due to the presence of Norwegian fjord herring in the transition-zone cluster, which show signatures of adaptation similar to the Baltic herring due to the brackish fjord conditions (**87**). Winter- and autumn-spawning specimens from the Atlantic cluster together. GSS and CBSS show substructure within the Baltic spring-spawning metapopulation and WBAS cluster away from CBAS.

Given the larger size of autumn-spawning herring, we expect that earlier fishing efforts prioritized these populations over the smaller spring-spawning aggregations. Each metapopulation is further separated into management stocks living in the western Baltic (ICES subdivisions 21 to 24), central Baltic (ICES subdivisions 25 to 27, 28.2, and 29), and gulfs (ICES subdivisions 28.1 and 30 to 32) (Fig. 1*A*) (30, 41). The western Baltic autumn-spawning stock was the population targeted by the Øresund fishery. Western Baltic herring (both spring and autumn spawners) spawn in coastal areas of the Kattegat and southwestern Baltic (31, 42), and are likely reproductively isolated populations from the rest of the Baltic (43, 44).

Herring remain an important cultural and commercial industry in the Baltic (7). As Europe attempts to shift toward a more "sustainable" diet based on aquaculture and marine resources, herring are becoming a key species in the proposed fight to reduce global carbon footprints (45–49). Baltic herring are facing an additional challenge in our attempts to reduce land-based meat consumption both through a regional increase in fishmeal production for aquaculture (50) as well as direct consumption. In the European Union, there have been calls to increase understanding of ocean ecosystems to assess the viability of feeding the world from the ocean (48). Ecologists have already noted the disconnect between the reality of modern ocean environments and governments' proposed sustainable new marine industries (49), although it is unclear whether this analysis holds true in all ecosystems or over time.

Here, we publish a long time series of genome-wide aDNA data for Baltic herring, analyzing 40 archaeological herring specimens from seven sites in Poland, Denmark, and Estonia that span the period from 750 to 1600 CE. Using *BAMscorer*, software specifically designed for population assignment using ultra-low-coverage sequence data (51), we identify the biological origin of each specimen, illustrating the change in fishery targets throughout time and evaluating the onset of long-range fish trade in the Baltic. We use modern whole-genome resequencing data—including 53 sequences generated for this study—to investigate population structure and model recent past demography for each of the Baltic herring populations in our data (Fig. 1*C*). We assess the difference in western Baltic autumn-spawning herring outcomes for the Øresund fishery in comparison with more recent fishing efforts and provide insight into the timing of herring population turnover in the Baltic. We compare the demographic trajectories of four Baltic herring populations with known historical events, including changed fishing practices and temporal changes in sea surface temperature, to assess the impact of these events on herring population size and the long-term sustainability of this iconic industry.

Results

Population Structure.

We resolved modern population structure in a principal-component analysis (PCA) based on 10 million genome-wide single-nucleotide polymorphisms (SNPs) in 68 modern individuals (Fig. 1 *A* and *B*), consistently separating populations based on geography (Baltic or Atlantic) and spawning season (Fig. 1*C*). We further observed fine-scaled population structure within spawning ecotypes in the Baltic, with central Baltic and gulf spring spawners, as well as central Baltic and western Baltic autumn spawners, clustering closer to their respective sample locations (Fig. 1*C*). This fine-scaled structure was also supported by higher levels of relatedness within each sample location and subsequently lowered levels of relatedness in these subpopulations when grouped as metapopulations (*SI Appendix*, *Text* and Fig. S9). The modern mitogenomes revealed three major clades [IQ-TREE (52, 53)] that exhibited no association with geography or environment across the Baltic and Atlantic, in accordance with previously published results (44). All archaeological mitogenomes clustered with the modern Atlantic and Baltic samples, identifying these as herring (*SI Appendix*, Fig. S11). Genetic diversity [π;

VCFtools v0.1.16 (54)] across the genome was significantly higher for autumn-spawning herring (*SI Appendix*, Fig. S12). This higher diversity indicates a larger effective population size over time for the autumn spawners relative to spring spawners.

Population Assignment.
All 40 archaeological samples were classified using *BAMscorer* as autumn-spawning herring. Autumn-spawning Baltic and Atlantic herring are genetically differentiated at the chromosome 12 inversion (37). All specimens could therefore be assigned to either the Atlantic or the Baltic using their inversion haplotype. Nearly all exhibited the chromosome 12 inversion haplotype (BB) associated with Baltic herring (Fig. 2). Spawning season and chromosome 12 inversion type could be assigned with as few as 50,000 reads, while salinity adaptation required 60,000 reads for accurate assignment (*SI Appendix*, Figs. S6–S8). Two specimens from Truso had too few reads to properly assign a salinity adaptation but could be assigned for chromosome 12 inversion type and spawning season (Fig. 2 and Dataset S2).

Salinity adaptation was mixed between all sites, with earlier sites showing some individuals with higher salinity adaptation, which is indicative of populations that spend part of their annual cycle in the Skagerrak and North Sea, such as the western Baltic autumn-spawning herring, and later sites showing more low salinity–adapted individuals (Fig. 2 and Dataset S2). Indeed, the oldest samples in the dataset—from Truso in Poland, dating to 800 to 850 CE (21)—showed nearly half of samples stemming from high salinity–adapted populations. In contrast, the sites dating to later periods were more dominated by low salinity–adapted autumn spawners (Table 1). This change indicates a potential spatial shift in target population for Baltic fisheries over time from western to central Baltic, although further studies with larger sample sizes are merited to confirm this conclusion.

Table 1 – Percentage of Western Baltic Autumn Spawning herring specimens present in sampled specimens from each archaeological assemblage

Site	*Date*	*Percentage WBAS*	*n*
Janów Pomorski (Truso)	800-850	45.5%	11
Kołobrzeg-Budzistowo	Late 8th-late 9th	55.6%	9
Giecz	9th-13th	20%	5
Selsø/Vestby	10th-late 14th	44.4%	9
Kałdus	12th	100%	1
Kadriorg Wreck	14th	0%	1
Mała Nieszawka	14th-15th	0%	4

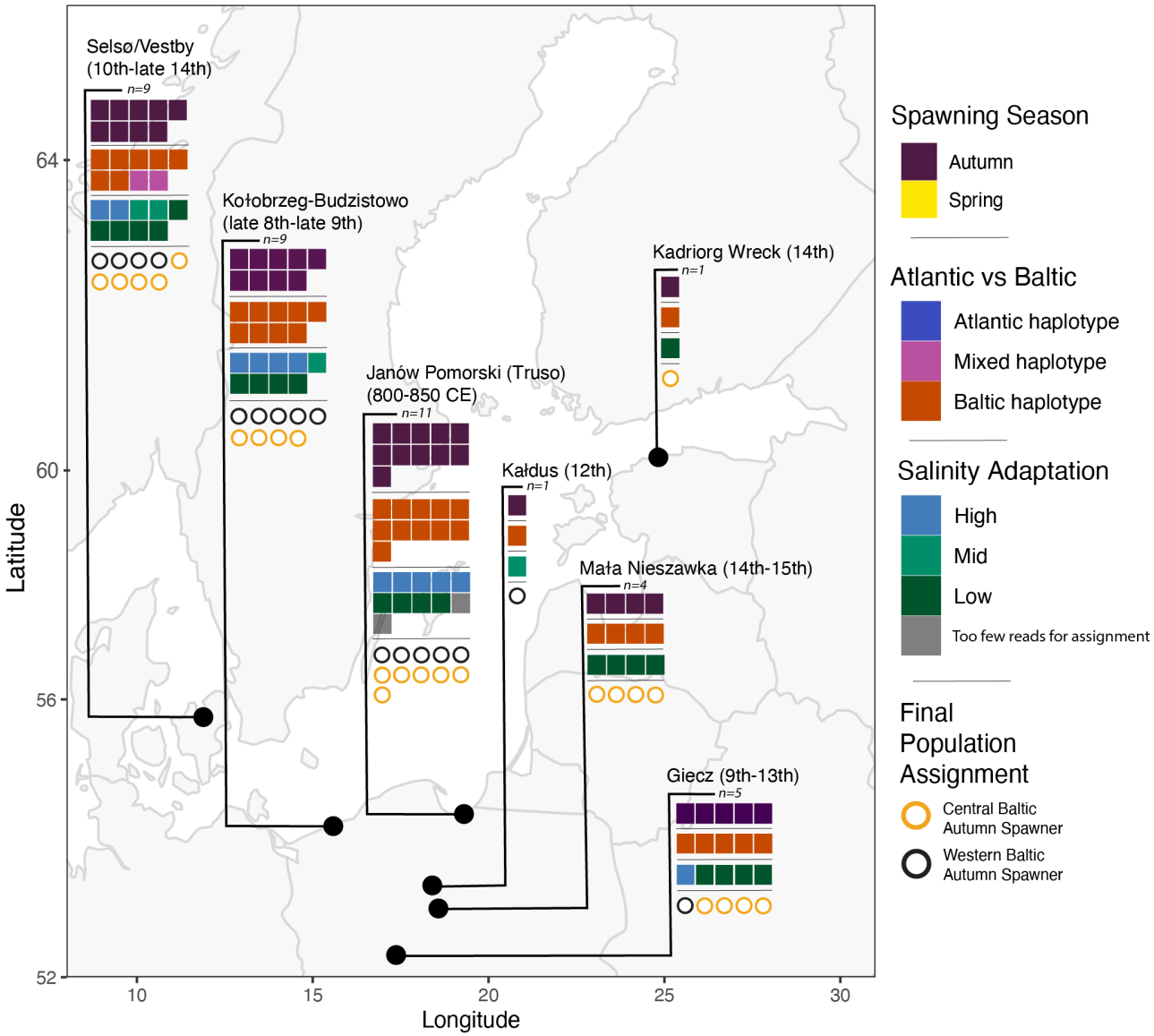

Figure 2 – Population assignment results for archaeological herring specimens. Each individual is represented by a single square for each assignment test. The three assignment tests (spawning season, chromosome 12 inversion type for autumn-spawning specimens, and salinity adaptation) are then combined to make a final population assignment illustrated by the row of circles for each site. Archaeological specimens were assigned as autumn or spring spawning across sites reported to be associated spawning season by Han et al.[38] Atlantic and Baltic chromosome 12 inversion types were assigned based on BAMscorer sensitivity analysis (see Methods; Fig S7). Salinity was assigned using SNPs reported by Han et al.[38] to be associated with adaptation to salinity. Some archaeological specimens had assignment values in between high and low (see Supplementary Dataset S1), therefore were classified as stemming from "mid"-level salinity. Western Baltic specimens are present in the earlier sites from around Poland whereas later sites and contexts trend towards central Baltic specimens. Context-specific results can be found in Supplementary Dataset S1.

Demographic Analysis.
Both Baltic autumn-spawning populations showed fewer and shorter runs of homozygosity (ROHs) across all time bins (*SI Appendix*, Fig. S13). This pattern indicates either a large effective population size or population growth (55). The Baltic spring-spawning populations exhibited signs of smaller effective population size and/or population decline across all time bins. No ROHs >330 kb were found in any population, likely due to the high recombination rate in herring (2.54 cM/Mb) (36) and small sample size for this analysis. The combined ROH for all time periods indicates a large effective population size in the past for autumn-spawning herring and smaller effective population size over time for spring-spawning herring.

Highly divergent demographic trajectories are obtained using *gone* (56) for each of the four Baltic populations under study (Fig. 3): central Baltic spring spawners (CBSS), central Baltic autumn spawners (CBAS), western Baltic autumn spawners (WBAS), and gulf spring spawners (GSS). These results illustrate long-term demographic independence of the four main herring stocks in the Baltic. The population size of WBAS started declining ∼800 y before present (YBP), which corresponds to the onset of the Øresund fishery (Fig. 3). The WBAS population never fully recovered from this decline, gradually dropping off until ∼100 to 150 y ago, at which point the decline became much more rapid. In contrast, the CBAS population exhibited a strong population increase ∼600 YBP and continued to climb until ∼100 YBP, at which point they rapidly declined. Coincident with the initial decline of WBAS, the central Baltic spring-spawning herring (CBSS) began to increase. They plateaued around 500 YBP and then started to decline ∼400 YBP. Gulf Spring-spawning herring (GSS) showed a strong population increase until very recently, only showing a decline in recent generations. Each population is plotted with relevant historical events noted, including the onset of particular fisheries as well as the duration of two key past climatic events: the MCA and the LIA, during which the Baltic was, respectively, warmer and much colder than today, albeit with short-term variability (32, 57).

Discussion

Using modern and historical whole-genome sequences from Atlantic and Baltic herring, we resolve herring population structure, reveal the earliest known large-distance herring trade, and propose that historical fishing operations had an observable genetic impact. We here contextualize our results with historical, ecological, and archaeological perspectives to situate our findings in a wider narrative of human–ocean interaction.

Population Assignment.
We find no evidence of spring spawners in the archaeological specimens analyzed. There are multiple reasons this might be the case. Historical evidence shows that the medieval fisheries, including the Øresund fishery, were often strictly regulated to target spawning aggregations, and thus a single herring stock (16, 27). Further, when population sizes of the autumn-spawning herring were high, it is likely that the spring-spawning herring were low; data from other oceanic basins show coexistence of the two metapopulations, but that one seasonal spawner appears to dominate (24, 25). All pre–20th century Baltic fisheries under consideration appear to have focused on autumn-spawning herring. This observation provides genetic evidence for historical arguments that autumn spawners were the dominant metapopulation in the Baltic in the Middle Ages. We further show that both past and present Baltic fisheries focused on Baltic herring rather than Atlantic herring as previously supposed (8).

Our archaeological specimens exhibited variation in adaptation to salinity. This result reflects the differentiation between herring that spawned within the Baltic Proper and those that spawned within the transition zone of the Kattegat and western Baltic (37). The higher salinity–adapted individuals appear in multiple sites in Poland, which would not have had high salinity–adapted communities spawning near the coast. While salinity conditions in the Baltic have changed slightly in the last 1,000 y (58), the changes were not significant enough that ocean-level salinity conditions would be present in the central Baltic. Strikingly, our samples from Truso, which date between 800 and 850 CE (21), show the presence of high salinity–adapted herring with Baltic-type inversions on chromosome 12 which could only stem from fishing operations in the Kattegat or western Baltic (37). Previous studies have shown that while there is currently a small degree of connectivity between the central and western Baltic, the majority of this is unidirectional toward the western Baltic (44). Furthermore, populations from the western Baltic have been shown to be genetically distinct from the central Baltic (43), and therefore it is highly unlikely that these herring were fished locally. Assemblages with later dates show a shift toward lower representation of high salinity–adapted herring (Fig. 2 and Table 1). This observation reflects the known historical decline of the Øresund fishery, and shows the existence of refugia for the Baltic autumn-spawning herring metapopulation during the Medieval Warm Period, when anoxic conditions were prevalent in the central Baltic (Fig. 3).

The discovery of high salinity–adapted herring at Truso provides genetic evidence that this assemblage stemmed from a product of trade, a hypothesis suggested by the type of bones retrieved (16). With an origin stemming from the western Baltic region, these fish thus show the existence of long-distance fish trade in the Baltic at an earlier date than previously believed. This observation expands the scale of earlier observations of long-distance cod trade during the Viking Age (59) and its scope, given that herring trade is technologically more complex (requiring salting rather than only drying) (5).

Demographic Analysis.

Demographic reconstruction showed distinct trajectories for each of the four populations under consideration. These reconstructions highlight the importance of management based on biological units, and indicate the differential effects of fishing pressure and climate change on specific populations of the same species within the same region. We here evaluate possible varying impacts of fishing pressure and climatic change (e.g., sea surface temperature) on each of the four stocks in turn to illustrate this point. We here illustrate how these population stock dynamics are not always consistent with expected change due to climate impacts alone.

Western Baltic Autumn Spawners.

WBAS exhibits a population decline coincident with the intensification of fishing in the Øresund region. High temperatures in the central Baltic have a limiting effect on autumn-spawning herring reproduction (41). We would therefore expect the end of the MCA (1,000 to 800 YBP) to result in increased population size for WBAS in the absence of fishing pressure. In contrast, WBAS begin to decline ∼800 YBP and continued to decline until the present day. We observe a small drop corresponding to the proposed collapse in the 16th century described by Lehmann et al. (16), yet the population did not suffer a drastic contraction until ∼100 YBP. We conclude that the 16th-century decline of the Øresund fishery was therefore not due to a complete population collapse, although the fishery clearly had a significant impact on the overall size of the WBAS stock.

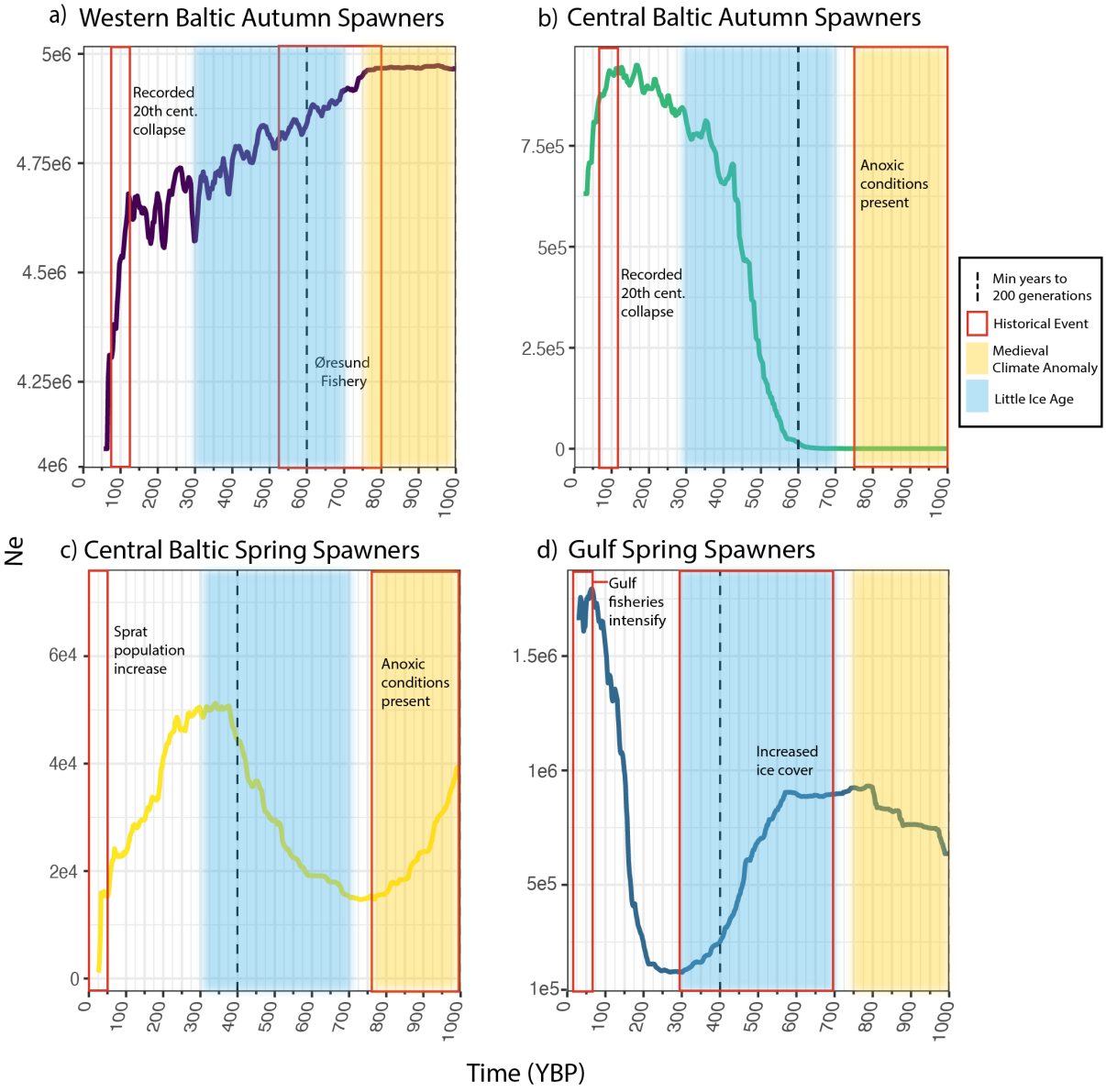

Figure 3 – Temporal reconstruction of recent effective population sizes in four Baltic herring stocks. Each stock is represented in a separate chart: WBAS, CBAS, CBSS, and GSS. The *y* axis indicates estimated N_e in the past while the *x* axis indicates time in years before present. Colored rectangles indicate key historical events. Yellow rectangles show the approximate duration of the MCA and blue rectangles indicate the LIA. The dashed vertical lines show the minimum date (YBP) at which 200 generations in the past is reached (calculated using minimum generation times of 3 y for autumn spawners and 2 y for spring spawners), the known accurate window for *gone*. Historical events are denoted by red boxes for each population. (*A*) Demographic trajectory of the western autumn spawners shows a decline starting shortly after the start of the Øresund herring. They show an additional severe decline corresponding to the reported 20th-century autumn-spawning fishery collapse in the Baltic. Please note that, for reasons of scale, the *y* axis does not start at zero for this panel. (*B*) Demographic trajectory of central autumn spawners, which appear limited during the MCA when anoxic conditions are present in the central Baltic. They increase during the LIA and rapidly decline during the period of known autumn-spawning population collapse coinciding with the increase of the sprat (*S. sprattus*) population. (*C*) Central spring spawners show an increase around the time of the decline of the western autumn spawners, and then a decrease again at the end of the LIA as well as another dramatic decrease around the time of the autumn spawners' collapse ~100 YBP. (*D*) GSS decreases during the LIA and then increases dramatically at the end of the LIA, starting to decline only in very recent generations when fisheries in the gulfs intensify. Please note that, for reasons of scale, the *y* axis does not start at zero for this panel.

Central Baltic Autumn Spawners.

CBAS exhibits extremely low effective population size until the onset of the LIA (~700 YBP), at which point it increases dramatically until ~100 YBP. Sediment cores have shown that temperatures in the central Baltic during the MCA were warm enough to produce algal blooms and anoxic conditions (32, 58, 60). Autumn-spawning herring spawn in deeper waters than spring spawners, and are therefore more vulnerable to anoxic conditions (61). Combined with temperature-limited reproductive success (41), CBAS was thus likely under limiting environmental conditions prior to the LIA. Our results show an expansion of this population that mirrors the timing of the WBAS decline and the onset of the LIA, a time when there would also have been low fishing pressure in the central Baltic. Subsequently, we see a dramatic reduction in CBAS effective population size corresponding to the historically documented 20th-century decline of autumn-spawning fisheries (26, 29). This decline has previously been shown to be caused by overfishing rather than direct or indirect climate impacts, including warming temperatures and eutrophication (30).

Central Baltic Spring Spawners.

CBSS shows dramatic changes in effective population size over time. We expected to see an increase in the CBSS population concurrent with the autumn-spawning decline ~100 YBP, as proposed by the hypothesis that a demographic transition occurred in the Baltic, yet this is not reflected in the results. It is unclear why CBSS shows a decline at the end of the LIA, although this is perhaps in response to the growing CBAS population. One would expect that CBSS would fill the niche left by the collapsing CBAS population in the 20th century, yet it does not appear to do so. Interestingly, sprat (*Sprattus sprattus*) operate in the same niche as Baltic herring, and the two species have been shown to limit each other's population sizes (62). Prior to the mid 20th century, sprat were rare in the fishing record in the Baltic (63), only appearing coincident with the decline of the CBAS herring. CBSS may therefore have been limited in taking over the niche left by the autumn spawners' decline due to an increase in the sprat population around the same time (64). Thus, the domination of spring spawners in the 20th- and 21st-century fisheries may reflect not a population expansion of the spring spawners but a population collapse of WBAS and CBAS. Since the industrial fishery began targeting the CBSS ~100 YBP, we see a significant reduction in effective population size. ICES reports also indicate a declining population over the last 50 y, with some variation in the past decade (65). There has not been a strong year-class for this stock since 2014 (66).

Gulf Spring Spawners.

GSS has been subject to low fishing pressure throughout history (67), and therefore its demographic trajectory is likely more determined by changing environmental factors than the other herring stocks. Low temperatures and increased ice cover in the gulfs have been shown to have a limiting effect on gulf herring recruitment (40), and therefore a rise in population size after the LIA is to be expected and we see this in our demographic reconstructions. Fishing pressure on the gulf herring was not significant until the latter half of the 20th century (67). Our results follow this trend, in that GSS starts to decline in recent generations, but it is possible that demographic changes could also be due to climate change and increasing temperatures in the gulfs. It is likely too early to tell which factors are the driving components in the changed demographic trend for gulf herring in recent generations.

Limitations.

While *gone* is stated as accurate for a minimum sample size of two individuals, it is possible that small sample sizes can affect the demographic calculations (56). The impact of small sample sizes is also reflected in the ROH analysis, which showed similar patterns to *gone* in

that autumn spawners had fewer coalescent events in the past compared with spring spawners, indicating larger effective population size in the past. However, the ROH for CBAS lacked expected coalescent events that would reflect the low effective population size prior to 600 YBP (*SI Appendix*, Fig. S13). Where *gone* can be used with as few as two samples, ROHs generally require four to six (68), and therefore we advise caution in interpreting these results further than supporting the general trend of autumn-spawner dominance prior to 200 YBP.

Additionally, we offset each trajectory by minimum generation time for each stock due to the difficulty of accounting for changes in year-class strength over time. This is likely a conservative estimate, as herring are known to spawn in overlapping generations, and both local climatic conditions (69, 70) and fishing pressure (71) have been demonstrated to impact generation time and life history in herring. However, given the cyclical nature of year-class strength, it is not possible to accurately model changes in generation time. Additionally, the strength of particular year-classes has been shown to change drastically in response to changes in sea surface temperature and ice cover (69), meaning it is not always the larger, older fish that are contributing the most to the next generation and complicating determination of average generation time. Given this uncertainty, the minimum generation time is the lowest common denominator we can use, as this is the earliest age fish in these populations are known to reproduce and therefore can impact the next generation. Demographic reconstructions using longer generation times revealed similar impacts of climate change and exploitation pressure and are included in *SI Appendix*, Fig. S14.

Further, *gone* is known to be highly sensitive to population structure, and therefore connectivity between populations and/or the existence of substructure can affect results (56). Given the possibility of connectivity between GSS and CBSS (44), we further assessed the demographic trajectory of the Baltic spring spawners as a single metapopulation (*SI Appendix*, Fig. S15). This estimate shows a mixture of the results from the CBSS and GSS analyses, but is consistent with our conclusion that both changing temperatures and fishing pressure have impacted Baltic herring demography. The combined analysis also shows a decline similar to the GSS in recent generations.

Any study exploring effective population size (N_e) reconstructions would be remiss not to include discussion of N_e and its complex relationship with census size (N). N_e is an estimate of levels of genetic diversity and capacity for genetic drift translated into a number that we often interpret as a proxy for population size (72). Thus, N_e is an important value for assessing the evolutionary health of a population, for example, estimating extinction risk. Reductions in N_e are indicative of events such as inbreeding depression, which means fewer adult individuals are contributing to the next generation (73, 74). Reductions in N_e can depress a population's resilience to overexploitation and adaptive capacity, thereby impacting the evolutionary health of a population (75).

The relationship between N_e and N is complex, particularly for fish species such as herring with enormous census sizes and sweepstakes-style reproduction strategies (76). Further, population substructure and differences in productivity can result in drastically different N_e/N ratios (77). As each of the herring populations here is demonstrably independent, it is possible that the N_e/N ratio is unique to each population. The facts that the N_e/N ratio is here unknown and that N_e is a marker of demographic change are crucial distinctions to understand in the interpretation of this study. It is the relative changes for each population that indicate the impact of exploitation and climate change rather than the absolute estimates of N_e. We show that herring stocks exhibit differential responses to both climate changes and fishing pressure, each of

which has an evolutionary impact on the species. Evolutionary risk is not often applied to conservation and fisheries management policies, but should be taken into account to maximize stock and species health (75).

Human and Environmental Impacts on the Baltic Herring.
We here present observations that are consistent with early human fisheries in the Baltic having an impact on herring population trajectories. To address growing demand for marine fish protein in Europe, the initial fishery (beginning by 850 CE and expanding especially ∼1200 CE) focused on the most economically relevant herring with the biggest spawning aggregations, the western Baltic autumn-spawning herring. The onset of this fishery corresponded to a negative population trajectory at a time when environmental conditions for the stock were improving. Fishing finally ceased in Øresund during the 1600s, which may have been associated with changes in stock size due to overfishing. Fishing pressure then focused on the next-largest herring stock, CBAS, and the pressure again intensified until it collapsed. After the highly valued autumn spawners were fully exhausted in the early 20th century, only then did the herring fishery turn to targeting spring spawners, the smaller, less-valued population. And finally, GSS, long disregarded as a potential commercial target, is now facing the pressures of industrial fishing (78–80).

The obtained demographic trajectories cannot be explained by changes in climate alone. Rather, they indicate that each stock has experienced trajectories in the past (or currently) that do not correspond to changes in climate. Moreover, for CBAS, the demographic trends reported here in the last 150 y can be directly attributable to fishing pressure rather than climate (30).

Baltic herring are currently facing the combined challenges of overfishing, eutrophication, rising temperatures, competition from expanding sprat populations, and further dilution of the Baltic by fresh water (10, 18, 24, 62, 81–83). In recent years, ICES has recommended an MSY of 0 for several Baltic herring stocks (7, 78, 84, 85). Yet, fishing continues and the stocks do not recover (86), because economic and cultural factors continue to drive exploitation. In the face of these issues, it may seem that the fate of the herring is sealed. However, modeling of Baltic ecosystem dynamics has shown that fisheries policy and conservation management are some of the most important factors in determining the long-term sustainability of the Baltic (10, 87). Therefore, it is crucial that we learn from the past, both recent and further-reaching, to better understand the real impact of various management and extraction policies on the demography of Baltic herring throughout time, as well as the differential responses of each herring stock to varying environmental pressures.

By bringing historical ecology methods into play, we here elucidate ecological and cultural thresholds (88) that were crossed as the Baltic fisheries changed over time, finally resulting in the Baltic we see today. These thresholds—including cultural change [e.g., increased demand for marine fish because of rising urbanism and religious requirements (2)], ecosystem change, and shifting exploitation patterns—can be used to inform management policies in the future by reframing what constitutes healthy population dynamics (size, migratory behavior, etc.) to better reflect long-term ecosystem dynamics. Further, the demonstrated demographic independence of each Baltic herring stock here assessed provides further support for managing each stock separately. Finally, our research highlights the interconnected nature of herring stock dynamics, climatic change, and fishing pressure. Given the challenges facing the Baltic due to ongoing climate change and exploitation pressure, it is crucial that management bodies take this interconnected feedback system into account.

The gulf spring-spawning herring are the last remaining healthy herring stocks in the Baltic (84, 89). They are also the stock most adapted to the brackish conditions which are expected to increase in the Baltic into the future. In order to preserve this stock, it is crucial that management bodies recognize this pattern of serial exploitation and climate impact in the Baltic, thereby providing avenues to identify specific exploitation strategies that have resulted in population collapse in the past (90) and avoid these for the future. Further, the differing responses to climate change as well as the evolutionary health for each stock should be considered when providing quotas and assessing stocks in the changing future. This could take the form of including genetic information such as effective population size in stock assessments rather than exclusively relying on estimated census size (75, 91)—which would entail further research on the relationship between N_e and N for the Baltic herring stocks—or assessing historical management strategies associated with fluctuations in stock size.

The demographic trajectories of CBAS and GSS provide further avenues of hope for the Baltic herring. Both stocks' demographic reconstructions indicate that in the absence of industrial fishing pressure, herring stocks can rapidly recover from long-term exposure to poor environmental conditions. If the changing climate in the Baltic can be addressed sufficiently to maintain suitable threshold conditions for herring and fishing reduced in line with ICES recommendations, we may yet be able to preserve a significant spring-spawning Baltic herring population.

Materials and Methods

Archaeological Material and Laboratory Methods.

Archaeological bone samples were obtained from seven sites in Poland, Denmark, and Estonia spanning 800 to 1600 CE (Fig. 1*A*). A full sampling table with site information can be found in Dataset S1. Samples were processed following the laboratory pipeline in Ferrari and Atmore et al. (51). Full laboratory methods can be found in *SI Appendix*, with library protocols detailed in Dataset S1. All laboratory protocols were carried out in the dedicated aDNA laboratory at the University of Oslo following regular decontamination and authentication protocols (92–94). Samples yielded 0.002 to 2.5× coverage.

Modern Genome Sampling.

Fifty-three tissue samples were collected around the Baltic and the Norwegian coast between 2002 and 2010 as reported in Ruzzante et al. (95) and André et al. (96) (Fig. 1*B*). DNA was extracted from the tissue samples using a DNeasy Blood and Tissue Kit. Library building and sequencing were carried out at the Norwegian Sequence Centre. Samples were sequenced on an Illumina HiSeq 4000, yielding coverage of 7 to 17×. An additional 22 individual whole-genome sequences were obtained from previously published data to ensure that all major herring populations were represented (37). Coverage from publicly available sequences ranged from ∼15 to 55×. Full metadata for all modern samples can be found in Dataset S2.

Alignment and SNP Calling.

Both modern and ancient sequences were aligned to herring reference genome Ch_v2.0.2 (36). All sequences were aligned using PALEOMIX v1.2.13 (97). Modern sequences were aligned using bwa-mem and ancient sequences were aligned using bwa-aln. mapDamage2.0 (98) plots for postmortem deamination were assessed to validate the ancient samples (*SI Appendix*, Fig. S1). Modern nuclear sequences were further processed following the GATK best practices pipeline with GATK4 (99). Full methods are described in *SI Appendix*. Several individuals from the modern dataset were removed due to suspected contamination and/or incorrect metadata from the publicly available dataset. The final modern dataset comprised 52 genomes

sequenced for this study and 16 publicly accessed genomes. The process of data cleaning and individual assessment is detailed in *SI Appendix*, Figs. S2–S4.

Determining Population Structure.
smartPCA (100, 101) (Eigensoft v7.2.1) was run on the entire modern nuclear sequence dataset to assess population structure. A maximum-likelihood phylogenetic tree was then built with IQ-TREE v1.6.12 (52, 53) using the mitogenome dataset including all modern and ancient samples to verify that the archaeological samples are Atlantic or Baltic herring. A Pacific herring (*Clupea pallasii*) mitogenome—obtained from Han et al. (37)—was used as an outgroup.

Genetic diversity (π) was estimated in 100-kb windows along the nuclear genome using VCFtools v0.1.16 (54) for each of the four Baltic populations: WBAS, CBAS, CBSS, and GSS, with two or three samples representing each population. Samples were chosen randomly to control for differences in sample sizes between populations, and the same subsets were used for ROH analysis (see below). Full metadata for modern sequences can be found in Dataset S2. Fine-scaled population structure was determined using KING v1 (102), which calculates individual pairwise kinship coefficients within each population. Baltic spring spawners and Baltic autumn spawners were assessed as metapopulations to determine the level of relatedness between possible subgroups (e.g., gulf vs. central Baltic spring spawners and western vs. central Baltic autumn spawners).

Population Assignment.
BAMscorer is capable of accurate genomic assignments using extremely low coverage data (51). Previous studies have reported that population structure in herring is driven by spawning season and adaptation to different levels of salinity (23, 34–37). Three sets of assignment tests were designed for *BAMscorer* using the following biological characteristics: spawning season, chromosome 12 inversion type (Atlantic vs. Baltic), and adaptation to salinity. These biological characteristics correspond to diagnostic genomic Atlantic herring population data (34, 36, 37), which shows strong genetic differentiation between spawning seasons (35, 37) and adaptation to salinity levels (23, 37), as well as differentiation between Baltic and Atlantic autumn-spawning herring at the chromosome 12 inversion (37).

To determine spawning season and salinity adaptation, we used databases of previously published diagnostic loci (37), containing 835 SNPs associated with spawning season and 2,303 SNPs associated with salinity. To determine the chromosome 12 inversion type, a database with 4,503 SNPs was created using the default parameters from the *BAMscorer* v1.4 *select_snps* module. We then performed a sensitivity analysis, following the methods from Ferrari and Atmore et al. (51), to assess the minimum required read depth for 100% classification of each assignment test. For this, we used whole-genome data of eight modern individuals with known metadata. These eight modern individuals were not included among those individuals used to obtain the 4,503 divergent SNPs. The aligned reads of those eight individuals were then randomly down-sampled to between 500 and 100k reads in increments of 500 reads up to 10,000 reads and then increments of 10,000 reads to simulate extreme low coverage data. For each increment and individual, the data were randomly bootstrapped (20 iterations). Assignment sensitivity was then assessed by running the assignment test on the down-sampled alignment files. Minimum required read depth was determined when all 20 randomly down-sampled files per individual were assigned correctly. Sensitivity tests were performed for each of the three assignment tests using the eight modern specimens with known metadata as test data. Full methods and results of assignment sensitivity analysis can be found

in *SI Appendix*. After determining assignment sensitivity and required read depth, each test was then applied to those ancient individuals for which sufficient reads were obtained for accurate assignment. Only autumn-spawning Baltic and Atlantic herring are differentiated on the chromosome 12 inversion type. Therefore, a hierarchical approach was applied, first assessing spawning season, then chromosome 12 inversion, and finally salinity adaptation. These were then combined to yield a final population assignment.

Demographic Reconstruction.
ROHs were analyzed per population to determine the general trends in effective population size for each population (complete methods can be found in *SI Appendix*). The timing of the coalescent events associated with specific windows of ROH length was determined using the formula $L = 100/2g$ cM (103), where L refers to the length and g is generation time (*SI Appendix*, Fig. S11). ROHs were subsequently binned into two groups corresponding to 650 to 400 YBP and 400 to 200 YBP. We compared the total sum and number of ROHs for each bin as well as across all bins.

The four Baltic datasets containing 2 to 10 individuals were input into the demographic software package *gone* (56), which estimates N_e in the recent past using linkage disequilibrium (LD) decay. N_e estimates are geometric means taken from 40 bootstrapping iterations that randomly sample 50,000 SNPs from each chromosome in each population to estimate LD decay. *gone* has been shown to be robust to natural selection (104) and viable for small sample sizes (56). No minor-allele frequency filter was applied to the dataset used for *gone*. Default parameters were used except for substituting the known population-wide recombination rate for Baltic herring of 2.54 cM/Mb (36). *gone* results were scaled by generation using the minimum generation time in herring [3 y for autumn spawners and 2 y for spring spawners (105)]. Each population trajectory was additionally offset by the year the sampling took place (1979 to 2016; Dataset S2). As previous studies have suggested some connectivity between GSSs and CBSSs (44), an additional analysis was undertaken using the Baltic spring spawners as a single population (*SI Appendix*, Fig. S15).

We reconstructed population demography using *gone* v1, which is found to be accurate up to at least 200 generations in the past (56). We show trajectories up to 1,000 YBP to reflect the possibility of overlapping and/or longer generation times. As herring spawn in overlapping generations with varying maturation times, and can live up to 18 y (106), generation times in 5- and 10-y increments are further shown in *SI Appendix*, Fig. S14 to illustrate the possible variations in trajectory for each population. Exact N_e estimates of *gone* are likely affected by large effective population sizes in the past and the small sample size used here (56). True N_e estimation is further confounded by fish spawning behaviors, including overlapping generations and batch spawning (107). Therefore, the key findings from the *gone* analysis are the divergent trajectories and their relative changes rather than the absolute values of N_e estimated for each population at a given date. All four populations showed unrealistic bottlenecks to near-zero N_e in the four most recent generations. The original *gone* paper reports similar patterns for recent generations when using small sample sizes ($n \leq 10$) and applications to other fish populations show a similar pattern (56, 107), and therefore these recent extreme bottlenecks were disregarded as an artifact of the calculations and removed from the results. Given the possibility of connectivity between the GSS and CBSS populations, *gone* was also used to estimate demographic history for the Baltic spring-spawning metapopulation as a whole (*SI Appendix*, Fig. S16)

Data, Materials, and Software Availability
The raw genome sequence data reported in this article have been deposited in the European Nucleotide Archive (accession no. PRJEB52723)

All study data are included in the article and/or supporting information. Additional raw datasets can be found as part of the supporting information at PNAS. They were not included here due to size of the tables.

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Chapter heading from Olaus Magnus (1555)

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Supplementary Information

Population dynamics of Baltic herring since the Viking Age revealed by ancient DNA and genomics

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Datasets S1 to S2

Supplementary Information Text

Full ancient DNA laboratory methods

Single-bone samples weighing 10-70mg were bathed in UV light to clean the exterior, then placed in digestion buffer (1 ml 0.5 M EDTA, 0.5 mg/ml proteinase K, 0.5% N-Lauryl sarcosine) and crushed in 1.5ml Eppendorf tubes using single-use plastic micro-pestles. DNA was extracted following the double-digest procedure from Damgaard et al.[1] with MinElute PB buffer (QIAGEN). Samples were then purified through MinElute columns using the QIAvac 24 Plus vacuum manifold system (QIAGEN) for a final elution volume of 65ul. Two types of libraries were built, with some samples built using the double-stranded library protocol from Meyer & Kircher[2] and some built with single-stranded libraries following the Santa Cruz protocol[3]. A full list of the library protocols used per sample can be found in Supplementary Dataset S1. All laboratory protocols were carried out in the dedicated ancient DNA laboratory at the University of Oslo following regular decontamination and authentication protocols[4–6]. Each library underwent 12-15 cycles of PCR amplification followed by purification using the Agencourt AMPure XP PCR purification kit using a 1:1 bead:sample ratio. Libraries were then assessed for quality on a Fragment Analyzer™ (Advanced Analytical) using the DNF-474 High Sensitivity Fragment Analysis Kit to determine suitability for sequencing. Samples of high enough quality were then sequenced on an Illumina HiSeq 4000 and/or NovaSeq 6000 at the Norwegian Sequencing Centre.

SNP calling and filtering

Modern samples were filtered using bcftools v1.3[7] (*FS<60.0 && SOR<4 && MQ>30.0 && QD > 2.0 && INFO/DP<415140' --SnpGap 10*) and VCFtools v0.1.16[8] (*--minGQ 15 --minDP 3 --remove-indels --maf 0.01*). Non-biallelic loci were removed. An additional dataset with no MAF frequency filtering was created for *gone* analyses known to be sensitive to removing minor alleles. Mitogenomes were called and filtered as above for ancient and modern samples. Individuals with >30% missingness were removed.

Removing outliers from the whole-genome dataset of modern herring specimens

Our dataset contains whole genome data of herring specimens obtained from a range of difference sources, collected over a number of years (see Supplementary Dataset S2). The dataset further combines publicly available data with data generated *de novo* for our study. Given this wide range of sources, we performed a number of exploratory analyses to ensure data integrity. First, we previously identified that two of the individuals from the Han et al.[9] dataset were technical duplicates[10] using KING[11] (Table S1). One of these individuals was chosen at random to be included in the dataset and the other was discarded. Second, exploratory population analyses using smartPCA[12,13] indicated that several individuals were significant outliers (Figure S2). To examine this pattern, levels of SNP-based levels of heterozygosity were calculated for the modern nuclear data using --het from VCFtools[8]. Three individuals (HER_Z12_IsleOfMan, HER_NSSH34, and M-HER004) showed unusually high levels of heterozygosity (Figure S3), which is a measure of possible poor read mapping and contamination[16]. These individuals therefore show unusually high similarity in genetic information and/or poor read mapping yet high heterozygosity. This could be the result of cross-contamination between specimens. We further assessed the possibility of contamination by analyzing levels of heterozygosity along the mitogenome (Figure S4). As a haploid sequence, expected heterozygosity for each locus is 0. For those individuals containing loci with heterozygosity >0 this is indicative of contamination. Again, three individuals (HER_NSSH33, HER_NSSH34, and HER_Z12_IsleOfMan) showed clear signs of contamination. Based on these results, four individuals (HER_Z12_IsleOfMan,

HER_NSSH33, HER_NSSH34, and M-HER004) were removed from the dataset. Finally, one individual (AAL1_CelticSea) from the Han et al. (2020) dataset was removed as it consistently clustered with individuals with non-matching metadata (Figure S5). Given other inconsistencies of the metadata, including the duplicated sample, this sample was also removed. The cleaned dataset was used for demographic (runs of homozygosity, KING, and *gone*) and for whole-genome PCA analyses.

BAMscorer sensitivity analysis

In order to determine which ancient sequences could reliably be scored, we first assessed the required read depth for accurate assignment in each of the three assignment tests (Chromosome 12 inversion, spawning season, and salinity adaptation). Required read depth per assignment was assessed following the down-sampling and bootstrap method of Ferrari & Atmore et al.[10]. We selected 8 samples as a training dataset from the modern reference dataset to independently test the power of assignment probability with known metadata. The alignment files of these eight samples were randomly down-sampled to between 1k and 100k reads 20 times and then used to assess BAMscorer sensitivity for each assignment test.

Given the relatively low number of autumn spawning herring, we investigated whether including the removed contaminated individuals (see above) impacted the biological patterns for each comparison. Although these outliers impact the whole genome population analyses, SmartPCA analysis on each of the three BAMscorer assignment databases showed that including these outliers associated with contamination bias did nothing to change the shape of the distributions. Due to limited sample size for the different categories in these assignment tests, several outliers were left in the BAMscorer databases apart from AAL1_CelticSea (suspected incorrect metadata) and Gavle54 (identical to Gavle98) from the Han et al.[9] dataset. Full information on which samples were used for each stage of analysis can be found in Supplementary Dataset S2.

The modern database showed strong differentiation between spring and autumn/winter spawning seasons, following previously reported results[9,17,18]. Sensitivity analysis showed that spawning season can be accurately determined in alignment files with as few as 50 000 reads (Fig S6). The chromosome 12 inversion could be confidently assigned with as few as 5 000 reads (Fig S7) using default parameters. Given that only samples for which spawning season could be assigned were analyzed, all samples still retained at least 50 000 reads. Salinity scores could be determined for samples with at least 60 000 reads (Fig S8).

Evaluating demographic independence

Demographic independence of the Baltic subpopulations was further assessed by calculating individual pairwise relatedness for each group with KING. To assess the substructure in the Baltic, metapopulations were grouped, resulting in two Baltic populations: autumn spawners and spring spawners (for full KING results, see Supplementary Dataset S2). In the Transition Zone (TZ) population, M-HER066 showed strongly negative kinship coefficients with the rest of the TZ individuals. This is a sign that there is population structure, thus M-HER066 is likely not actually a TZ individual, but could be part of the Atlantic spring spawners population, which is where it clusters on the PCA. M-HER066 was removed from analysis to eliminate outlier bias. Kinship coefficients were plotted per population to visualize the distribution of relatedness in each population (See Fig S9).

A one-way ANOVA showed that metapopulation ID was significantly associated with kinship coefficient mean and variance (p=8.16e-14, DF=4). Baltic spring spawners had the lowest

average kinship coefficient (0.0336). Baltic autumn spawners had the second highest kinship coefficient (0.05827), likely due to the effect of the Fehmarn individuals, which showed high relatedness to each other. The Fehmarn within-group average was 0.08. This illustrates there is some substructure in the herring metapopulations, although it is likely that there is a degree of connectivity, as strong substructure would result in negative kinship coefficients. It should be noted that all individuals showed low levels of relatedness and there was some variance in relatedness between all groups. The Transition Zone showed the highest variation in kinship coefficient, likely due to the presence of subpopulations such as the Idefjørd herring.

Runs of Homozygosity

Initial ROH results showed that the variation in ROH length, count, and total sum were largely determined by differences in sample size. Therefore, a subset of each population was chosen randomly, with 2-3 individuals per population (samples used can be found in Supplementary Dataset S1). PLINK files were generated from the modern nuclear sequence data and then assessed for differences in runs of homozygosity (ROH) using PLINK 1.9[19] following previously-published recommendations[20–22]. The following command was used: *plink –bfile herring --chr-set 26 --double-id --homozyg-snp 50 –homozyg-kb 90 –homozyg-density 50 –homozyg-gap 1000 –homozyg-window-snp 50 –homozyg-window-het 3 –homozyg-window-missing 10 –homozyg-window-threshold 0.05 --out herring_roh.* ROHs were compared by length, count, and total sum between the 4 Baltic populations.

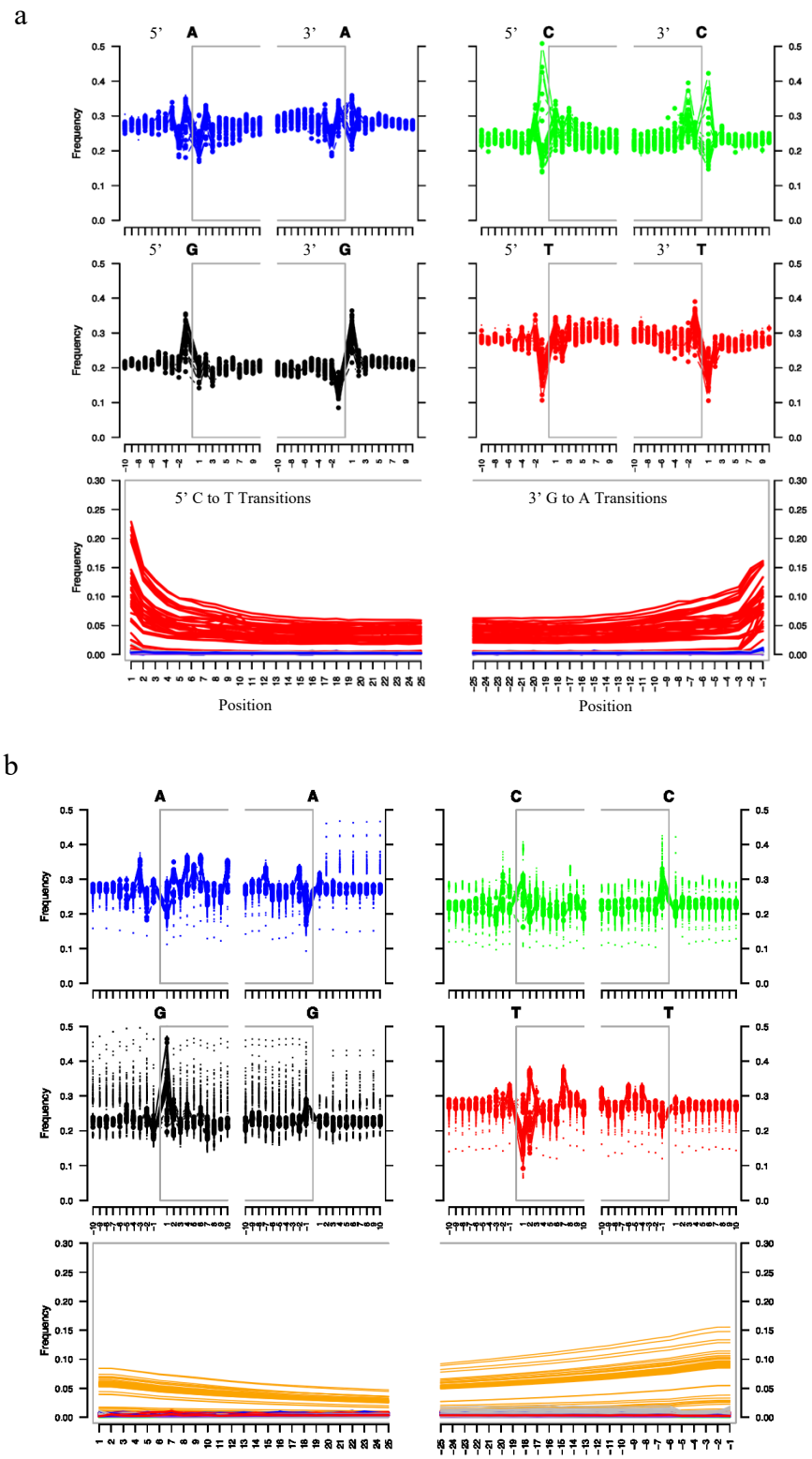

Figure S1 – Fragment Misincorporation and ancient DNA Damage plots. a) Patterns were obtained by using MapDamage v. 2.0.6 after down-sampling BAM files to 1 million reads. These plots show the typical fragmentation and the post-mortem deamination patterns that are characteristic of those associated with ancient DNA[23]. We simultaneously plot all archaeological herring specimens (n=40) b) Fragment misincorporation and damage plots for modern herring sequence data (n=72).

Table S1 – KING output for technical duplicates/relatedness in modern herring reference data. Concordance values above 0.8 are indicative of duplicates according to the KING documentation. Table from Ferrari et al[8]. Gavle54 and Gavle98 (Han et al.[9], PRJNA642736) are therefore considered technical duplicates. We removed Gavle54 from the dataset (see also Supplementary Dataset S2.

FID1	ID1	FID2	ID2	N	N_IBS0	N_IBS1	N_IBS2	Concord	HomConc	HetConc
Gavle54	Gavle54	Gavle98	Gavle98	11314407	187	4995	11309225	0.99954	0.99998	0.99674

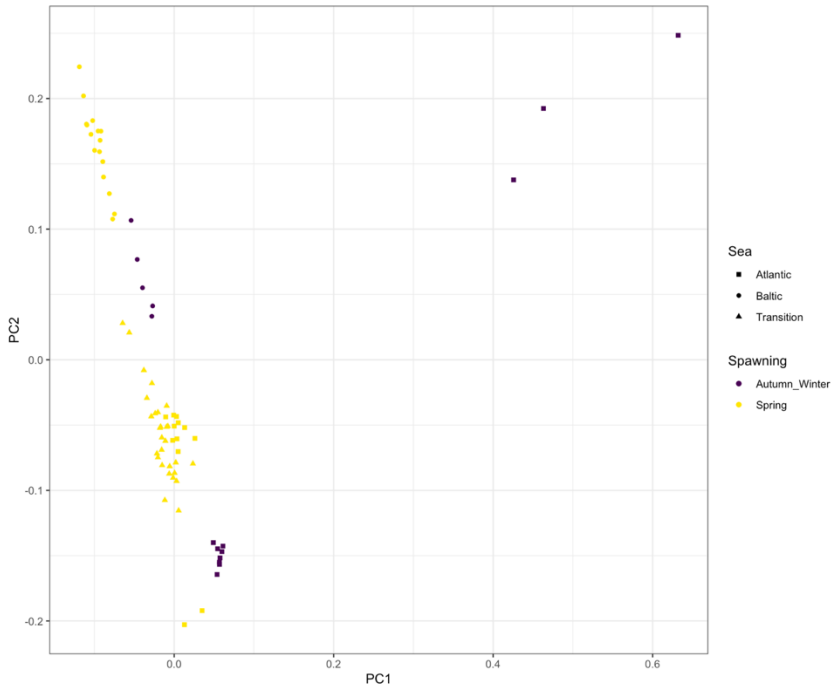

Figure S2 – Exploratory population analyses of Atlantic herring using genome-wide data. The PCA is based on 10,368,446 SNPs. The color indicates the spawning season and the shape indicates the sea in which the sample was collected. "Transition" refers to the area between Norway, Sweden, and Denmark that spans the transition between the North Sea and the Baltic Sea. Three Atlantic herring specimens are located away from the main herring clusters. This pattern is driven by inclusion of a single specimen (HER_Z12_IsleOfMan) that is contaminated (see also Figure S3, S4, and S5).

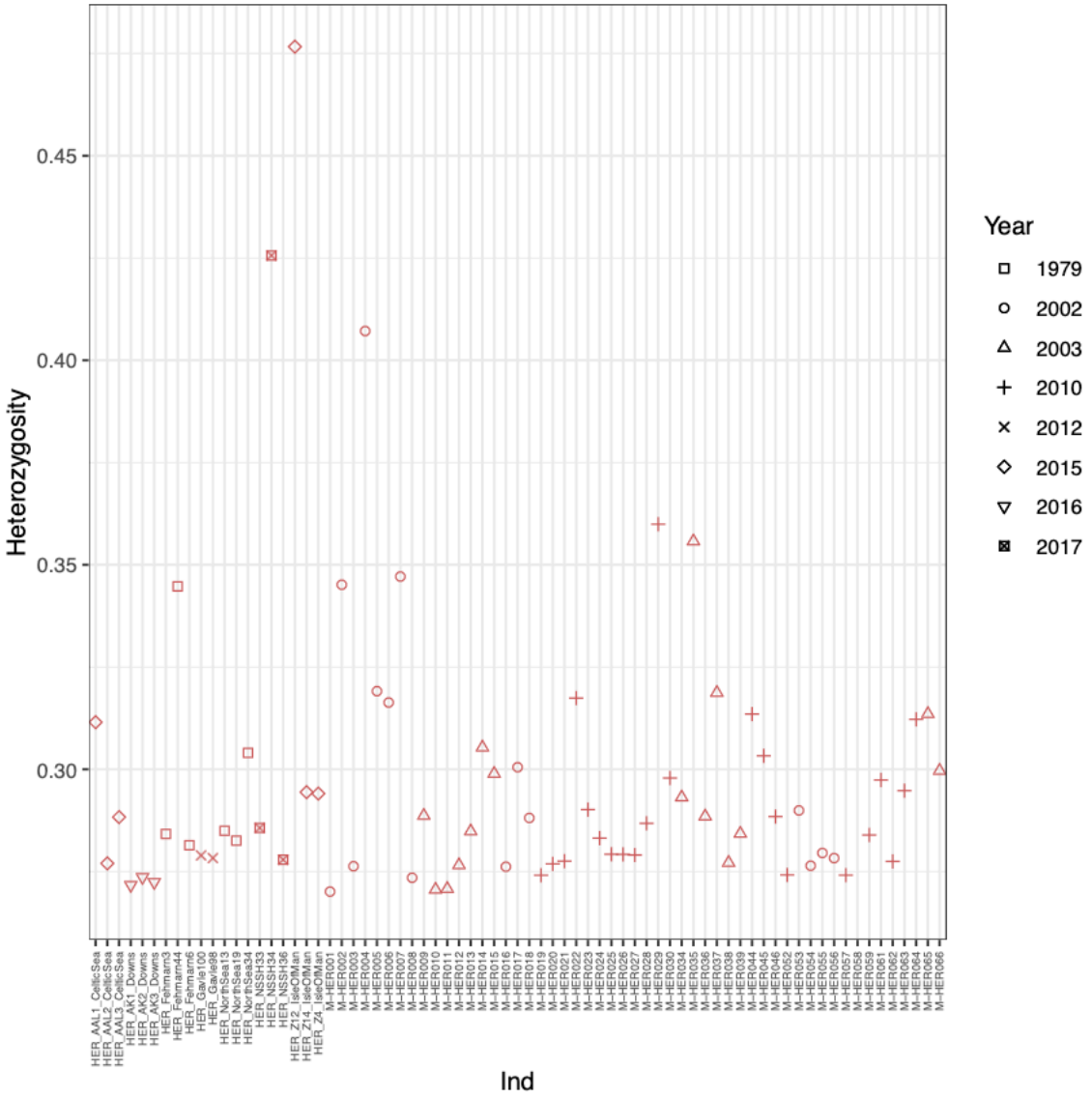

Figure S3 – Observed vs expected heterozygosity in modern herring specimens. Individual herring specimens are ordered along the x-axis. Shapes indicate the year in which the sample was obtained. Three individuals are clear outliers – HER_Z12_IsleOfMan, HER_NSSH34, and M-HER004.

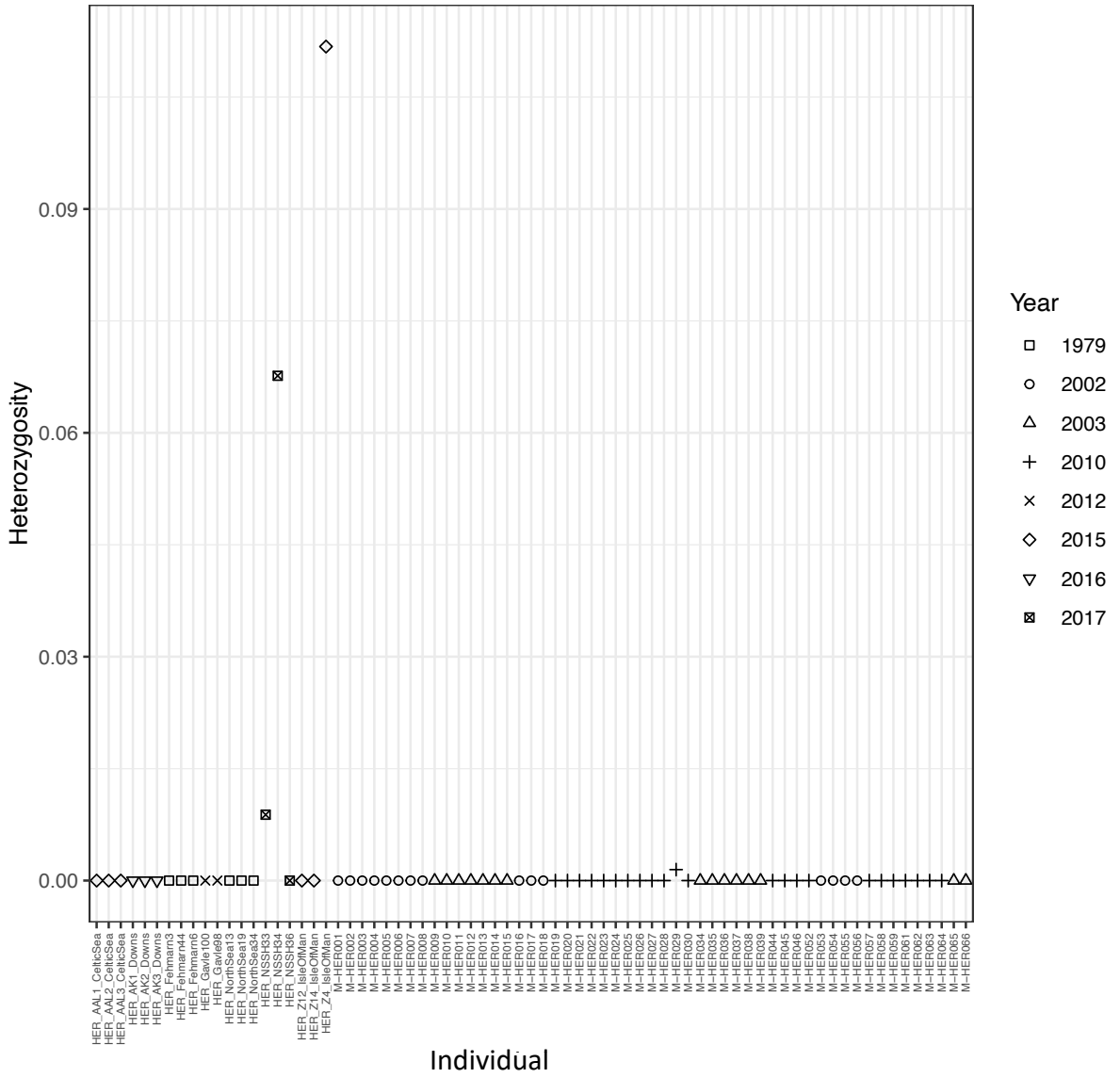

Figure S4 – Levels of heterozygosity observed in the mitogenome of modern herring specimens. SNPs were called on the mitogenome while allowing diploid genotype calls. Heterozygosity was calculated using VCFtools. We would expect the mitogenome to yield homozygote genotypes only. We observe three individuals with heterozygosity values >0, which is indicative of intraspecific contamination. These individuals are HER_NSSH33, HER_NSSH34, and HER_Z12_IsleOfMan. M-HER004 does not exhibit signs of contamination in the mitogenome, but was still removed from the dataset due to its high levels of heterozygosity.

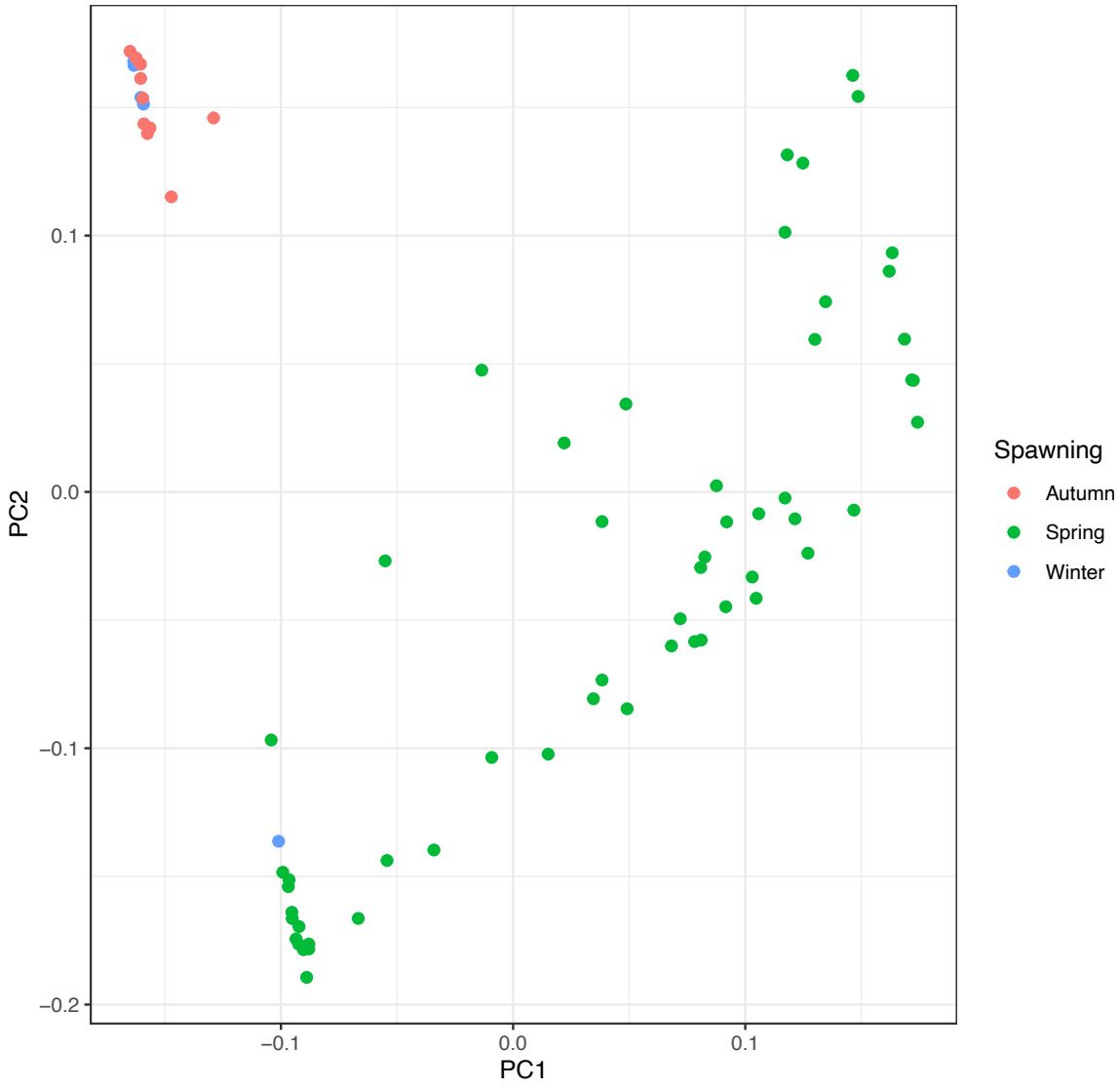

Figure S5 – PCA of spawning loci including all modern herring specimens. The PCA was run on whole genome data from 68 herring specimens (Supplementary Dataset S2) using a selection of spawning loci (n=835) that were obtained from Han et al[9]. We obtained a clear distinction between spring spawners and autumn/winter spawners. HER_AAL1_CelticSea from the Celtic Sea is coded as a winter spawner but consistently clustered with spring spawners in smartPCA analysis.

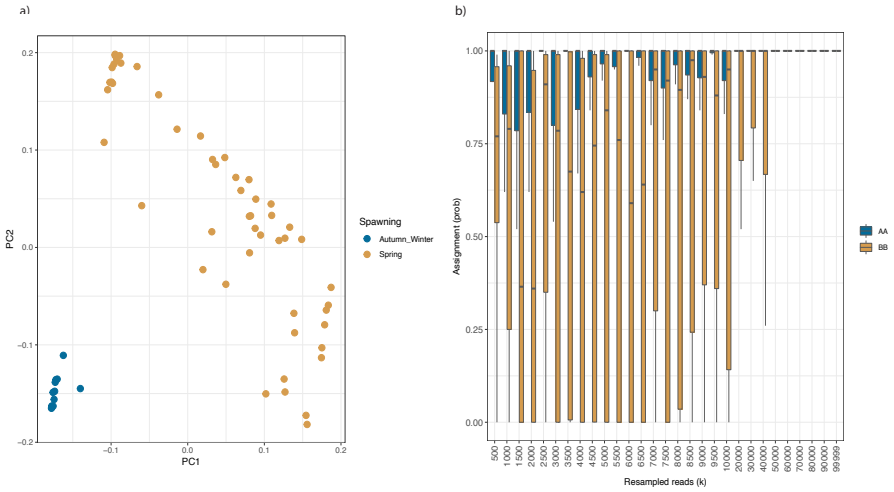

Figure S6 – Spawning season assignment sensitivity. a) PCA of the 835 diagnostic loci that identify spawning season from Han et al[9]. The PCA is based on whole genome data of 60 modern herring specimens (Supplementary Dataset S2), excluding eight modern herring specimens that were used as a test dataset with known metadata. b) Application of BAMscorer to the bootstrapped down-sampled alignment files of the eight test specimens with known metadata show greater difficulty assigning spring spawning than autumn spawning. Each individual BAM file from the training dataset was randomly down-sampled to between 500 and 100,000 reads for 20 iterations. The y-axis represents the proportion of those files which are known to be type AA or type BB that were assigned correctly using BAMscorer. Both seasons can be assigned with no error with a minimum of 50,000 reads. AA is autumn spawning and BB type is spring spawning.

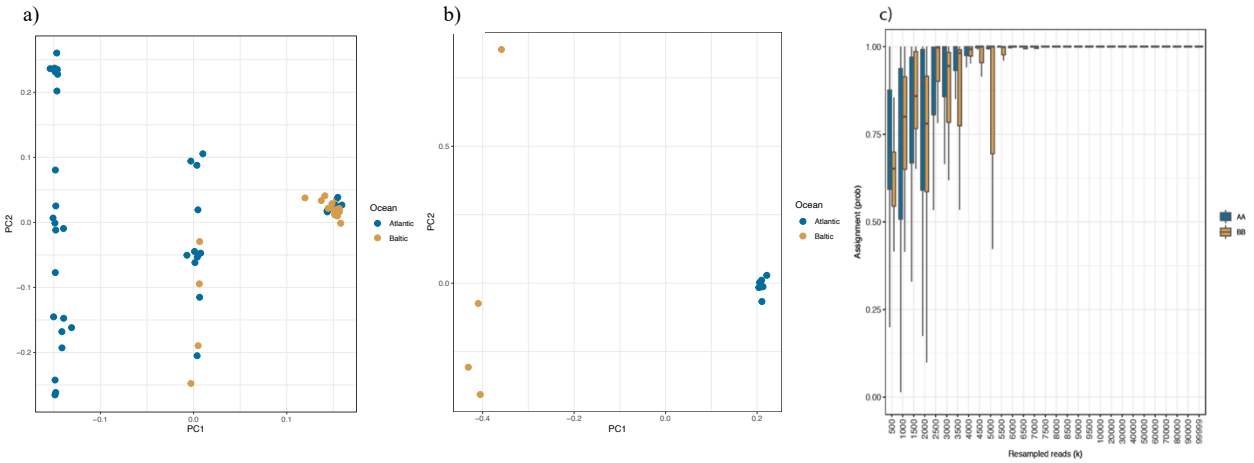

Figure S7 – Chromosome 12 inversion split between Atlantic and Baltic haplotypes. a) Chr12 inversion for all herring individuals in the modern dataset (n=68, Supplementary Dataset S2). b) Chr12 inversion distribution for only autumn spawners only (n=9). This PCA is based up 4503 divergent SNPs. The autumn spawners show a split between the Atlantic and Baltic type inversions, as reported by Han et al.[9] c) The inversion locus within autumn spawners can be scored with as few as 5000 reads. Three of the eight test specimens were autumn spawners, one Baltic type and two Atlantic type (Supplementary Dataset S2). Each individual BAM file from the was randomly down-sampled to between 500 and 100,000 reads for 20 iterations. The y-axis represents the proportion of those files which are known to be type AA or type BB that were assigned correctly using BAMscorer.

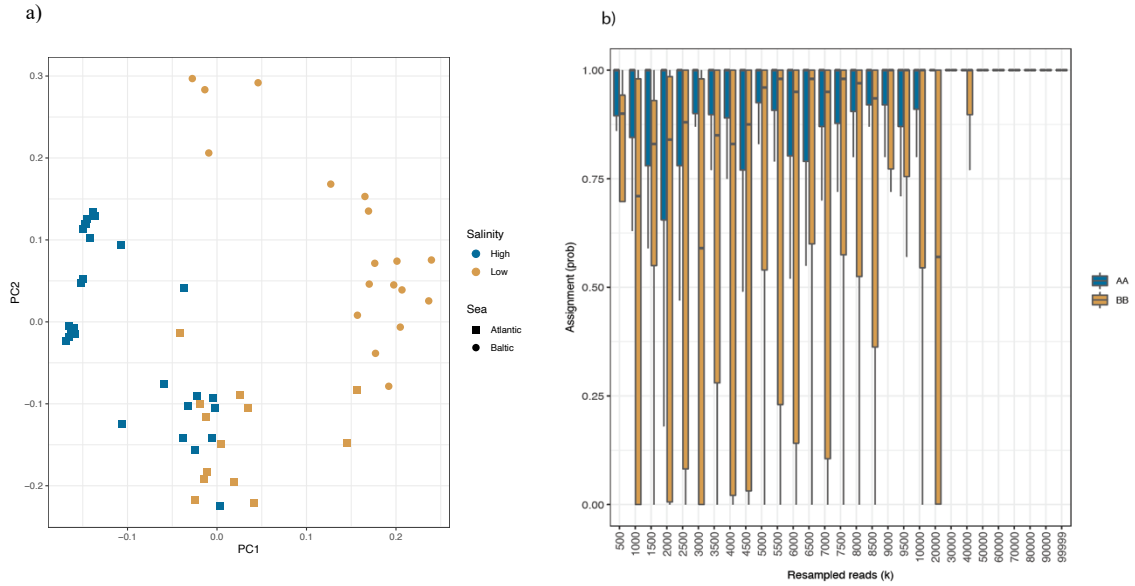

Figure S8 – Salinity adaptation loci BAMscorer database creation and parameter testing. a) PCA generated with smartPCA. Color indicates ocean basin as a proxy for salinity (high being Atlantic, low being Baltic) and shape indicates population of origin. The PCA is based on 2303 divergent SNPs obtained from Han et al.[9] Some Atlantic samples were from Norwegian fjords with low salinity therefore they fell between the two groups. These samples were removed from the dataset for the final scoring. b) Eight individuals with known metadata were removed from the reference dataset for sensitivity analysis (Supplementary Dataset S2). Iterative application of BAMscorer to down-sampled alignment files showing a minimum of 50,000 reads is required for determining salinity adaptation. Each individual BAM file from the eight test specimens training dataset was randomly down-sampled to between 500 and 100,000 reads for 20 iterations. The y-axis represents the proportion of those files which are known to be type AA or type BB that were assigned correctly using BAMscorer.

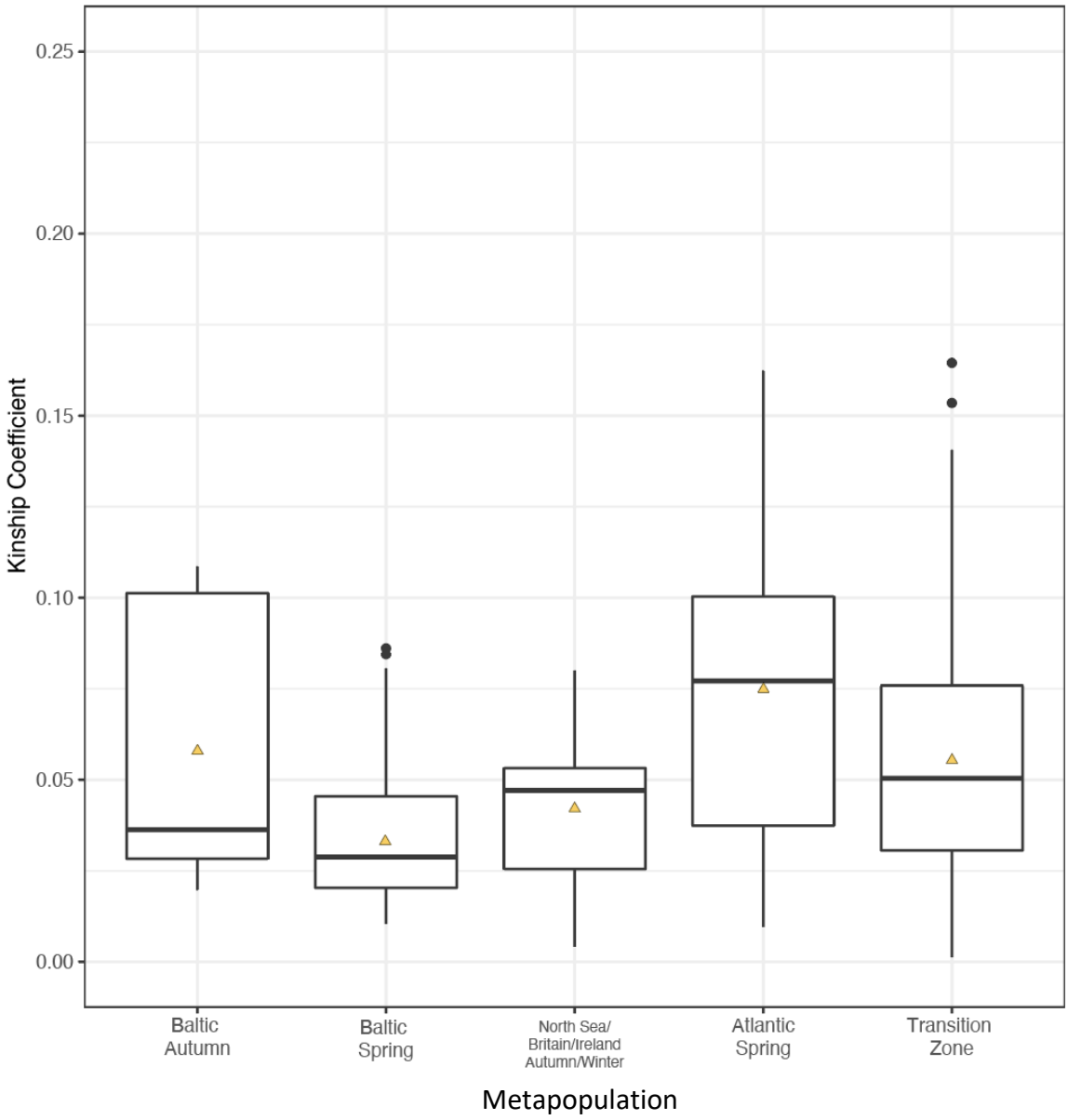

Fig S9 – Individual pairwise relatedness by metapopulation. Each boxplot shows the distribution of kinship coefficients across the metapopulation along the x-axis. Baltic autumn and Baltic spring populations were grouped as two metapopulations to assess the level of substructure in each population; a lower kinship coefficient is indicative of higher substructure. Lines indicate median kinship coefficient and yellow triangles indicate the mean kinship coefficient per metapopulation. Baltic spring spawners show the lowest median and mean kinship coefficient. Baltic autumn show the second-lowest median kinship coefficient, but the mean is dragged higher by the high degree of relatedness in the Fehmarn population.

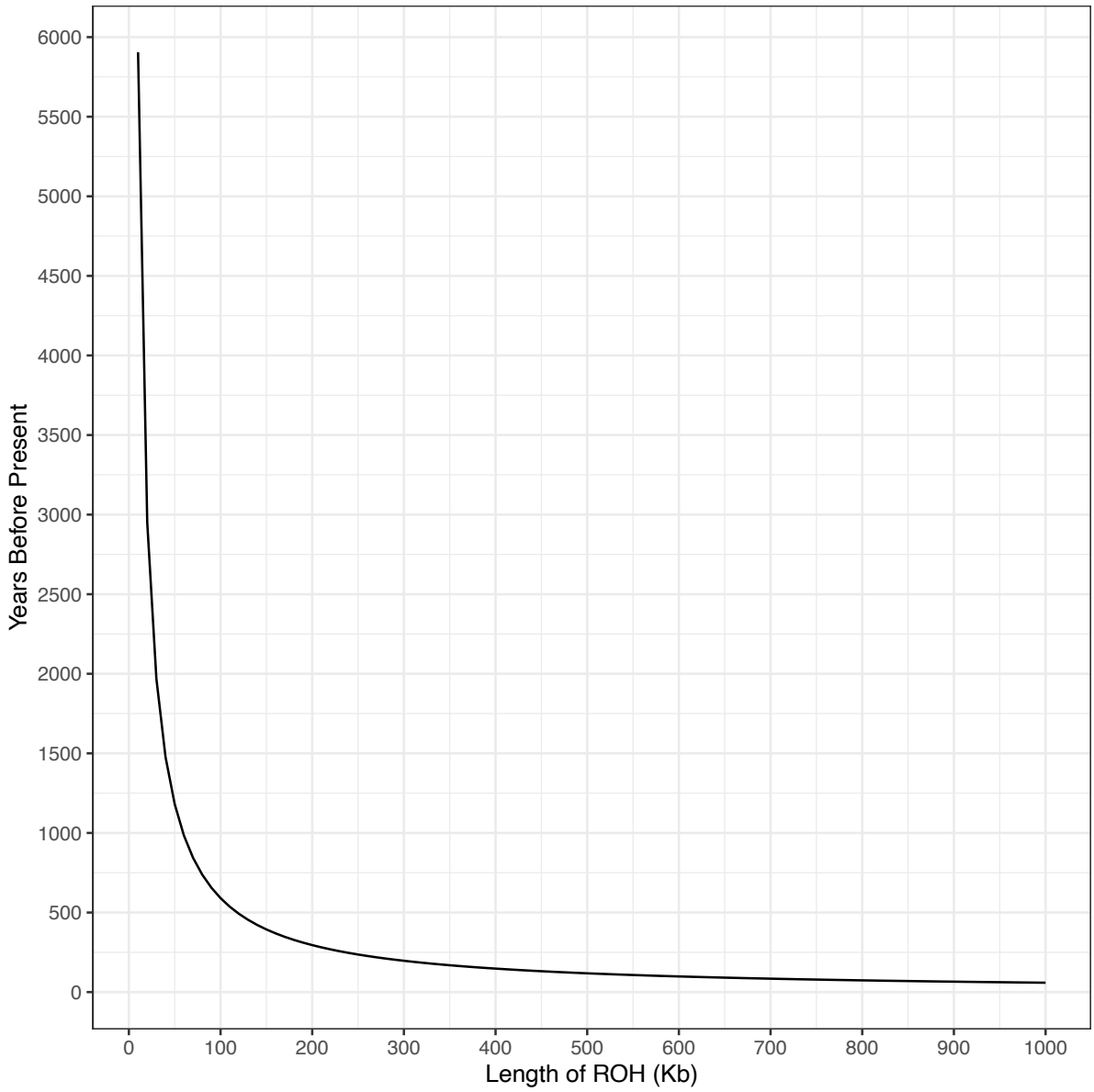

Figure S10 – Time to coalescent event according to length of run of homozygosity. Using the formula 100/2g cM/Mb = L^{24} and the known herring recombination rate of 2.54 cM/Mb, we calculated the relationship between length of ROH and timing of coalescent event. The curve indicates the length and its associated coalescent event in years before present.

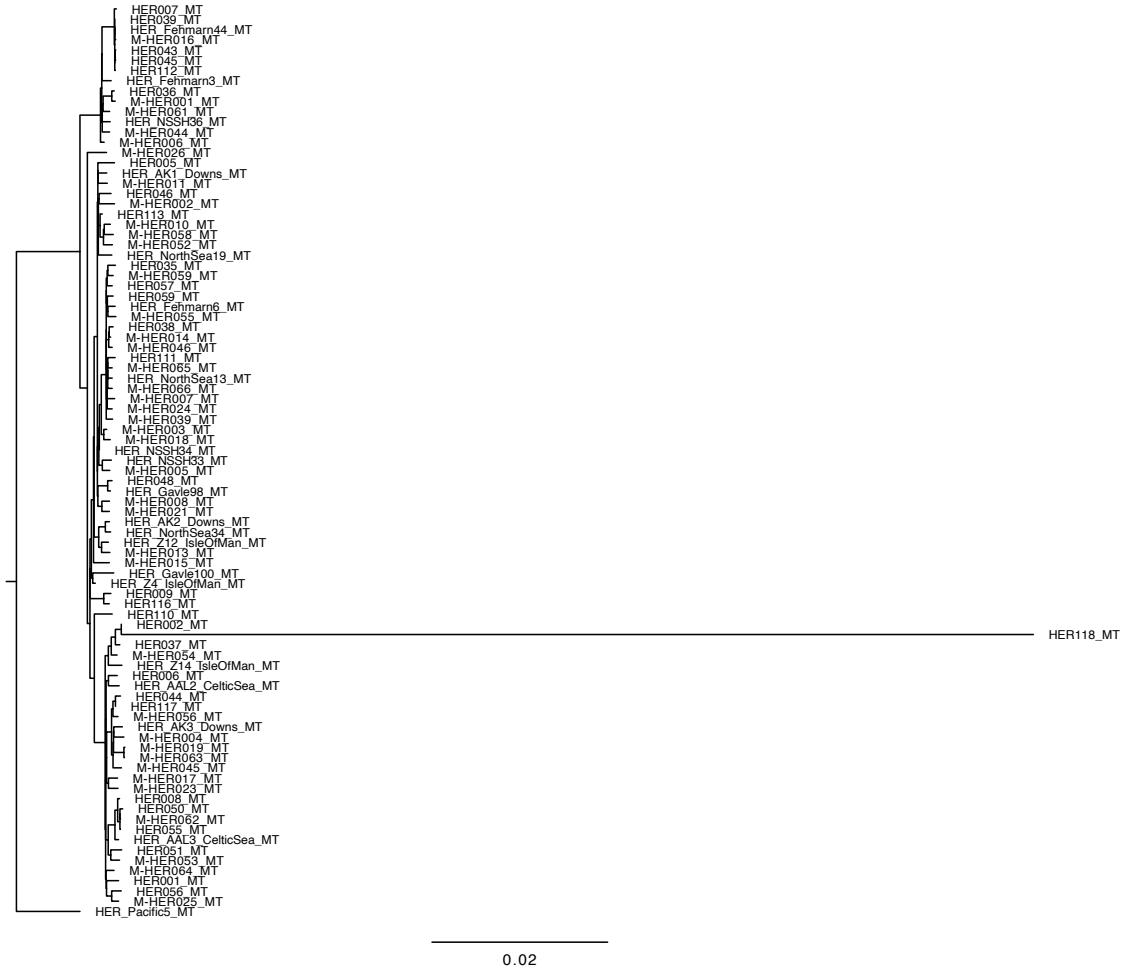

Figure S11 – IQtree phylogeny of the herring mitogenome. All ancient samples clustered with the modern samples and exhibited the structure reported by Teacher et al.[25] that is not associated with geography. HER118 appears quite differentiated from the rest of the samples. BLAST showed that it is indeed a Baltic herring, therefore it was left in the analysis for BAMscorer assignment.

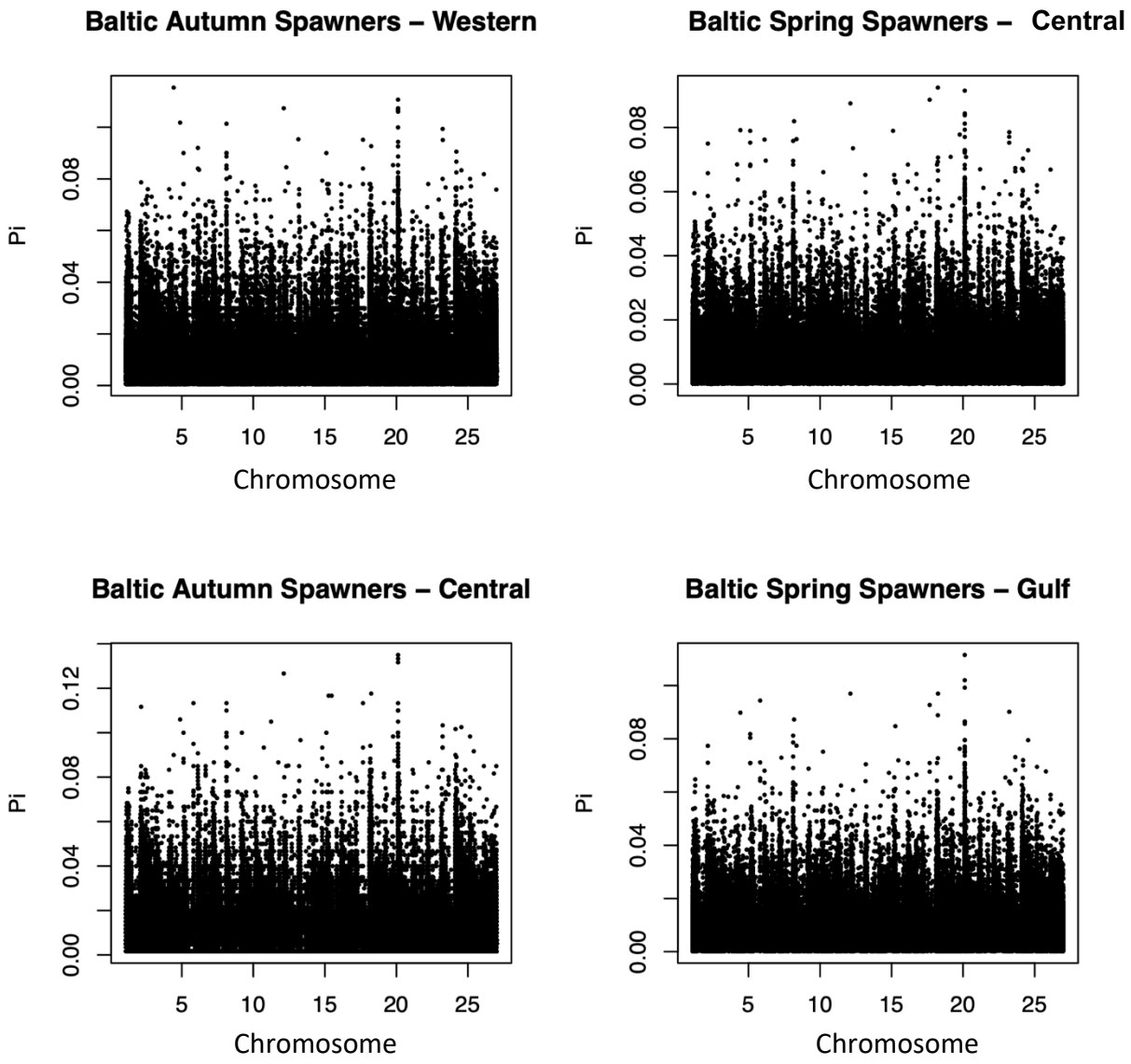

Figure S12 – Estimates of pi for four different Baltic herring populations. The western Baltic autumn spawners had an average pi of 0.006; the central Baltic autumn spawners had an average of 0.008; the central Baltic spring spawners had an average of 0.004; and the gulf spring spawners an average of 0.005.

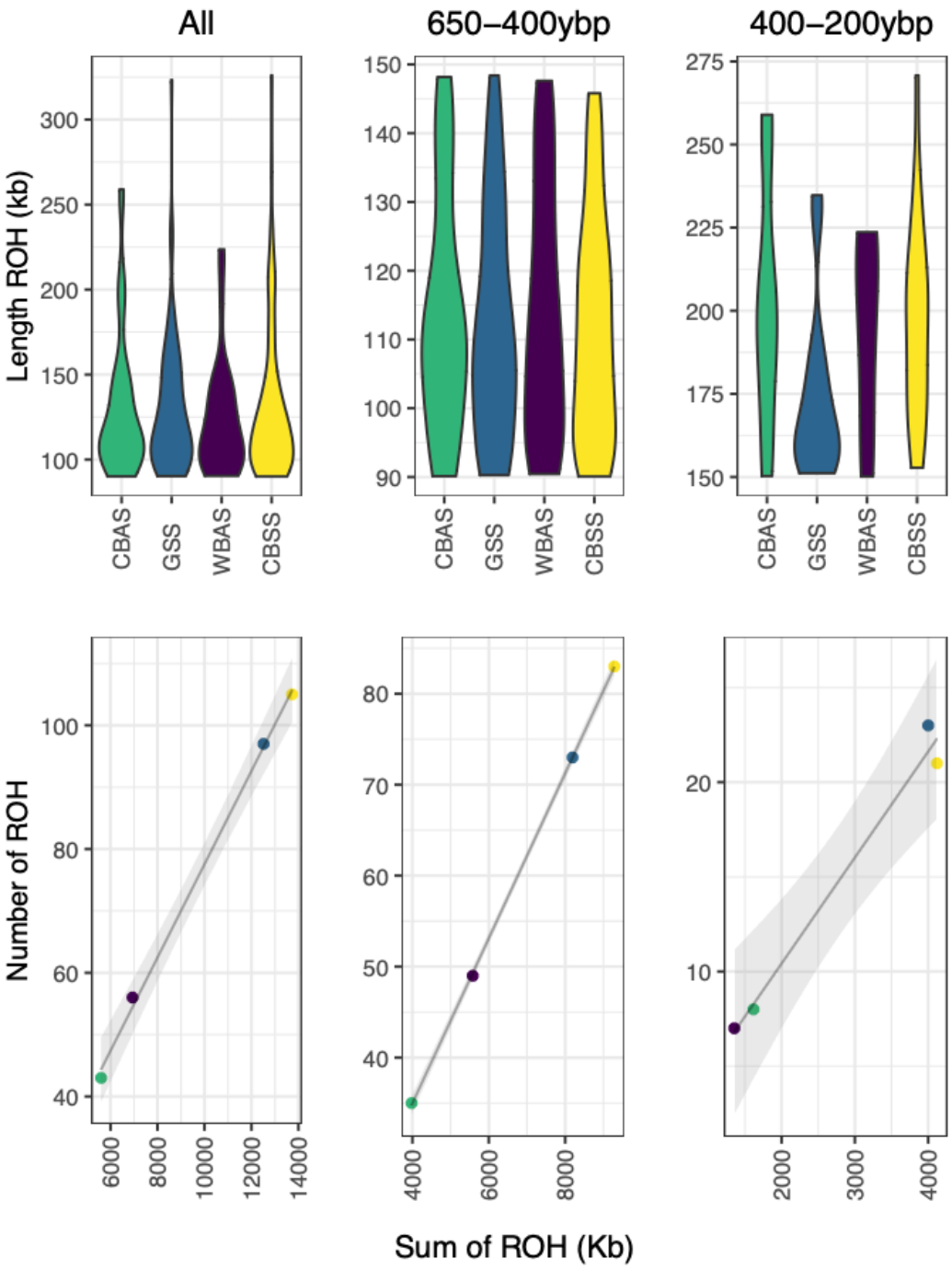

Fig S13 – Runs of homozygosity in four modern Baltic herring stocks. The top panel shows the distribution of ROH across the genome for the entire genome and two bins associated with coalescent events 650-400YBP and 400-200YBP. The bottom panel illustrates the summed ROH for each population compared to the total number of ROH. These results indicate a larger effective population size in the past for both autumn spawning herring populations and a smaller effective population size in the past for both spring spawning herring populations.

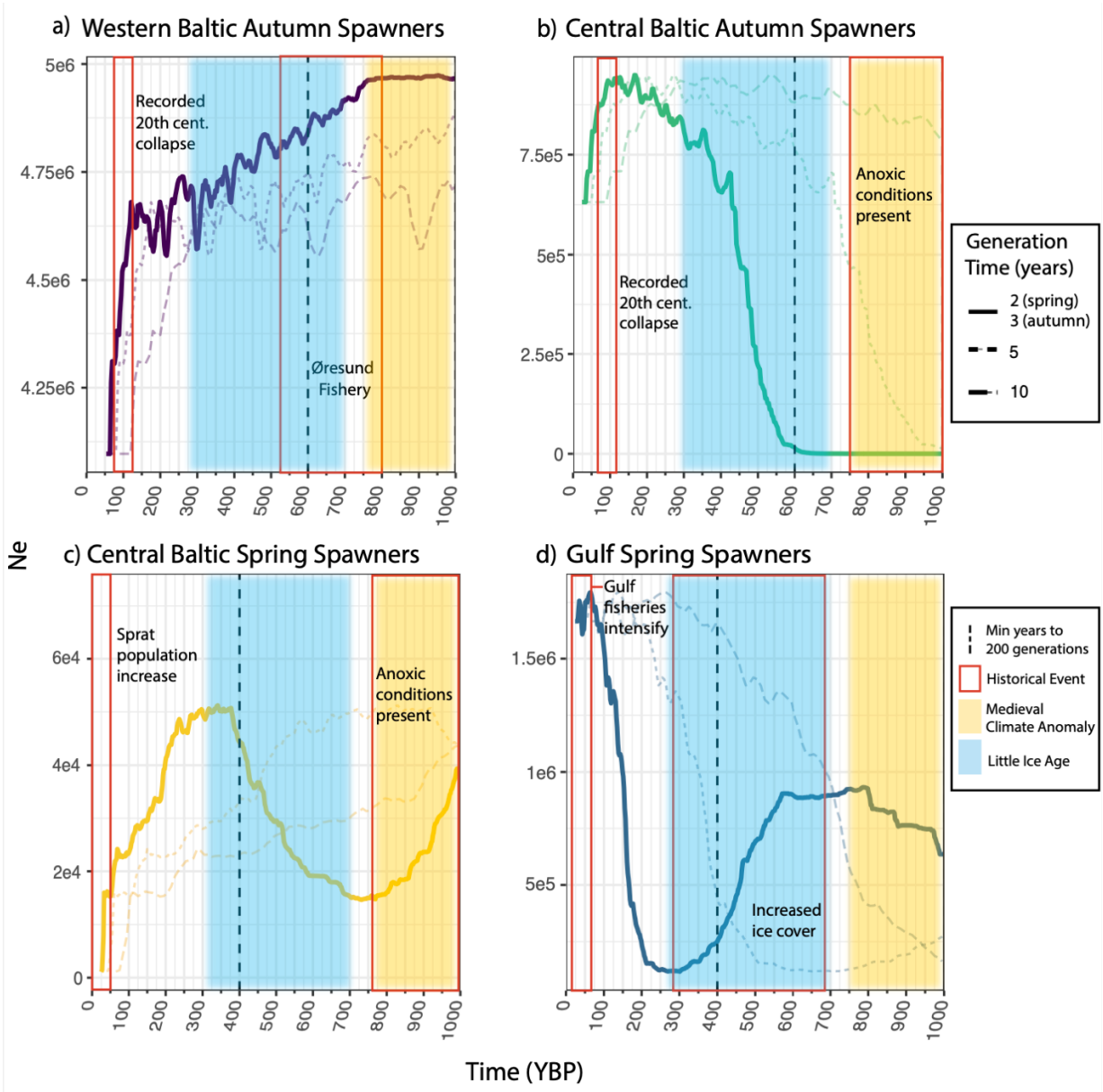
a) Western Baltic Autumn Spawners
b) Central Baltic Autumn Spawners
c) Central Baltic Spring Spawners
d) Gulf Spring Spawners
Ne
Time (YBP)
Recorded 20th cent. collapse
Øresund Fishery
Anoxic conditions present
Sprat population increase
Gulf fisheries intensify
Increased ice cover
Generation Time (years)
2 (spring) 3 (autumn)
5
10
Min years to 200 generations
Historical Event
Medieval Climate Anomaly
Little Ice Age

Fig S14 – gone analysis with generation time uncertainty. Each stock is represented in a separate chart: western Baltic autumn spawners (WBAS); central Baltic autumn spawners (CBAS); central Baltic spring spawners (CBSS); gulf spring spawners (GSS). Colored rectangles indicate key historical events. Yellow rectangles show the approximate duration of the Medieval Climate Anomaly (MCA) and blue rectangles the Little Ice Age (LIA). The dashed vertical lines show the minimum date (YBP) at which 200 generations in the past is reached (calculated using minimum generation times of 3 years for autumn spawners and 2 years for spring spawners), the known accurate window for *gone*. Historical events are denoted by red boxes for each population. As herring reproduce over their lifespan in overlapping generations, additional generation times were used to scale the demography for each population. These are visualized here as dashed lines. a) Demographic trajectory of the western autumn spawners shows a decline starting shortly after the start of the Øresund herring. They show an additional severe decline corresponding to the reported autumn spawning fishery collapse in the Baltic; b) Demographic trajectory of central autumn spawners, which appear limited during the MCA when anoxic conditions are present in the central Baltic. They increase during the LIA and rapidly decline during the period of known autumn spawning population collapse coinciding with the increase of the sprat (*Sprattus sprattus*) population; c) Central spring spawners show an increase around the time of the decline of the western autumn spawners, then a decrease again at the end of the LIA as well as another dramatic decrease around the time of the autumn spawners' collapse ~100YBP; d) Gulf spring spawners decrease during the LIA and then increase dramatically at the end of the LIA, starting to decline only in very recent generations when fisheries in the gulfs intensify. This panel illustrates the accuracy of using generation times between 2-5 years for Baltic herring. GSS were not intensively fished until very recently, therefore their demographic trajectory should reflect changes in environmental factors such as sea surface temperature and presence of ice cover. Generation times 2 and 5 reflect best the expected changes in population size given known responses to such environmental changes in Baltic herring.

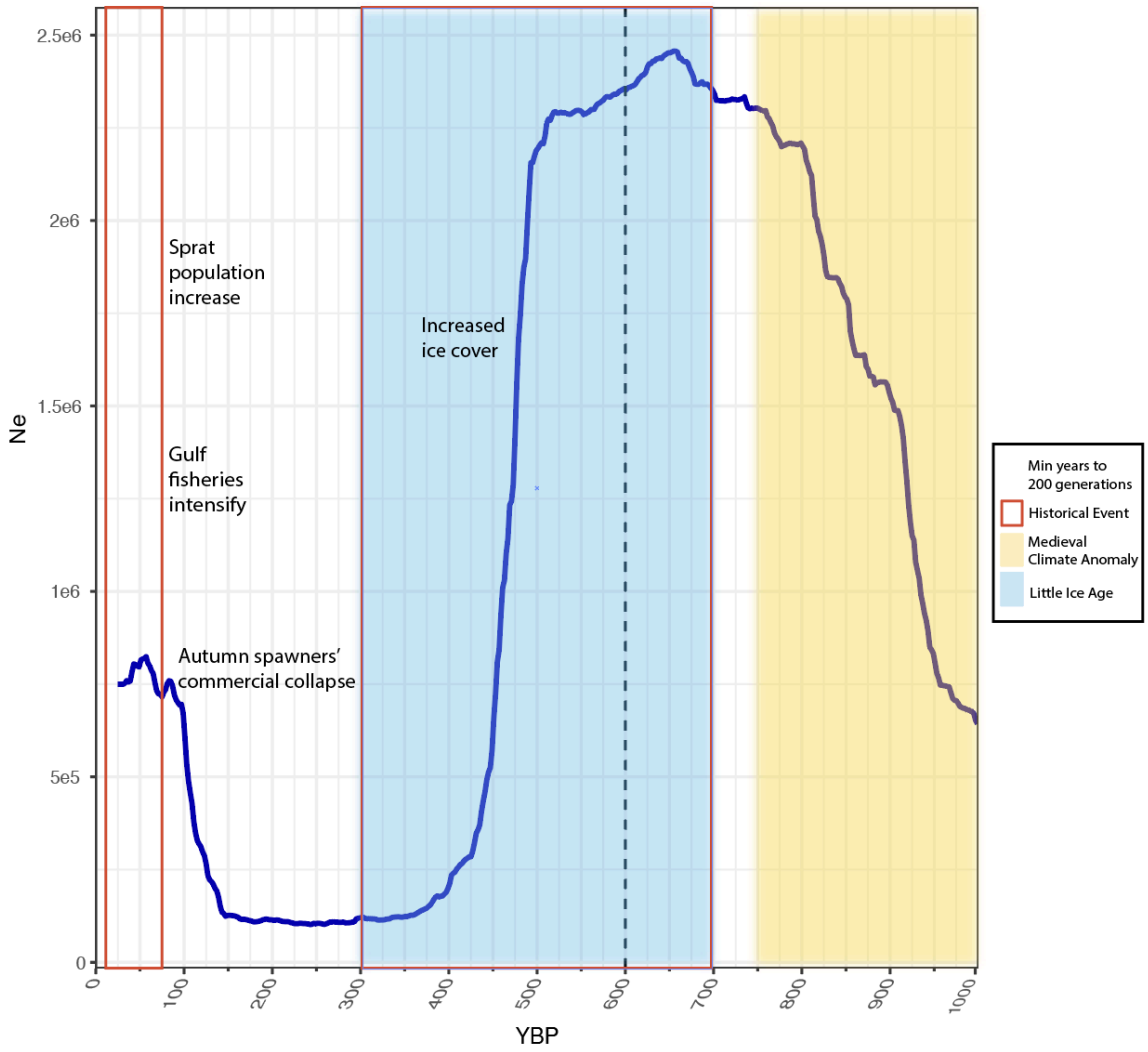

Fig S15 – gone analysis for all Baltic spring spawners. Here, Baltic spring spawners were grouped as a single metapopulation (n=10) and *gone* was run with the same parameters as before. This figure shows the projected demographic trajectory when both the GSS and CBSS are treated as a single population. Historical events are again marked, illustrating the relationship between fishing pressure, changing climate, and demographic trends in Baltic herring.

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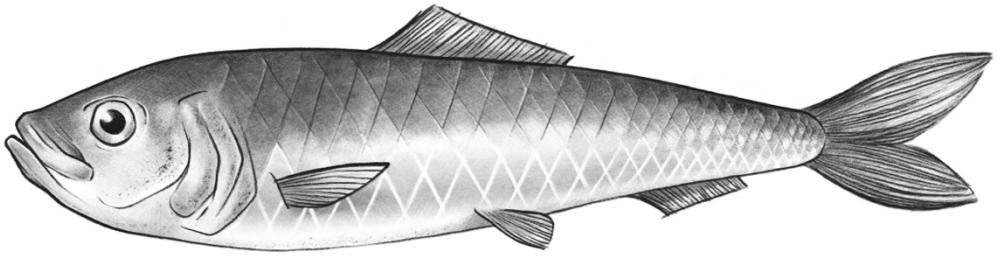
Discussion



The chapters in this thesis can be seen as a case study in marine historical ecology work. In Chapter 1, I laid the theoretical groundwork for how to incorporate ancient biomolecular research into the interdisciplinary framework of historical ecology, from project design to contextualization of results. In Chapter 2, I illustrated the ways in which one must challenge assumptions to push a field forward – why were we ignoring small fish bones? Here, I showed that the size of the bone was not a significant factor in the capacity of that bone to yield biologically meaningful DNA sequences.

Despite my ability to sequence these genomes, many of them yielded extremely sparse, low-coverage data. Therefore, in Chapter 3 we developed a software package that can handle ultra-low-quality sequences. By applying this software package to my ancient herring database, I was able to identify populations of origin for 64% of my specimens. This then provided the basis for Chapter 4, in which changing population of origin over time for Baltic assemblages hinted at an impact on herring ecology due to changing fishing pressure. By comparing the ancient samples and population genomics of modern herring, I was then able to demonstrate how genomic results can be seamlessly integrated with previously-published research in the fields of history, palaeoclimate, archaeology, ecology, fisheries science, and marine biology.

Assessing Research Aims

In the introduction to this thesis, I outlined a series of four overarching research aims. These were designed to address knowledge gaps surrounding Atlantic herring ecology and the development of the herring industry in Europe. I here assess whether or not each research aim was achieved and, if so, how this has contributed to our knowledge surrounding herring, European history, the field of ancient DNA, and possible avenues for the future.

a) Create a representative database of modern and ancient herring genomes for the east Atlantic and the Baltic. Despite accessibility limitations due to COVID and the vagaries of ownership of archaeological material, I managed to put together a dataset of 121 herring specimens from 16 sites across Europe. These sites spanned eight centuries and seven (contemporary) nations. Not all of these specimens yielded high quality DNA sequences, yet a large proportion yielded biologically meaningful information; Using *BAMscorer* (Chapter 3) I was able to assign a population of origin to 64% of these samples from 15/16 sites. In Chapter 4 we only scratched the surface of the questions that can be answered with this dataset. Additional questions – about the impact of the Dutch and East Anglian herring industries on the North Sea, the evolution of the Norwegian spring spawning herring, and the intriguing origin of the bones from Switzerland – had to be postponed after the onset of the repetitive strain injuries in my hand. I also spent three months conducting stable isotope extraction from herring bone collagen in the Dorothy Garrod lab at the University of Cambridge.

Every time I explained my project to an archaeologist or historian I was asked, "Oh, well why don't you use *this* site …" There are so many sites of import across Europe that it was impossible to sample from all of them. The main country left out of my sampling was Ireland, which had a herring industry distinct from that in Scotland and England. Despite my efforts, it was not possible to obtain these specimens, in part because the archaeologists involved were not entirely sure where they were or who had the power to send them to me for analysis. The truth of the matter is that there are always more specimens that can be added to a dataset. At some point one has to determine that sample collection has to stop in favor of analysis. But perhaps future researchers will be able to use and expand this dataset for additional questions that address fisheries I was not able to incorporate into this PhD.

As for the modern sequences, we have demonstrated thoroughly in Chapter 4 that our dataset – 53 samples sequenced for this thesis in conjunction with 19 publicly available samples – resolves herring population structure to an unprecedented degree. With this dataset, we were able to determine that metadata errors and possible sequence contamination/duplication were confounding the structure signals in previous studies (e.g., Han et al., 2020, see the Supplement to Chapter 4). The conclusion in Han et al. (2020) was that herring were really only differentiated when using a subset of loci under putative selective pressure. I suspect this is due to two factors; 1) combining SNP panels and poolseq with whole-genome sequencing in principal components analysis; 2) the inclusion of samples we have now found to be contaminated and/or technical duplicates.

My initial PCA results showed a strange pattern with many outliers (see Chapter 4, Supplementary Figure S2). After evaluating these PCA outliers, it was determined that they were biasing the PCA and after removal the population structure made perfect sense with what we know about Atlantic herring ecology. However, we found that the original studies were, despite their biases regarding whole-genome structuring, correct in their conclusions that the strongest segregating factors for herring were environmental adaptation (e.g., salinity and brackish conditions) and spawning season (Han et al., 2020; Lamichhaney et al., 2017, 2012; Pettersson et al., 2019). In fact, this structure appears strong enough in our cleaned dataset to drive whole-genome differentiation on a PCA (see Chapter 4, Figure 1C).

Thus, this thesis has succeeded in establishing a representative database of modern and ancient herring genomes in the eastern Atlantic and the Baltic. The majority of this database is publicly available on the European Nucleotide Archive and therefore free for use by other researchers. The remainder will be made available as future research projects are published. The hope is that myself and future scholars will continue to build on this public database and use it to answer additional questions regarding herring evolution and the development and impact of fisheries.

b) Characterize human impact and the development of intensive exploitation on the Atlantic herring populations through zooarchaeological analysis and whole-genome sequencing on a long time-series. The intention of this Research Aim was to be able to characterize human impact on herring around Europe, including the North Sea, the Norwegian Sea, and the Baltic. Unfortunately, injuries ensured that this was not to be the case. That being said, the Baltic study in Chapter 4 provides an excellent case study for understanding human impact. The Baltic has a long, well-documented history of exploitation and a highly structured herring population. Given known population collapses and currently high levels of fishing pressure in the region, it also provides a good example of how historical ecology work can inform management for both fisheries and adaptations to climate change.

The main potential critique of Chapter 4 is the question of migration patterns in herring. It has been documented that herring exhibit dramatic changes in migration routes in response to unknown factors that influence things like availability of zooplankton and population size. For example, the Bohuslän herring periods of the 17th and 19th centuries are thought to be the result of a changed migration pattern from the North Sea herring after a population boom (Alheit and Hagen, 1996; Corten, 1999). Similarly, the Norwegian spring spawning herring famously changed their migration patterns several decades ago after the commercial collapse of the stock (Claireaux et al., 2021). Herring do not instinctually know where to migrate; they learn where to feed and spawn from adult individuals in the stock (Corten, 2002). Thus, when a population collapse occurs this knowledge of spawning and feeding grounds is lost between generations

and a new migration route appears (Eliasen et al., 2021). Thus, it has been suggested to me that perhaps the fish migrated rather than declined due to exploitation pressure.

There are several reasons why this explanation is unlikely. First, herring do not appear to systematically change their migration patterns to avoid fishing pressure. The migratory patterns of the herring stocks in the North Sea have been documented for centuries and appear little changed today despite over 1000 years of exploitation (Barrett et al., 2004; Kowaleski, 2016; Poulsen, 2008; Trueman et al., 2017). Herring migrations have only been demonstrated to change when there is severe population contraction (as in the case of the Norwegian spring spawning herring in the 20th and 21st centuries) or when there is an outbreak population dynamic, as in the case of the Bohuslän periods. During the Bohuslän periods, the original spawning grounds were not vacated, instead additional grounds were found to accommodate the increased population size. Thus, in order for the western Baltic autumn-spawning herring to have changed its spawning grounds, there would have to have initially been a severe population decline in the region. We know from Chapter 4 that a population decline did begin around 1200 CE with the onset of the Øresund fishery and that palaeoclimate indicators suggest there should have been a population increase at this time (see Chapter 4; Discussion). Thus, there is a possibility that the western Baltic autumn-spawning herring would have shifted its migration and spawning grounds. However, the genetic evidence indicates this was not the case.

Coincidentally with the decline of the western Baltic autumn-spawning herring population illustrated in Chapter 4, the central Baltic autumn-spawning herring population began to increase (Chapter 4; Figure 3A,B). Is it possible that this is because the western Baltic herring began to colonize the central Baltic? This hypothesis has been suggested to me as an alternate explanation to the overfishing hypothesis – the herring weren't overexploited, they simply moved! As demonstrated in the previous paragraph, a migratory shift of this magnitude would actually *support* the hypothesis of overfishing. Further, the central Baltic autumn spawners have a distinct genetic signal in comparison to their extant western counterparts. The central Baltic is far more brackish than the Kattegat and Øresund regions, which are near-ocean salinity. Therefore, any western Baltic herring attempted to colonize the central Baltic would be at a distinct fitness disadvantage in comparison with the brackish-adapted herring that already inhabited those waters. Further, the western Baltic autumn spawning herring do still exist in the Kattegat, simply not in enough numbers to support a fishery, indicating they likely did not migrate away from the region.

This chapter, therefore, highlights the ways in which (archaeo)genomic data can answer long-held questions regarding the true impact of exploitation on marine species. There are, of course, many more case studies to be undertaken to fully comprehend human impact on the Atlantic herring. Because of the databases created to address Research Aim 1, these future studies can now be carried out. Using Chapter 4 as an example, it will be possible to conduct similar research on other fisheries using the same techniques and theory developed in Chapters 1,2, and 3. Thus, this Research Aim has still been addressed sufficiently to provide concrete avenues for continued study of this issue. I have also provided a case study that illustrates how human exploitation can impact herring populations.

c) Provide sustainable measures for informing fisheries policy in multiple countries relating to Atlantic herring. This was an ambitious aim for a PhD thesis, yet I believe it has – at least in part – been met. In Chapter 4, I demonstrated the utility of historical ecology in assessing fisheries impacts over a long time-scale. The analysis in this chapter shows that by the early

20th century herring stocks across the Baltic were already majorly impacted by fishing pressure. This highlights the false assumption implicit in policy recommendations for MSY in many fish stocks; namely, that stock sizes from 50-100 years ago are a good baseline for determining population health (Jackson, 2001; Pauly, 1995). Research in marine historical ecology such as this chapter has increasingly been used in policy formation to provide more holistic, sustainable management practices (Engelhard et al., 2016).

This chapter also illustrates the power of genomics in determining demographic independence and the question of interpopulation connectivity, which was a standing question in herring ecology (Aro, 2002; Bekkevold et al., 2005; Ruzzante et al., 2006; André et al., 2011). The results showing demographic independence for each stock strengthen the ICES recommendations to manage each biological unit separately. Through comparison with palaeoclimate data, I've further demonstrated the importance of understanding the different impacts various climatic factors can have on Baltic herring stocks, which is crucial for sustainable management.

The challenge inherent in providing scientific advice for fisheries policy is the question of quantification. Industry and economy want a number they can aim for; how many tonnes of fish can be taken from the environment? Research that highlights changes in effective population size (which, let's be honest, even many biologists don't fully understand) don't help much in directly determining fishing policies (although see Hare et al., 2011 for discussion of using Ne in management policies). Even when numbers are reached, such as the recommended MSY from ICES, there is no guarantee that industry will take these into account when determining total allowable catch (TAC) (Baltic Eye 2021).

Truly providing sustainable measures for fishery policy is a much larger question than can be addressed by one PhD student. That being said, coincident with the publication of Chapter 4, the EU was determining its TAC for the Baltic herring stocks. Given existing concern regarding the health of Baltic herring stocks, there was much attention on this decision, particularly in countries like Sweden (Carl André, pers. comm.). When Chapter 4 was published, the results were of high interest to media outlets in Sweden and Germany. While I can't say much about how this has impacted public opinion, what this does tell us is that people are hungry for these sorts of results. Perhaps part of the power of historical ecology, then, is in driving public opinion. While not always included as a component in scientific impact, public opinion is actually a key factor in conservation policy (Niemiec et al., 2022; Nilsson et al., 2020; Reddy et al., 2017), and therefore should not be overlooked. Modeling has shown that while herring are at risk from various factors, including climate change, fishing, and pollution, management strategy is the driving force behind long-term viability of the species (Froese et al., 2022; Niiranen et al., 2013).

d) Gain understanding of herring responses to climate change in the past to inform management policies for the future. This aim was again addressed in Chapter 4. While I originally intended to be able to look into more evolutionary impacts such as adaptation and natural selection, I instead assessed changes in effective population size in various stocks in relation to past climate change. Future efforts will have to assess the question of selection and more direct environmental modeling. Looking at demography in relation to past climate as a first step makes sense for several reasons. First, this highlights general trends we can use to inform both management and future research. Second, it illustrates the high demographic complexity in herring; different herring populations are affected quite differently by climate change despite all belonging to the same species. Recent research into the effects of climate change have

largely focused on comparatively short-term studies and lab experiments (Casini et al., 2010; Polte et al., 2021, 2017, 2014), which illustrate potentially worrying trends in various herring stocks' capacity to deal with warming temperatures and, for the Baltic, increasing brackishness. This study provides a first window into how these trends might play out over time.

The problem with using the past to understand possible future climate impacts is that we are rapidly exiting the climate window in which humans have ever lived (IPCC, 2021). Fully understanding how this might impact certain species will not be able to rely on historical ecology alone. Instead, results such as ours must be placed in the coming new context, which will rely on experimental study and modeling. Addressing climate change, then, highlights the necessity of crossing academic boundaries and incorporating all we know about a species into our predictions.

Unintended consequences of this thesis

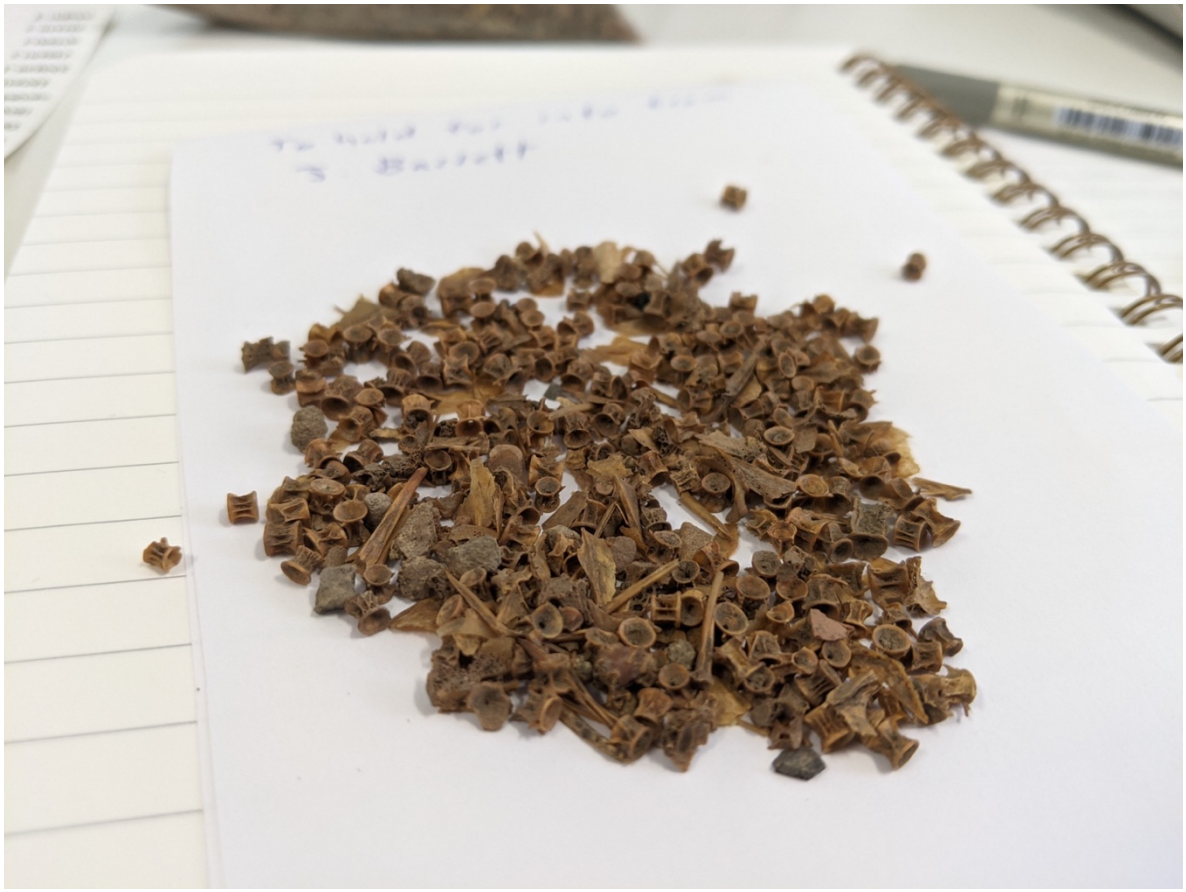
It will not escape the attention of the reader that many of my research aims were only addressed in Chapter 4. I quite clearly set out to do research on Atlantic herring three years ago, but what I failed to anticipate was the steps I would need to take along the way before these aims could be reached. I didn't intend to work on a new software program, interrogate aDNA laboratory practices, or evaluate theoretical approaches. But often, the unintended consequences are some of the most valuable aspects of a project. For me, this resulted in a chance to dive into the theoretical side of ecology as well as provide pathways to mitigate some of the ongoing ethical issues inherent to destructive analysis.

In Chapter 1, I set out to write a chapter for a Briefing Document put together for my International Doctoral Training Network (ITN), SeaChanges. By luck of the draw (literally), I ended up working with Fabricio Furni and Magie Aiken, and we managed to leave that first meeting with a solid question regarding what constituted an "industrial" fishery. Months of theory development ultimately led to the system of thresholds we introduced in Chapter 1, with the concept of "industrial" ultimately playing a minor role. This paper provided a theoretical backbone I used to guide the rest of the PhD.

In early 2021, just as Chapter 1 was concluding, I finally started receiving crucial archaeological samples. The initial results I was getting from lab work indicated that a lot of my data would be of very low quality. This was an issue that Bastiaan had long been considering, as it was not unique to herring. It was established that some biological characteristics could be determined with very small amounts of genomic data, for example determining sex (e.g., Barrett et al., 2020). Could we use this principle and apply it more widely than sex determination? How could we minimize the number of specimens that were discarded in the standard aDNA pipeline? Developing this software package with Bastiaan allowed me to exercise (and improve!) my computational skills. Further, it has allowed me to interrogate what I think of as being "good" data, something that has been reiterated to me by attendees of talks and workshops I've now given in how to use the software. Several researchers have reached out to me to state they are thrilled that they can use *BAMscorer* on their samples, which may otherwise not have been usable.

The winter-spring of 2021 was a long, strict lockdown in Oslo. My time was divided between programming *BAMscorer*, cross-country skiing, and weeks spent in the lab processing archaeological specimens. My samples had finally all arrived! I was able to extract DNA from 121 herring bones, which I used to assess the viability of tiny bones for aDNA analysis in Chapter 2. The argument for using small bones was thoroughly made in this chapter. All I will

state here is that I hope this study is of use to other researchers. aDNA research deals with many ethical issues and specimen waste is certainly not a small problem for the field. Chapter 2 is part of a growing area of development in ancient biomolecular research in which scientists work to minimize destructive impacts (e.g., Pinhasi et al., 2015; Scarsbrook et al., 2022; Sirak et al., 2017).



Herring bones ready to be sorted for biomolecular analysis.
Credit: Lane Atmore

Thus, Chapter 4 could not have been completed without the three steps developed in Chapters 1-3. The basis of Chapter 4 lies on samples that would traditionally not have been selected for whole-genome sequencing, that produced DNA sequences that were so low quality as to be nearly unusable. Yet, because of the work laid out in my initial chapters, I was able to demonstrate that important biological information could be gleaned from these specimens. This thesis, therefore, serves as a case study in reevaluating our relationship to waste in the aDNA pipeline.

Conclusions

At times during this thesis, I had the eerie sensation of what I've come to call "time vertigo." I read a paper on protests against the invention of bottom trawling in England that prompted a petition to King Edward III in 1376 (Jones, 2018). It read like a statement from Greenpeace. But that wasn't the wildest thing, that was the fact that trawling was *banned* in England and across Europe in response to this activism from fishers in the 14th century. The bans were repealed only when it became clear that governments would never be able to monitor fishing practices well enough. It wasn't until the 19th century, it appears, that governments stopped

listening to their fishermen (Roberts, 2007). It certainly makes one question the narrative of constant progress we've apparently made from the so-called "Dark Ages."

I was also struck by the similarities between medieval fisheries management and modern management practices. With the increase in technology after WWII (as discussed in the Introduction) (Holm, 2012; Pitcher and Lam, 2015), fisheries were increasingly able to target pelagic feeding aggregations of herring (which comprise multiple biological populations at once) in great numbers rather than spawning aggregations (consisting of one population) (Ruzzante et al., 2006). These fisheries often target multiple species at once (ICES 2019). This complicates management; smaller stocks are more vulnerable to overfishing yet the impact of exploitation on individual stocks is masked by management policies that fail to address biological populations (Moore et al., 2021). As a result, policies have increasingly been developed to target stocks as biological units as a component in a wider ecosystem rather than resources to be harvested merely for economic gains (FAO 1997; FAO 2020). Intriguingly, medieval fisheries typically targeted biological populations of herring, mostly in the form of spawning aggregations. Indeed, this was tightly controlled in terms of access to fishing grounds only during the spawning season – for example, both the Øresund and Dutch herring industries only allowed fishing during the autumn spawning aggregations (Poulsen, 2008; Jahnke, 2009). Some have argued these fisheries were more sustainable (e.g., Poulsen, 2008), although Chapter 4 obviously throws that assumption into question. Future research will have to focus on specific management techniques including regulations on fish size and TAC throughout time to better understand the nuances of sustainability in fishing.

We can't assume that improved technology will solve all our problems. As a case in point, despite seemingly-increasing returns due to new technology, the catch per unit effort continues to decline for fisheries across the world (Link and Watson, 2019). Our technology allows us to take more fish from the ocean, but we have to work ever harder for the same level of catch that would have been easy to pull from the sea only a few hundred years ago. Yet, increased technology without the proper management policies in place will result in overexploitation and ecosystem degradation (Hilborn et al., 2020). The question of marine ecology preservation through management was already a concern in 1387 when fishers petitioned King Edward III to ban trawling and when Øresund fishers raised concerns about overfishing in the 15th century. Today, we have much the same concerns as our ancestors – the destruction of trawling, the dangers of overfishing, weighing the economy as more important than the environment.

Now, all this is not to say that fisheries in the Middle Ages were always better managed than modern industries, nor that people in the past knew more than we do now. This is merely to highlight the importance of historical literacy. Learning from (or even returning to) past management strategies because they worked better than what we are currently doing is not a failure. Refusing to recognize that we have made mistakes and previous generations may have created better solutions, however, *is* a failure. It's the unfortunate fate of the contemporary historical ecologist that we are conducting such work during the anthropogenic sixth mass extinction and devastating climate change. Our research is inherently depressing. There is something twisted about the sense of achievement one feels when their results show fisheries-induced devastation and impending climate-induced destruction. It makes for a compelling story – people are thirsty for stories about how we've trashed the world these days. The ability to reach back through millennia and uncover the impact of management techniques across space and time is the unparalleled strength of historical ecology. We have so many examples of different relationships with nature, management techniques, and modes of extraction. In the current fight against the biospheric emergency, why would we not take advantage of that?

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